

Solid-Phase Extraction for Cleanup of Pesticide Residues Suspected as Endocrine Disruptors in Foods

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Solid-phase extraction cartridges, Silica, Florisil, ethylenediamine-*N*-propylsilane-bonded silica (PSA), trimethylaminopropylsilane-bonded silica (SAX) and graphitized nonporous carbon (ENVI-Carb) were investigated for cleanup of pesticides residues suspected as endocrine disruptors in food extracts. Elution patterns and recoveries were examined for 38 pesticides with 2 types of eluting solvents: acetone/*n*-hexane and diethyl ether/*n*-hexane. A 2% acetone-*n*-hexane eluant used with the Silica cartridge gave the best recovery for 33 pesticides and minimized interference from coextractants. The remaining 5 pesticides were eluted successively with 20% acetone-*n*-hexane from tandem connected Silica and PSA cartridges. Thirty-eight pesticides were analyzed in 30 duplicate meals with the limits of detection ranging from 0.01 to 0.5 ppb. *trans*-Chlordane, *trans*-nonachlor and *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) were found frequently (Tr.-1.1 ppb). Dietary daily intakes were 0.27 $\mu\text{g}/\text{d}$ for total chlordane, 0.85 $\mu\text{g}/\text{d}$ for total dichlorodiphenyltrichloroethane (DDT), which were significantly below the acceptable daily intake (ADI) values.

Key words — solid-phase extraction, pesticide residue, duplicate meal, endocrine disruptor, ultramicroanalysis

INTRODUCTION

Solid phase extraction (SPE) cartridges are widely used for extract cleanup prior to GC

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analysis of pesticide residues in foods.¹⁻⁶⁾ SPE procedures save analytical time, reagent costs, and reduce the use of hazardous solvents. We previously developed a multiresidue pesticide method by GC/MS using octadecylsilane-bonded silica (ODS) and ethylenediamine-*N*-propylsilane-bonded silica (PSA) cartridges^{7,8)} and have applied it to the monitoring of pesticide residues at the level of 0.01 ppm in agricultural products.

Recently, endocrine disrupting chemicals have been noted all over the world, and pesticides account for about two-thirds of chemicals which are suspected as endocrine disruptors (EDs).⁹⁾ EDs can act at an extremely low concentration, so ultramicroanalyses are required to clarify the exposure from foods.

Thirty-eight pesticides containing not only organochlorine pesticides, but also other polar ones were selected as the analytical objects of this study. Aiming at detection of 0.1 ppb level in foods, 5 kinds of SPE cartridges (Silica, Florisil, PSA, trimethylaminopropylsilane-bonded silica (SAX) and graphitized nonporous carbon (ENVI-Carb)) were tested to develop more effective cleanup procedures. Cleanup of organochlorine pesticides in foods using Silica,⁵⁾ Florisil^{4,5)} and ENVI-Carb⁶⁾ cartridges with the combination of non- and low- polar eluting solvents has already been reported. On the other hand, ENVI-Carb and anion-exchange cartridges such as SAX (strong), PSA and NH₂ (weak) have been used with relative polar eluting solvents for multiresidue pesticide analyses at the level of 0.01 ppm.^{2,7,8,10)}

In this study, fractionation in the cleanup process with Silica and PSA cartridges, and 20 times concentration of the test solutions for GC/MS (selected ion monitoring (SIM) mode) analysis were introduced, which enabled measurement of the residues with detection limits ranging from 0.01 to 0.5 ppb.

MATERIALS AND METHODS

Food Samples — Thirty duplicate meals for adults were collected at hospital cafeterias (10 of each breakfast, lunch and dinner). Nine of these 10 breakfasts included bread and the other meals included rice. Average weight was 445 \pm 108 g for breakfast, 682 \pm 183 g for lunch and 796 \pm 157 g for dinner.

Analytical Standards — Pesticide standards were purchased from Kanto Chemical (Tokyo, Japan),

Hayashi Pure Chemical Industries (Osaka, Japan) and Wako Pure Chemical Industries (Osaka, Japan).

