研究成果の刊行に関する一覧

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
なし							

雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
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研究成果に関する刊行物

論文

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Original Article

Rapid Method for the Determination of 180 Pesticide Residues in Foods by Gas Chromatography/Mass Spectrometry and Flame Photometric Detection

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A method was established for the determination of 180 pesticide residues in fruits and vegetables. The procedure involved extraction with acetonitrile, followed by a salting-out step with anhydrous $MgSO_4$ and NaCl. Removal of sediment and water was performed simultaneously by centrifugation. Co-extractives were removed with a double-layered SPE column, and graphitized carbon black and primary secondary amine (GCB/PSA) solid phase extraction cleanup cartridge. The eluate was determined by GC/FPD and GC/MS without further cleanup. Recovery data were obtained by fortifying 9 matrices at 0.05–0.1 μg/g. Recoveries of 180 pesticides were mainly 70–110% and the relative standard deviation (RSD) was below 25%. Limits of detection ranged between 0.01 and 0.05 μg/g for tested pesticides. © Pesticide Science Society of Japan

Keywords: pesticide, residue analysis, multiresidue, graphitized carbon black / primary secondary amine.

INTRODUCTION

In Japan, maximum residue levels (MRLs) have been set for over 200 pesticides in the last decade, and this number will be increased to over 400 in 2006. In addition, agricultural products that contain pesticides not on MRL lists will be excluded from the market as illegal, with a positive-list system to be introduced in the fiscal year of 2006.

Pesticide residue analysis of foods has been performed by numerous governments and private laboratories throughout the world.[1–3] Regulatory agencies involved in the monitoring of pesticide residues in foods require fast and efficient multiresidue methods with a broad scope of application in order to maximize the coverage of their monitoring activities. Modern residue monitoring programs are expected to be responsive to the latest developments in agriculture and new legislation.

To date, many multiresidue analytic methods have been reported.[4–6] Some of them require special instruments for extraction or cleanup. The system for supercritical fluid extraction (SFE),[7,8] accelerated solvent extraction (ASE),[9–11] and gel permeation chromatography (GPC)[12,13] operate automatically. But only one sample is processed at a time, and the settings must be changed for each sample, consequently the cost is high. The aim of this study was to develop a simple and efficient multiresidue analysis that takes just one day and does not require expensive instruments for sample preparation. The main focus was to shorten the analytical process during extraction and cleanup. Anastassiades *et al.* reported a rapid approach to the analysis of pesticide residues in fruits and vegetables, named QuEChERS.[14] We examined QuEChERS and found that the method contained a respective point, small size liquid-liquid partitioning, and also found two negative aspects, weak extraction potency (shake) and insufficient cleanup (batch). In this study, we developed a more efficient method, adapting a cleanup cartridge using a graphitized carbon black (GCB) and primary secondary amine (PSA) double-layered (GCB/PSA) solid phase extraction (SPE) for the analysis of 180 pesticides in fruit and vegetable samples. These pesticides were detected by gas chromatography mass spectrometry (GC/MS) in the electronic ionization mode (EI) and negative chemical ionization mode (NCI), and by GC with a flame photometric detector (GC/FPD). The newly described method would compensate for the negative aspects of the QuEChERS method.

MATERIALS AND METHODS

1. Apparatus

1.1. Electron ionization (EI) mode GC/MS

A POLARIS Q ion trap mass spectrometer (Thermo Electron

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Corp., USA) equipped with a TRACE GC Ultra; column, Rtx-5 ms capillary column 30 m×0.25 mm×0.25 μm (Restek Corp., USA); a helium carrier gas flow, 1.5 ml/min; injection temperature, 250°C; transfer line temperature, 280°C; ion source temperature, 200°C; ion mode, electronic ionization / scan mode; oven temperature program: 60°C for 1 min, 8°C/min to 280°C and held for 5 min; splitless injection at a volume of 1 μl by a AS-2000 auto sampler.

1.2. Negative chemical ionization (NCI) mode GC/MS

A GCMS-QP2010 gas chromatograph / mass spectrometer (Shimadzu, Japan); column, DB-5 capillary column 30 m×0.25 mm×0.25 μm (J & W Scientific, USA); helium carrier gas flow, 1.7 ml/min; injection temperature, 250°C; interface temperature, 250°C; ion source temperature, 200°C; ion mode, negative chemical ionization / selected ion monitoring mode; reaction gas, methane; oven temperature program: 60°C for 1 min, 20°C/min to 170°C, then 6°C/min to 300°C and held for 7 min; splitless injection at a volume of 1 μl by a Shimadzu AOC-20i auto injector.

