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# Survey of Diphenyl Ether Herbicides in Dietary Foods by the Total Diet Study in Osaka, Japan

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Diphenyl ether herbicides have been scattered mainly to paddy fields from May to June. The compounds used in large quantities in Japan are 2,4-dichlorophenyl-4'-nitrophenyl ether (NIP), 2,4,6-trichlorophenyl-4'-nitrophenyl ether (CNP) and 2,4-dichlorophenyl-3'-methoxy-4'-nitrophenyl ether (X-52). They have been detected recentry in some fishes and shellfishes (Gretch et al. 1979; Yamagishi et al. 1979; Ishikawa et al. 1980; Watanabe et al. 1981). Therefore, we examined whether or not other foods were contaminated with them by total diet study method (also referred to as market basket program), and their maximum daily dietary intakes in Japan are presented in this paper.

## MATERIALS AND METHODS

NIP, CNP, X-52, n-hexane, ethanol, anhydrous sodium sulfate, acetonitrile, ethyl acetate and sodium chloride of pesticide grade and silver nitrate of special reagent grade were used. Florisil (80-100 mesh, Floridin Co.) was activated at 130°C overnight. Gas chromatography was Shimadzu GC-7A, equipped with a Ni electron capture detector (ECD). Operating conditions were as follows : column; 2% OV-17, 2% DEGS, on Gas chrom Q (100-120 mesh), Glass column size; 2 mm X 1.8 m column temperature; 235°C, injector and detector temperature; 280°C. Gas chromatography-mass spectrometer (GC-MS) GC: Hewlett packard 5790 A. Operating conditions: column; 2% OV-1 on Gas Chrom Q (100-120 mesh), glass column size; 2 mm x 1.8 m, column temperature; 200°C. MS: Single ion monitor (SIM) mass spectra were measured with a JEOL DX-300 connected with a data system JEOL JMA-3500. The mass spectrometer was operated at 70 eV of electron energy, 2.5 kV of accelerating voltage and 250° C of source temperature.The choice of foods collected for the market basket samples and their consumption values are based on a food consumption survey conducted by the Ministry of Health and Welfare of Japan and the Health Department of Osaka Prefecture (1981). The samples collected by means of the market basket sampling method in June 1982 were separated into 14 food classes (Table-1 ). Twenty grams of the homogenized sample (food groups 1-3, 5-14) were placed in a 500 ml separatory funnel and extracted

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with acetonitrile (40 ml X 3). The obtained acetonitrile layer was poured into 300 ml of 3% sodium chloride solution and shaken with n-hexane (200 ml X 1). After the n-hexane layer was washed with 100 ml of water, the organic layer was dried with anhydrous sodium sulfate and concentrated to 5 ml. Five grams of sample (4th group) was poured into 200 ml of 5% sodium chloride solution and shaked with n-hexane (100 ml X 3). After the n-hexane layer was washed with 100 ml of water, the organic layer was dried and concentrated, and its fat content was measured. Two grams of the fat was placed in a 100 ml separatory funnel, mixed with n-hexane (15 ml) and extracted with acetonitrile saturated with n-hexane (30 ml X 3). The acetonitrile layer was poured into 600 ml of 5% sodium chroride solution and shaked with n-hexane (100 ml). Then the n-hexane layer was washed with water (100 ml), dried and concentrated to 5 ml. Silver nitrated florisil (Suzuki et al. 1979) was packed into glass column (15 mm id) with n-hexane and 2 g of anhydrous sodium sulfate was put on the top. After flowing 50 ml of n-hexane, the fraction containing diphenyl ether herbicides were eluted with 100 ml of 2% ethyl acetate in n-hexane. The eluted solution was again concentrated to an appropriate volume and used as a sample for ECD gas chromatography.

## RESULTS AND DISCUSSION

First, gas chromatography columns were examined to get good seperation of the diphenyl ether herbicides from organochlorine pesticides. On an OV-17 column, CNP and NIP were found to overlap on p,p'- and o,p'-DDTs, respectively. DEGS column gave a fairly good separation between those three compounds (Ishikawa et al. 1980).

In order to obtain cleaner samples for gas chromatography, we sought appropriate clean up conditions. NIP, CNP and X-52 were injected onto the silver- nitrated florisil column (3 g). They were fractionated into 10 ml with 2% ethyl acetate in n-hexane and analysed by ECD-GC (Fig.1). NIP and CNP were eluted at 10-20 ml and X-52 was eluted at 20-60 ml. Coloring materials of both vegetables and fishes (carotenes or organic sulfides) were satisfactorily eliminated through the silver- nitrated florisil column. For the recovery determination of samples, we selected two low fat sample groups (1st and 7th) and two high fat sample groups (4th and 10th). Recovery tests were carried out by spiking samples with 1 μg each of NIP, CNP and X-52 prior to the application on the silver nitrated florisil column (table 2). Overall recoveries (%) of NIP, CNP and X-52 were between 79.7 and 95.5 ,between 82.4 and 99.9 and between 82.6 and 101.4 respectively. We deduced those recovery values to be satisfactory and adopted these analytical conditions to the food analysis mentioned below. Food groups (No.1-13) were collected at June, 1982 and analysed. Food contamination with CNP (Table 3) was found in the 7th group (0.02 μg) and 10th group (1.00 μg). X-52 was found in the 7th group (0.08 μg). The reason of detection of CNP and X- 52 in those food groups was inferred as follows. In the case of the 7th group, the pesticides (CNP and X-52) mainly spread to paddy fields in May and June was inferred to be the origin of contamination at vegetable

Table 1. Constitution of the food groups

| No. | Food composition |
|---|---|
| 1 | Rice and processing rice |
| 2 | Cereals except group 1 |
| 3 | Sugars and confectionaries |
| 4 | Oils and fats |
| 5 | Beans |
| 6 | Fruits |
| 7 | Vegetables ( green and yellow ) |
| 8 | Vegetables except group 7 and seaweeds |
| 9 | Seasonings and beverages |
| 10 | Fishes and shellfishes |
| 11 | Meats and eggs |
| 12 | Milks |
| 13 | Prepared foods |
| 14 | Drinking water |

Total dietary intake of food is 1.4 kg / day.