Stock standard solutions (500 $\mu\text{g}/\text{ml}$) were prepared in *n*-hexane for each organochlorine and pyrethroid pesticide (heptachlor, heptachlor epoxide (*endo*-, *exo*-), dichlorodiphenyltrichloroethane (DDT) (*p,p'*-, *o,p'*-), dichlorodiphenyldichloroethane (DDD) (*p,p'*-), dichlorodiphenyldichloroethylene (DDE) (*p,p'*-), endosulfan (α -, β -), endosulfan sulfate, benzene hexachloride (BHC) (α -, β -, γ -, δ -), ardrin, dieldrin, endrin, dicofol, methoxychlor, mirex, hexachlorobenzene, permethrin, cypermethrin and fenvalerate), and in acetone for the others (trifluralin, vinclozolin, alachlor, nitrofen, malathion, parathion, simazine, atrazine, metribuzin and carbaryl). The stock standard solutions were admixed and diluted with acetone to make a mixed standard solution (10 $\mu\text{g}/\text{ml}$). The mixed standard solution and chlordane standard solutions (10 $\mu\text{g}/\text{ml}$) were admixed and diluted with acetone to make a mixed fortification solution (1 $\mu\text{g}/\text{ml}$). Working standard solutions 0.004, 0.04 and 0.4 $\mu\text{g}/\text{ml}$ for GC/MS analysis were freshly prepared in 20% acetone-*n*-hexane from the mixed fortification solution.

SPE Cartridges — ODS: Isolute C18 (endcapped), 6 ml, 1 g (International Sorbent Technology (IST), Hengoed, U.K.); Silica and Florisil: Sep-pak Vac RC, 500 mg (Waters, Milford, U.S.A.); PSA and SAX: Isolute, 3 ml, 500 mg (IST); ENVI-Carb: Supelclean, 6 ml, 500 mg (Supelco, Bellefonte, U.S.A.)

Instrumentation — Gas chromatography with mass-selective detection was performed on a HP5890 Series II GC system with a HP5972 MSD system (Hewlett Packard, Palo Alto, U.S.A.). The following analytical conditions were used: a 5% phenyl-methyl silicone column (DB-5ms, 30 m \times 0.25 mm i.d., 0.10 μm film thickness (J&W Scientific, Folsom, U.S.A.)); initial oven temperature 80°C, hold 3 min, linear gradient 30°C/min to 170°C, hold 4 min, then linear gradient 10°C/min to 270°C, hold 15 min; injector temperature 250°C; interface temperature 280°C; initial carrier gas (He) pressure 30 psi (4 ml/min), hold 1 min, decrease immediately to 8 psi, keep constant flow (0.9 ml/min); injection volume 4 μl (splitless mode); purge off time 1 min; ionization energy 70 eV. The pesticides were detected in the SIM mode.

Elution of Pesticide Standard Solutions through the SPE Cartridges — Silica, Florisil, PSA, SAX and ENVI-Carb SPE cartridges were tested.

Eluting solvents were acetone and *n*-hexane: The mixed fortification solution (0.5 ml) was evaporated under a stream of nitrogen, the residue was

dissolved in 2 ml of *n*-hexane and then transferred to each cartridge conditioned with 10 ml of 20% acetone-*n*-hexane and *n*-hexane. Eight ml of *n*-hexane was added first and eluates were collected together (1st fraction). Then, successive elution with 10 ml of 1, 2, 5, 10 and 20% acetone-*n*-hexane was performed on each cartridge and eluates were collected separately (2nd to 6th fraction).

Eluting solvents were diethyl ether and *n*-hexane: Pesticides were transferred to each cartridge conditioned with 10 ml of 30% diethyl ether-*n*-hexane and *n*-hexane and eluted with *n*-hexane as described above. Then, successive elution with 10 ml of 2, 5, 10, 15 and 30% diethyl ether in *n*-hexane was performed. Collected eluates were evaporated and adjusted to 2 ml with 20% acetone-*n*-hexane for GC/MS analysis, respectively.

To investigate the recovery from PSA cartridge with 4 eluting solvents (2, 5, 10 and 20% acetone-*n*-hexane), the cartridges were conditioned with 10 ml of each solvent. The mixed fortification solution (0.1 ml) was evaporated under a stream of nitrogen. The residue was dissolved in 2 ml of each eluting solvent and transferred to the cartridge, then eluted with 8 ml of the same solvent. Collected eluates were submitted to GC/MS analysis as described above.