1.3. GC/FPD

A GC-17A (Shimadzu, Japan) equipped with a flame photometric detector (FPD); column, DB-1701 capillary column 30 m×0.32 mm×0.25 μm (J & W Scientific, USA); helium carrier gas flow, 2.0 ml/min; injection temperature, 250°C; detector temperature, 280°C; oven temperature program: 80°C for 2 min, 20°C/min to 180°C, then 4°C/min to 260°C, then 10°C/min to 280°C for 5 min, splitless injection at a volume of 2 μl by a Shimadzu AOC-14 auto injector.

2. Chemicals

Acetonitrile, toluene, acetone and *n*-hexane were of pesticide analysis grade from Wako Pure Chemical Ind. (Japan). Anhydrous magnesium sulfate, sodium chloride and acetic acid were of analytical grade from Wako. SPE tubes, GCB; Supelclean ENVI-Carb (250 mg) and GCB/PSA; Supelclean ENVI-Carb/PSA (500 mg/500 mg), were purchased from Supelco (USA). GCB/PSA SPE was preconditioned with a 30 ml mixture of acetonitrile-toluene (3 : 1) containing 0.5% acetic acid.

Pesticide standards were obtained from Wako, Kanto Kagaku (Japan), Riedel de Haën (Germany), Hayashi Pure Chemical (Japan) and Dr. Ehrenstorfer G.m.b.H. (Germany). Each compound was dissolved in acetone to make a 1000 μg/ml stock standard solution. Mixed-compound intermediate solutions were prepared from stock solutions at concentrations ranging from 40 to 100 μg/ml. Spiking solutions were prepared from intermediate solutions containing approximately 100 or 200 compounds at concentrations of 5 μg/ml. Spiking solutions were used for fortifying the samples and also for the calculation after appropriate dilution.

3. Sample Preparation

All crops were purchased at a local market in Osaka and we confirmed that the concentrations of pesticide residues in foods were below detectable levels with the proposed method.

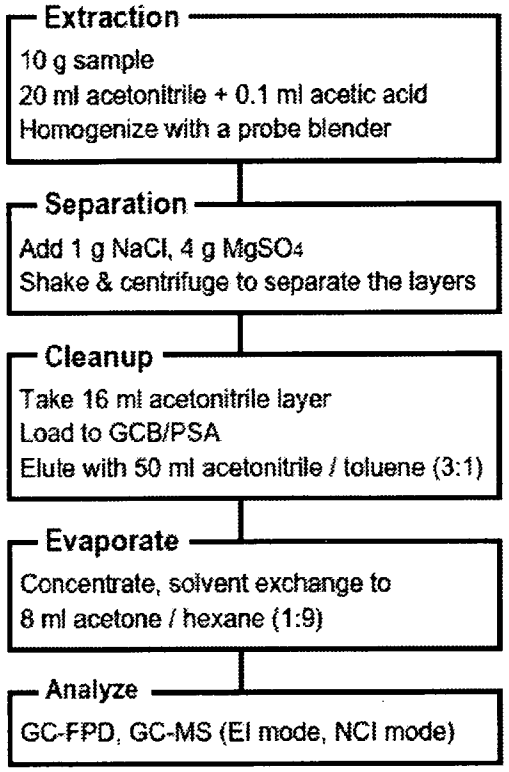

Fig. 1. Flow chart of the multiresidue method.

About 500–1000 g of food was chopped in a QS-7 food processor (Toshiba, Japan) for more than 1 min to obtain thoroughly mixed homogenates.