Table 2. Recovery of diphenyl ether herbicides from fortified samples

| Sample | Recovery ( % )* | | |
|---|---|---|---|
| | NIP | CNP | X-52 |
| 1st food group (20 g) | 95.5 ±4.28 | 93.5± 2.59 | 101.4 ±2.99 |
| 4th food group (5 g) | 79.7 ±1.25 | 82.4± 0.87 | 82.6 ±1.60 |
| 7th food group (20 g) | 81.7 ±1.40 | 87.3± 2.22 | 99.9 ±0.85 |
| 10th food group (5 g) | 78.6 ±1.32 | 99.9± 0.82 | 100.8 ±1.31 |

*Average of five determinations ( AV ± SD ); 1 μg of each herbicide was added to each food group prior to the clean up.

Table 3. Maximum daily Intake (μg/day) of the herbicides calculated from the data on June, 1982

| | Max. daily intake (μg/day) | | | Max. daily intake/weight | ADI |
|---|---|---|---|---|---|
| | Food group 7 | 10 | total | (μg/day/kg)** | (μg/day/kg)** |
| CNP | 0.02 | 1.00 | 1.02 | 0.02 | 2.04 |
| X-52 | 0.08 | ND | 0.08 | ND | NA* |
| NIP | ND* | ND | ND | ND | NA |

*ND: not detected, *NA: not available. The other food groups were all ND. **The average weight of the consumer was designated to be 50 kg (Ministry of Health and Welfare, Japan). Food tolerance for CNP is 0.1 ppm in vegetables by the Environmental Agency, Japan.

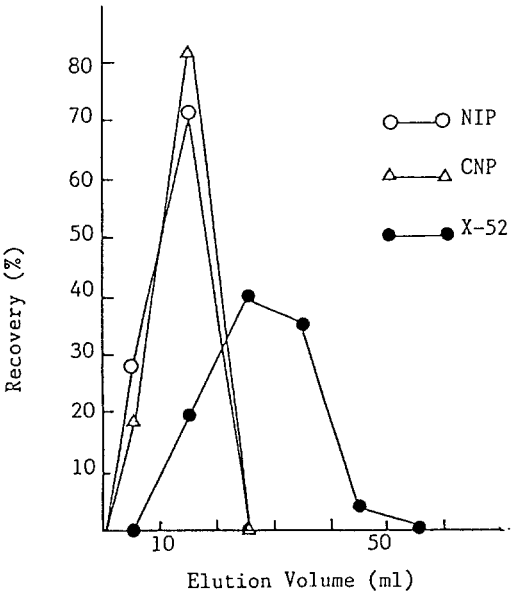


Figure 1. Elution profiles of herbicides from silver nitrated florisil column by 2% ethyl acetate in n-hexane for clean-up of samples

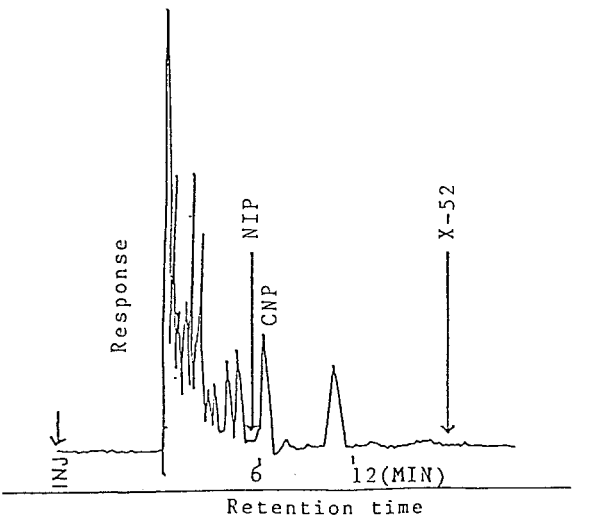


Figure 2. Gas chromatogram of the 10 th food group
Clean up: Silver nitrated florisil column/2% ethyl acetate in n-hexane (100 ml) Column: 2 mm x 1.8 m, Packing 2% DEGS on gas-chrom Q,Column temp 235°C, Injecter temp and Detecter temp 280°C, Detecter: Electron capture detecter

gardens (Murakami and Tanaka. 1985). The 10th food group was composed of fishes, and the origin of the contamination was inferred to be the same as the pesticide (CNP) mainly spread to paddy fields in May and June (Yamagishi et al. 1979). We deduce the importance of time of sampling for the detection of those herbicides in vegetables and fishes. Since diphenyl ether herbicides were not detected in both samples collected on December, 1980 and September, 1981 (unpublished data). Hence, the value of daily intake obtained at this time (1982, June) should be lowered for the value of one year. The gas chromatogram of CNP from the 10th food group is shown in Fig 2. As can be seen in the figure, no interferring peak was found in the extract. CNP and X-52 in food groups were confirmed by the retention time and relative intensity of mass fragmentgraphy ( M/Z 317 and 319 and M/Z 313, 283) by GC-MS (SIM).

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