Elution of Interfering Materials in Food Extracts through the SPE Cartridges — Crude extracted solutions of various agricultural products (rice, lettuce, banana *etc.*) obtained by acetonitrile extraction and ODS purification⁷⁾ were mixed, evaporated and dissolved in *n*-hexane to make a 1 ml aliquot correspond to *ca.* 10 g matrix. After conditioning of Silica, Florisil, PSA, ENVI-Carb and SAX SPE cartridges with 10 ml of 20% acetone-*n*-hexane and *n*-hexane, the *n*-hexane extracts (1 ml) were transferred to each cartridge and 9 ml of *n*-hexane was added. Then, successive elutions with 10 ml of 2, 5, 10 and 20% acetone-*n*-hexane were performed and eluates were collected separately. Collected eluates were evaporated and adjusted to 0.5 ml with 20% acetone-*n*-hexane for GC/MS analysis (scan mode), respectively.

Sample Preparation Method for Duplicate Meals and Recovery Test — Fifteen gram portions of homogenized samples were extracted with 60 ml acetonitrile and 10 ml water, then the extracts were cleaned up through an ODS cartridge conditioned with 10 ml of acetonitrile and water. The acetonitrile was separated by salting-out with NaCl and 40 ml of the acetonitrile layer was collected. After evaporating to dryness, the residue was adjusted to 2.5 ml with 2% acetone-*n*-hexane. A 2 ml aliquot was loaded on

a Silica cartridge conditioned with 10 ml of 2% acetone-*n*-hexane. The first fraction was eluted with 13 ml of 2% acetone-*n*-hexane, then a PSA cartridge conditioned with 10 ml of 20% acetone-*n*-hexane was attached below the Silica cartridge. The second fraction was eluted from the tandem cartridge with 10 ml of 20% acetone-*n*-hexane. The eluates of each fraction were evaporated and adjusted to 0.4 ml with 20% acetone-*n*-hexane for GC/MS analysis. A 1ml aliquot of the final 20% acetone-*n*-hexane solution corresponds to 20 g matrix (Fig. 1).

For recovery test of 38 pesticides from duplicate meals, 15 g portions of homogenized samples (bread

type breakfast and rice type dinner) were fortified with 30 μ l of the mixed fortification solution, and analyzed as described above.

RESULTS AND DISCUSSION

Cleanup with SPE Cartridges

Elution patterns of pesticide standard solutions from 5 kinds of SPE cartridges were examined with 2 types of eluting solvents. The Silica cartridge with acetone-*n*-hexane eluants gave the most efficient recovery, shown in Table 1.

Of the 38 compounds, 34 were eluted by the

CH₃CN Extraction

Sample 15g
CH₃CN 60ml + H₂O 10ml
homogenize and filter

ODS Cleanup

pass through ODS(1g) cartridge

Salting-out

shake eluate with NaCl 6g at pH7
collect CH₃CN layer 40ml
(equal to sample 10g)
dehydrate and evaporate
adj. to 2.5ml with
2% acetone in *n*-hexane

Silica Cleanup

load 2ml aliquot on
Silica(500mg) cartridge
add 2% acetone in *n*-hexane 13ml
collect all eluate and evaporate
→[First fraction]

Silica+PSA Cleanup

attach PSA(500mg) cartridge
below Silica(500mg) cartridge
add 20% acetone in *n*-hexane 10ml
collect eluate and evaporate
→[Second fraction]

GC/MS(SIM) analysis

adj. each fraction to 0.4ml with
20% acetone in *n*-hexane
(equal to sample 20g/ml)

Table 1. Recoveries of Pesticides Eluted into Each Fraction from Silica SPE Cartridge