An aliquot of 10 g of sample homogenate was weighed into a BLUE MAX 50 ml polypropylene conical tube (Becton Dickinson, USA) and 100–200 μl of spiking solution (5–10 ng/μl for all compounds) was added. The mixture was left to stand for more than 30 min before extraction. The spiked sample was extracted with a mixture of 20 ml of acetonitrile and 0.1 ml of acetic acid by a HG30 homogenizer (Hitachi, Japan) for 1 min. One gram of NaCl and 4 g of anhydrous $MgSO_4$ were further added and shaken immediately for about 30 s with the screw cap on.[14] The extract was centrifuged for 10 min at 6000 rpm using a Himac SCR 20B (Hitachi, Japan) to separate the sediment and water from the acetonitrile. Next, 16 ml (equivalent to 8 g of sample) of the acetonitrile layer obtained after salting out was loaded into a GCB/PSA SPE tube. Pesticides were eluted with 50 ml of acetonitrile-toluene (3 : 1). The eluate was evaporated and the residue was dissolved in 8 ml of acetone-hexane (1 : 9) for GC/FPD and GC/MS analysis. The concentration of the sample represented by the test solution was 1 g/ml. Figure 1 summarizes the procedure.

Calibration was achieved by preparing matrix matched calibration standards from the extracts of blank samples in order to compensate for the matrix effect. Analytes were quantified

by using a 3-point calibration with those matrix matched calibration standards corresponding to the spiked concentration.

RESULTS AND DISCUSSION

1. *Method Development*

Anastassiades *et al.* reported a quick, easy, cheap, effective, rugged and safe method named QuEChERS.[14] Pesticides were extracted by acetonitrile using a vortex mixer, the cleanup procedure was performed by dispersive-SPE using PSA particles, and the final extract was injected directly into the GC/MS system. They avoided the solvent evaporation and reconstitution steps to save time and labor. But we found that this procedure was not sufficient in removing food colorings such as chlorophyll, carotene, and water soluble materials, such as sugars and sodium chloride, when we used the QuEChERS method. We have introduced the positive aspects of QuEChERS, such as a small extraction scale, and phase separation with $MgSO_4$ and NaCl, to a conventional acetonitrile extraction and further improved the cleanup step. Duplicated analyses were performed for the comparison of extraction between the QuEChERS method (shaking) and our proposed method (homogenizing) using samples containing incurred pesticide residues. As shown in Table 1, the five detected pesticides, especially organochlorine pesticides, had lower values after shaking with QuEChERS than after vigorous mechanical homogenization with the newly proposed method.

Anastassiades *et al.* and Schenck *et al.* reported that the results with the vortexing procedure were similar to those with the blender for incurred pesticides.[14,15] It was suspected that the results might be affected by the difference in pesticides detected and the capability of our food processor. In any case, the probe homogenizer has an advantage over a shaker to break down foods into particles. The homogenizer needed a certain volume of solvent. Extraction was conducted with 10 g sample+20 ml acetonitrile. The homogenized extracts, 1 : 2 / sample : solvent ratio, were a darker color than the shaken extracts, 1 : 1 / sample : solvent ratio. The conclusion was reached that homogenizing was superior to shaking as an extraction method. Moreover, we obtained broad peaks of weak intensity on GC/MS and GC/FPD chromatograms with direct injection of the acetonitrile solution.

We chose traditional SPE involving evaporation and reconstitution for removing hexane-insoluble sugars and salts. We did not use internal standards because triphenylphosphate, which is used in the QuEChERS method, was trapped in GCB. The separated acetonitrile contained a small amount of water.[16] We disregarded the change in volume because we considered it to have little effect.

2. *Measurement*

Almost all of the targeted pesticides were measured by EI mode GC/MS, but food matrices were frequently detected and sometimes interfered with the results. To aid with identification, GC/FPD for organophosphorous pesticides and NCI mode GC/MS for organochlorin and pyrethroid pesticides were adopted. Organophosphorous pesticides were detected using GC/FPD with a DB-1701, a mid-polarity phase column. Acephate and methamidophos were little detected using GC/MS with a DB-5, a low polarity phase column. Azinphos-methyl and monocrotophos were not detected by GC/MS under the proposed conditions. Organochlorine and pyrethroid pesticides were detected with NCI mode GC/MS, which could detect halogenated compounds with high sensitivity.[17] Serious interference was not observed with NCI mode GC/MS and pyrethroids could be detected at lower levels than with GC/MS in the EI mode or GC with an electron capture detector (Fig. 2). Matrix enhancement effects were sometimes observed especially in EI mode GC/MS chromatograms. About 60% of pesticides showed unacceptably high responses (>120%) with an orange matrix. Pesticide concentrations calculated with standards in solvent alone may be much higher than expected. Calibration was achieved by preparing matrix matched calibration standards from the extracts of blank samples, in order to compensate for the matrix effect.