Pesticide	Recovery (%)						Total
	% of acetone in <i>n</i> -hexane ^{a)}						
	0%	1%	2%	5%	10%	20%	
<i>trans</i> -Chlordane	123	0	0	0	0	0	123
<i>cis</i> -Chlordane	122	0	0	0	0	0	122
<i>trans</i> -Nonachlor	124	0	0	0	0	0	124
<i>cis</i> -Nonachlor	118	0	0	0	0	0	118
Oxychlordane	124	0	0	0	0	0	124
Heptachlor	119	0	0	0	0	0	119
Heptachlorepoxide	117	0	0	0	0	0	117
<i>p,p'</i> -DDE	124	0	0	0	0	0	124
<i>p,p'</i> -DDD + <i>o,p'</i> -DDT	124	0	0	0	0	0	124
<i>p,p'</i> -DDT	128	0	0	0	0	0	128
α -Endosulfan	124	0	0	0	0	0	124
β -Endosulfan	0	0	117	0	0	0	117
Endosulfan sulfate	0	0	107	6	0	0	114
α -BHC	108	0	0	0	0	0	108
β -BHC + γ -BHC	115	0	0	0	0	0	115
δ -BHC	0	95	0	0	0	0	95
Aldrin	119	0	0	0	0	0	119
Dieldrin	0	118	0	0	0	0	118
Endrin	41	87	0	0	0	0	128
Dicofol	0	122	0	0	0	0	122
Methoxychlor	0	133	0	0	0	0	133
Mirex	120	0	0	0	0	0	120
Hexachlorobenzene	117	0	0	0	0	0	117
Permethrin	0	137	0	0	0	0	137
Cypermethrin	0	143	0	0	0	0	143
Fenvalerate	0	141	16	0	0	0	156
Trifluralin	118	0	0	0	0	0	118
Vinclozolin	0	110	0	0	0	0	110
Alachlor	0	0	107	0	0	0	107
Nitrofen	0	139	0	0	0	0	139
Malathion	0	0	115	0	0	0	115
Parathion	0	122	0	0	0	0	122
Simazine	0	0	0	118	0	0	118
Atrazine	0	0	0	126	0	0	126
Metribuzin	0	0	0	125	0	0	125
Carbaryl	0	0	0	77	45	0	121

a) Pesticides were loaded with *n*-hexane and eluted successively with 10 ml of each solvent.

Fig. 1. Sample Preparation Method for Duplicate Meals by Extraction and Cleanup with SPE Cartridges

Table 2. Recoveries of Pesticides from PSA SPE Cartridge Loaded with 0–20% Acetone in *n*-Hexane

Pesticide	Recovery (%)										
	% of acetone in <i>n</i> -hexane ^{a)}						Total	% of acetone in <i>n</i> -hexane ^{b)}			
	0%	1%	2%	5%	10%	20%		2%	5%	10%	20%
Endosulfan sulfate	0	0	42	3	0	0	45	12	108	121	118
δ -BHC	0	0	12	0	0	0	12	93	91	117	111
Vinclozolin	0	0	0	0	0	0	0	81	93	114	116
Malathion	0	0	0	0	0	0	0	89	112	121	129
Metribuzin	0	0	15	0	0	0	15	27	47	69	102
Carbaryl	0	0	0	18	0	0	18	0	15	126	139

a) Pesticides were loaded with *n*-hexane and eluted successively with 10 ml of each solvent.

b) Pesticides were loaded and eluted with 10 ml of each solvent.

fraction of 0–2% acetone in *n*-hexane. Simazine, atrazine, metribuzin and carbaryl were retrievable with the 5% acetone–*n*-hexane fraction. Almost the same results were obtained with diethyl ether–*n*-hexane eluants, but the elution of alachlor and malathion was somewhat delayed. Except for the low recovery of β -endosulfan and delayed elution of endosulfan sulfate, elution patterns from the Florisil cartridge bore a close resemblance to those of the Silica cartridge. The SAX cartridge also showed similar elution patterns, which were somewhat retarded in comparison with the Silica cartridge.

As a result of elution tests of interfering materials in food extracts, comparatively little was eluted from these cartridges into the fractions with low-polar solvents. The Silica cartridge showed a better cleanup effect than other SPE cartridges in the 0–2% acetone–*n*-hexane fraction. However, by switching to more polar eluting solvents, interfering peaks were increased. The peaks of fatty acids (palmitic acid and linoleic acid), which were the main interference in GC/MS analysis, were eluted in the 10% acetone–*n*-hexane fraction from the Silica and SAX cartridges, and in the 20% acetone fraction from the Florisil cartridge.