Table 1. Comparison of analytical results obtained using two extraction methods

Food	Pesticide	Shaking	Homogenizing
			(ppm)
Pumpkin	Dieldrin	0.011	0.013
Pumpkin	Endrin	0.009	0.012
String bean	Dicofol	0.14	0.34
String bean	*o,p'*-DDT	0.011	0.025
String bean	Methamidophos	0.83	0.93

3. *Cleanup*

GCB with 30 ml of acetonitrile-toluene (3 : 1) was compared with GCB/PSA. The GCB column was effective at eliminating pigment and a primary secondary amine column could remove polar matrices and fatty acids. Extraction tests using both columns were conducted 5 times for each sample of lettuce, orange, and paprika. All extracts became clear after SPE, but the eluate from GCB contained some sediment and was dark. Next, 6 ml of extract (equivalent to 6 g of sample) was dried in a preweighed test tube, and the amount of coextracted material was determined from the difference in weight after the extract had dried. Figure 3 shows that the double-layered SPE column showed about 40% or more cleanup for the residual weight of dried matrices in all samples, compared with single GCB. Figure 4 shows the total ion chromatogram of banana extracts eluted from GCB and GCB/PSA.

These chromatograms indicated that PSA reduced GC-detectable matrices. Saito *et al.* also reported that the combination of GCB and PSA provided excellent cleanup for removal

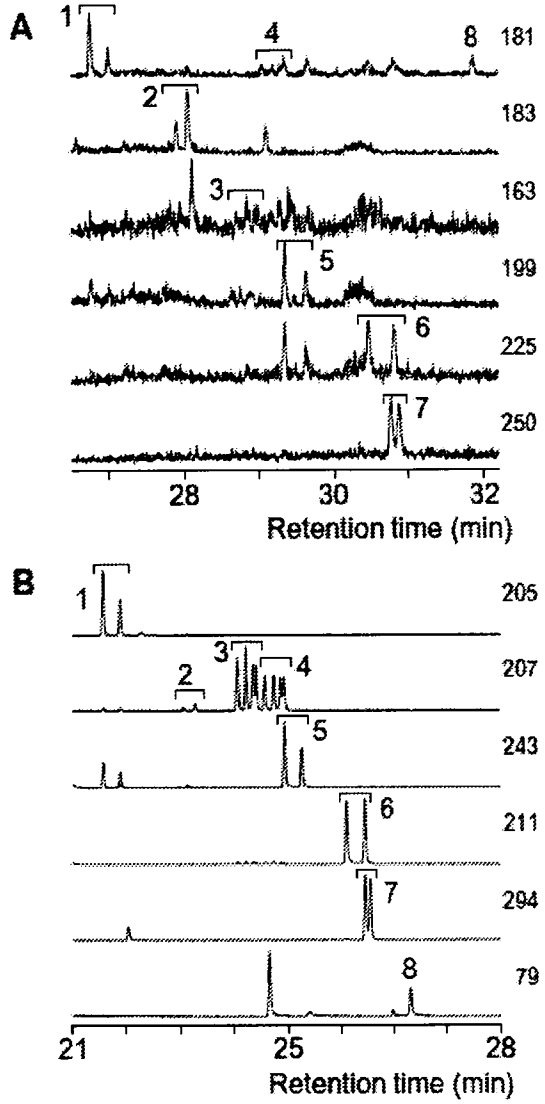

Fig. 2. GC/MS chromatograms of pyrethroid pesticides at 0.2 μg/ml. A: EI mode (scan); B: NCI mode (SIM). 1: Cyhalothrin, 2: Permethrin, 3: Cyfluthrin, 4: Cypermethrin, 5: Flucythrinate, 6: Fenvalerate, 7: Flavalinate, 8: Deltamethrin.

of matrix materials.[19] Almost all of the targeted pesticides were recovered sufficiently from GCB/PSA with the proposed procedure except chinomethionate and chlorothalonil. They were not recovered from GCB/PSA with acetonitrile-toluene (3 : 1), though both pesticides were well recovered from GCB. The potency of the PSA column was examined using a mixed-pesticide solution and the results showed that these pesticides were captured by PSA. PSA was capable of removing of fatty acids, and these pesticides might be captured as a result. We tried to weaken the effect of PSA by adding ethyl acetate or acetic acid to the mixture of acetonitrile-toluene. We found that the addition of 0.5% acetic acid improved the recovery of both pesticides from the double-layered SPE column. The recovery of chlorothalonil was over 70%, but the recovery of chinomethionate was still below 50%.