The fatty acids were retained on the PSA cartridge and not eluted in the 0–20% acetone in *n*-hexane fraction. However, many other interfering peaks, which were not so large as the fatty acid peaks, were detected, even in the fractions with low-polar solvents. Loaded with *n*-hexane, 32 pesticides were recovered from the PSA cartridge in the 0–5% acetone in *n*-hexane fraction. Endosulfan sulfate, δ -BHC, metribuzin and carbaryl showed low total recoveries. Vinclozolin and malathion were not recovered, even at a higher acetone ratio in the eluant. On the other hand, increasing solvent polarity for loading

Table 3. Recoveries of Fortified Pesticide Residues from Duplicate Meals by Extraction and Cleanup with SPE Cartridges

Pesticide (2 ppb ad.)	Recovery (%; mean \pm S.D., <i>n</i> =3)			
	Breakfast		Dinner	
	I	II	I	II
<i>trans</i> -Chlordane	64 \pm 4	0 \pm 0	66 \pm 3	0 \pm 0
<i>cis</i> -Chlordane	64 \pm 3	0 \pm 0	65 \pm 4	0 \pm 0
<i>trans</i> -Nonachlor	61 \pm 4	0 \pm 0	63 \pm 3	0 \pm 0
<i>cis</i> -Nonachlor	68 \pm 4	0 \pm 0	70 \pm 1	0 \pm 0
Oxychlordane	66 \pm 5	0 \pm 0	66 \pm 4	0 \pm 0
Heptachlor	57 \pm 3	0 \pm 0	57 \pm 4	0 \pm 0
Heptachlorepoxide	70 \pm 4	0 \pm 0	69 \pm 4	0 \pm 0
<i>p,p'</i> -DDE	63 \pm 2	0 \pm 0	72 \pm 1	0 \pm 0
<i>p,p'</i> -DDD	72 \pm 6	0 \pm 0	84 \pm 4	0 \pm 0
<i>p,p'</i> -DDT	67 \pm 2	0 \pm 0	75 \pm 2	0 \pm 0
<i>o,p'</i> -DDT	56 \pm 6	0 \pm 0	77 \pm 4	0 \pm 0
α -Endosulfan	85 \pm 6	0 \pm 0	88 \pm 6	0 \pm 0
β -Endosulfan	98 \pm 3	0 \pm 0	98 \pm 8	0 \pm 0
Endosulfan sulfate	16 \pm 12	65 \pm 16	11 \pm 7	67 \pm 10
α -BHC	71 \pm 3	0 \pm 0	67 \pm 6	0 \pm 0
β -BHC	81 \pm 4	0 \pm 0	80 \pm 1	0 \pm 0
γ -BHC	64 \pm 5	0 \pm 0	57 \pm 5	0 \pm 0
δ -BHC	69 \pm 2	0 \pm 0	76 \pm 5	0 \pm 0
Aldrin	43 \pm 3	0 \pm 0	46 \pm 4	0 \pm 0
Dieldrin	66 \pm 5	0 \pm 0	76 \pm 3	0 \pm 0
Endrin	70 \pm 1	0 \pm 0	70 \pm 2	0 \pm 0
Dicofol	63 \pm 4	0 \pm 0	75 \pm 6	0 \pm 0
Methoxychlor	95 \pm 1	0 \pm 0	96 \pm 4	0 \pm 0
Mirex	36 \pm 1	0 \pm 0	44 \pm 3	0 \pm 0
Hexachlorobenzene	36 \pm 2	0 \pm 0	40 \pm 1	0 \pm 0
Permethrin	81 \pm 1	0 \pm 0	111 \pm 6	0 \pm 0
Cypermethrin	107 \pm 4	0 \pm 0	146 \pm 19	0 \pm 0
Fenvalerate	89 \pm 12	0 \pm 0	67 \pm 6	0 \pm 0
Trifluralin	82 \pm 1	0 \pm 0	76 \pm 5	0 \pm 0
Vinclozolin	75 \pm 2	0 \pm 0	72 \pm 4	0 \pm 0
Alachlor	85 \pm 8	0 \pm 0	82 \pm 7	0 \pm 0
Nitrofen	100 \pm 4	0 \pm 0	122 \pm 7	0 \pm 0
Malathion	88 \pm 7	4 \pm 4	86 \pm 6	4 \pm 4
Parathion	111 \pm 13	0 \pm 0	117 \pm 5	0 \pm 0
Simazine	0 \pm 0	94 \pm 4	0 \pm 0	92 \pm 13
Atrazine	0 \pm 0	76 \pm 7	0 \pm 0	74 \pm 8
Metribuzin	0 \pm 0	48 \pm 3	0 \pm 0	37 \pm 4
Carbaryl	0 \pm 0	145 \pm 8	0 \pm 0	91 \pm 14

I : Fraction eluted with 15 ml of 2% acetone in *n*-hexane from Silica (500 mg).