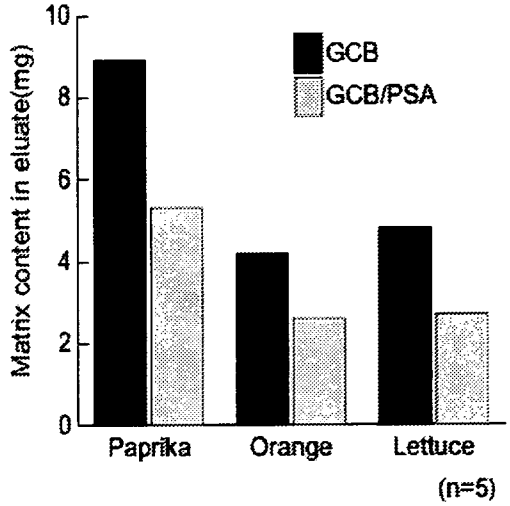

Fig. 3. Comparison of residual matrices in eluates obtained from two mini columns.

4. Recovery Test

The recovery tests were conducted 5 times for each sample of tomato, lettuce (n=5×2), orange and paprika at a level of 0.05 μg/g, and apple, banana, broccoli, spinach and grapefruit at a level of 0.1 μg/g. The data are summarized in Table 2.

Recoveries of 180 pesticides were between 70 and 110% and the relative standard deviation (RSD) was below 25% at each spiked level except for some pesticides in spinach and broccoli. Organophosphorous pesticides had lower RSDs than other pesticides. It was speculated that GC/FPD was more accurate than GC/MS. In routine analysis, it is easy to recognize the negative results for organophosphorous pesticides from one copy of a flat chromatogram. The data from GC/MS is composed of many mass chromatograms and takes some time to confirm. GC/FPD is useful to shorten the time needed for

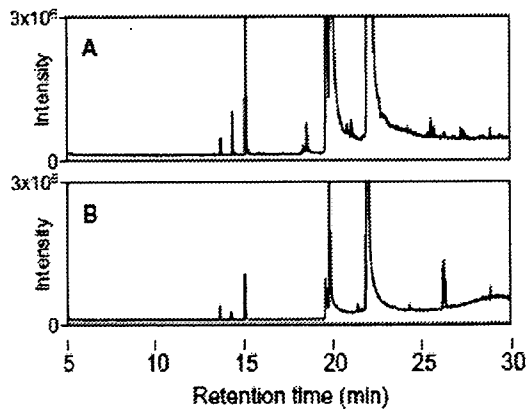

Fig. 4. Comparison of total ion chromatogram of banana extracts. A: GCB; B: GCB/PSA.