II : Fraction eluted with 10 ml of 20% acetone in *n*-hexane from Silica (500 mg)+PSA (500mg).

enhanced recoveries and 20% acetone-*n*-hexane was required for metribuzin (Table 2).

The ENVI-Carb cartridge was effective for the removal of the pigment, but most of interfering peaks, including fatty acids, were eluted into the first *n*-hexane fraction, together with most pesticides. Hexachlorobenzene, simazine, atrazine and carbaryl were adsorbed on it and eluted slowly.

Sample Preparation for Duplicate Meals

The first half of the sample preparation method shown in Fig. 1 is the same as the California Department of Food and Agriculture (CDFA) method,¹⁾ and has been applied to agricultural products. In the ODS cleanup step, the acetonitrile ratio had to be kept at *ca.* 75% to get good recoveries of pesticides and to remove interferences detected by GC/MS at long retention times.

Table 4. Pesticide Residues in Duplicate Meals

Pesticide	Breakfast		Lunch		Dinner		LOD (ppb)	LOQ (ppb)
	No. ^{a)}	Conc.(ppb)	No. ^{a)}	Conc.(ppb)	No. ^{a)}	Conc.(ppb)		
<i>trans</i> -Chlordane	8	Tr.(8) ^{b)}	9	Tr.(6) ^{b)} -0.09	10	Tr.(6) ^{b)} -0.34	0.01	0.03
<i>cis</i> -Chlordane	3	Tr.(3)	6	Tr.(3)-0.09	8	Tr.(4)-0.23	0.01	0.03
<i>trans</i> -Nonachlor	7	Tr.(7)	7	Tr.(3)-0.09	9	Tr.(5)-0.34	0.01	0.03
<i>cis</i> -Nonachlor	0		5	Tr.(3)-0.08	5	Tr.(3)-0.15	0.01	0.03
Oxychlordane	0		1	Tr.(1)	2	Tr.(2)	0.03	0.1
total Chlordane	9	Tr.(5)-0.06	9	Tr.(3)-0.44	10	Tr.(2)-1.11		
Heptachlor	0		1	0.07	2	0.06	0.01	0.03
Heptachlorepoxyde	9	Tr.(8)-0.08	6	Tr.(5)-0.15	6	Tr.(2)-0.14	0.01	0.03
<i>p,p'</i> -DDE	10	0.11-0.26	10	0.03-0.33	10	0.06-1.01	0.01	0.03
<i>p,p'</i> -DDD	1	Tr.(1)	2	Tr.(1)-0.1	5	Tr.(1)-0.8	0.03	0.1
<i>p,p'</i> -DDT	3	Tr.(3)	5	Tr.(4)-0.1	8	Tr.(2)-0.6	0.03	0.1
<i>o,p'</i> -DDT	1	Tr.(1)	2	Tr.(2)	5	Tr.(1)-0.2	0.03	0.1
total DDT	10	0.1-0.4	10	0.03-0.6	10	0.1-2.5		
α -Endosulfan	0		0		2	Tr.(2)	0.5	1.5
β -Endosulfan	0		0		0		0.5	1.5
Endosulfan sulfate	0		0		3	Tr.(1)-1.9	0.1	0.3
total Endosulfan	0		0		3	Tr.(1)-2.6		
α -BHC	1	Tr.(1)	0		0		0.1	0.3
β -BHC	3	Tr.(3)	0		1	Tr.(1)	0.1	0.3
γ -BHC	0		0		0		0.1	0.3
δ -BHC	0		0		0		0.1	0.3
total BHC	3	Tr.(2)-0.3	0		1	Tr.(1)		
Aldrin	0		0		0		0.1	0.3
Dieldrin	0		0		0		0.2	0.6
Endrin	0		0		0		0.1	0.3
Dicofol	4	Tr.(4)	4	Tr.(1)-0.8	1	89.8	0.1	0.3
Methoxychlor	0		0		0		0.1	0.3
Mirex	1	Tr.(1)	1	0.05	2	Tr.(1)-0.08	0.01	0.03
Hexachlorobenzene	9	Tr.(9)	4	Tr.(1)-0.04	8	Tr.(4)-0.31	0.01	0.03
Permethrin	0		4	Tr.(3)-0.2	5	Tr.(3)-1.6	0.06	0.2
Cypermethrin	0		3	Tr.(1)-2.4	3	1.2-57.9	0.3	1.0
Fenvalerate	0		2	Tr.(1)-13.7	1	4.9	0.5	1.5
Trifluralin	0		1	0.08	2	Tr.(1)-1.02	0.01	0.03
Vinclozolin	0		0		0		0.1	0.3
Alachlor	0		0		0		0.1	0.3
Nitrofen	0		0		0		0.1	0.3
Malathion	9	Tr.(4)-0.7	0		1	0.3	0.1	0.3
Parathion	0		0		0		0.5	1.5
Simazine	0		0		0		0.2	0.6
Atrazine	0		0		0		0.2	0.6
Metribuzin	0		0		0		0.2	0.6
Carbaryl	0		0		1	Tr.(1)	0.5	1.5

^{a)} Number detected among 10 samples. ^{b)} Sample numbers with trace level residues are shown in parentheses. LOD : Limit of detection, LOQ : Limit of quantitation.

Table 5. Dietary Daily Intake of Pesticides Calculated from Duplicate Portion Study

Pesticide	Dietary daily intakes ($\mu\text{g}/\text{d}$)				ADI ($\mu\text{g}/\text{kg}$ b.w./d)
	Breakfast	Lunch	Dinner	Total	
Total chlordane	0.01 \pm 0.01	0.08 \pm 0.11	0.18 \pm 0.26	0.27 \pm 0.30	0.5
Total DDT	0.09 \pm 0.04	0.14 \pm 0.12	0.62 \pm 0.71	0.85 \pm 0.72	5
<i>p,p'</i> -DDE	0.08 \pm 0.03	0.08 \pm 0.05	0.28 \pm 0.31	0.45 \pm 0.31	
Dicofol	Tr.	Tr.	6.2 \pm 19.7		25
Cypermethrin	N.D.	Tr.	6.2 \pm 17.1		50
Malathion	0.16 \pm 0.09	N.D.	Tr.		20

n=10 for each meal.

The latter part was modified to improve the purification effect. Considering the results described above, the Silica cartridge cleanup with 2% or 5% acetone-*n*-hexane eluant was the best choice. However, in case of overloading, the fatty acids were eluted into the 5% acetone-*n*-hexane fraction. In order to remove the fatty acids from the second fraction, the PSA cartridge was attached below the Silica, and the tandem cartridges were eluted with 20% acetone-*n*-hexane to prevent the adsorption of pesticides on the PSA cartridge.

Recoveries of Fortified Pesticide Residues from Duplicate Meals

Recoveries of pesticides from fortified meals at 2 ppb are shown in Table 3. Most pesticides were recovered from the first fraction, and simazine, atrazine, metribuzin and carbaryl were from the second fraction, and endosulfan sulfate was from both fractions. Low recoveries of aldrin, hexachlorobenzene and mirex, which are low-polarity compounds, were considered to be due to adsorption on the ODS cartridge. The chromatographic peaks of cypermethrin, carbaryl, parathion and permethrin were interfered by large matrix peaks. Therefore, these peaks showed big dispersion and high recoveries, even if compensated with control samples. Recoveries of nitrofen may be increased by the matrix-induced chromatographic response enhancement,¹¹⁾ as it is easy to adsorb to the GC analysis column. Acceptable recoveries 57–98% were obtained for the other pesticides.

Duplicate Portion Study for Pesticide Residues

Residual concentrations of pesticides are shown in Table 4. The dietary intakes were calculated using zero for under the detection limit and estimated values for trace levels, and aver-

age intakes that exceeded the product of quantitation limit and average weight of meals are listed in Table 5. Chlordane was detected from almost all samples, since detection of 0.01 ppb was possible, and intake of total chlordane was 0.27 $\mu\text{g}/\text{d}$. *p,p'*-DDE was also detected from all meals at 0.03–1.0 ppb, and intake of DDE was over half the total DDT. Malathion was detected from the bread type breakfast at *ca.* 0.2–0.7 ppb, and appears to derive from the wheat.

The dietary daily intakes in this study were similar to the data of previous work using market basket method.^{12,13)} The data from this study indicates that the exposure to pesticide residues from the diet is very low in comparison with the acceptable daily intake (ADI) values.

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