Table 2. Recovery data for compounds determined by the multiresidue method

Spiking level (μg/g)				0.05			0.1		
Compound	Detector	Monitor ion (*m/z*)	LOD (μg/g)	n	Mean recovery (%)	RSD (%)	n	Mean recovery (%)	RSD (%)
α-BHC	NCI	71	0.01	25[a]	91	8	25[a]	85	10
β-BHC	NCI	71	0.01	25[a]	93	9	25[a]	86	8
γ-BHC	NCI	71	0.01	25[a]	93	8	25[a]	86	8
δ-BHC	NCI	71	0.01	25[a]	93	8	25[a]	85	12
Tefluthrin	NCI	241	0.01	25[a]	93	11	25[a]	87	9
Chlorothalonil	NCI	266	0.01	25[a]	73	30	25[a]	72	22
Heptachlor	NCI	300	0.01	25[a]	100	11	25[a]	85	7
Aldrin	NCI	237	0.02	25[a]	91	9	25[a]	83	6
Dicofol	NCI	250	0.01	25[a]	91	12	25[a]	90	16
Heptachlor-epoxide	NCI	282	0.01	10[b]	86	6	10[b]	84	6
Captan	NCI	150	0.02	23[a]	87	19	10[a]	ND	----
Procymidone	NCI	282	0.02	25[a]	93	11	25[a]	86	9
pp'-DDE	NCI	35	0.01	25[a]	91	10	25[a]	84	9
Dieldrin	NCI	237	0.01	25[a]	92	11	25[a]	87	9
Endrin	NCI	237	0.01	25[a]	96	11	25[a]	87	12
Chlorobenzilate	NCI	278	0.01	10[b]	95	6	25[a]	81	23
pp'-DDD	NCI	71	0.01	25[a]	96	10	25[a]	85	7
op'-DDT	NCI	71	0.01	25[a]	99	10	25[a]	85	9
pp'-DDT	NCI	71	0.01	25[a]	100	11	25[a]	84	11
Captafol	NCI	150	0.01	23[a]	73	27	5[a]	43	20
Cyhalothrin	NCI	205	0.01	25[a]	100	12	25[a]	88	15
Permethrin	NCI	207	0.02	25[a]	98	14	25[a]	87	19
Cyfluthrin	NCI	207	0.01	25[a]	101	14	25[a]	87	12
Cypermethrin	NCI	207	0.01	25[a]	101	14	25[a]	89	12
Flucythrinate	NCI	243	0.01	25[a]	101	14	25[a]	82	18
Fenvalerate	NCI	211	0.01	25[a]	99	14	25[a]	83	13
Fluvalinate	NCI	294	0.01	25[a]	105	13	25[a]	82	17
Deltamethrin	NCI	79	0.01	25[a]	97	16	10[a]	78	12
Dichlorvos	FPD	----	0.01	25[a]	89	7	25[a]	85	6
Methamidophos	FPD	----	0.01	25[a]	71	14	25[a]	62	24
Acephate	FPD	(136)*	0.01	10[b]	80	6	22[a]	59	34
Ethoprophos	FPD	(158)*	0.01	25[a]	99	12	25[a]	92	8
Dioxabenzofos	FPD	(216)*	0.01	25[a]	93	7	25[a]	90	4
Terbufos	FPD	(231)*	0.01	25[a]	92	6	25[a]	87	8
Diazinon	FPD	(179)*	0.01	25[a]	95	6	25[a]	90	5
Iprobenfos	FPD	(204)*	0.01	25[a]	94	12	25[a]	91	12
Dichlofenthion	FPD	(279)*	0.01	25[a]	96	7	25[a]	88	6
Isazophos	FPD	(172)*	0.01	10[b]	85	12	10[a]	85	10
Monocrotophos	FPD	----	0.01	10[b]	89	5	10[b]	84	4
Cyanophos	FPD	(243)*	0.01	10[b]	84	9	10[a]	87	6
Dimethoate	FPD	(87)*	0.01	10[b]	93	16	25[a]	77	29

Table 2. (Continued)

Spiking level (μg/g)				0.05			0.1		
Compound	Detector	Monitor ion (m/z)	LOD (μg/g)	n	Mean recovery (%)	RSD (%)	n	Mean recovery (%)	RSD (%)
Chlorpyrifos-methyl	FPD	(286)*	0.01	10[b]	86	8	25[d]	85	7
Tolclofos-methyl	FPD	(265)*	0.01	25[d]	94	6	25[d]	91	4
Pirimiphos-methyl	FPD	(290)*	0.01	25[d]	95	6	25[d]	92	4
Chlorpyrifos	FPD	(258)*	0.01	25[d]	95	6	25[d]	89	6
Phosphamidon	FPD	(127)*	0.02	10[b]	91	3	10[c]	89	4
Fenthion	FPD	(278)*	0.01	25[d]	91	9	25[d]	86	7
Malathion	FPD	(127)*	0.01	25[d]	93	7	24[d]	87	17
Bromophos-methyl	FPD	(331)*	0.01	10[b]	86	8	10[d]	84	9
Fenitrothion	FPD	(260)*	0.01	25[d]	94	7	24[d]	86	11
Dimethylvinphos	FPD	(295)*	0.01	10[b]	90	3	10[c]	88	5
Isofenphos	FPD	(213)*	0.01	25[d]	96	7	25[d]	93	4
Phenthoate	FPD	(274)*	0.01	25[d]	95	7	25[d]	92	4
Fosthiazate	FPD	(227)*	0.02	10[b]	91	3	10[c]	84	11
Prothiofos	FPD	(309)*	0.01	25[d]	95	6	25[d]	91	6
Tetrachlorvinphos	FPD	(329)*	0.01	10[b]	90	4	10[c]	82	12
Methidathion	FPD	(145)*	0.01	25[d]	94	7	25[d]	94	10
Profenofos	FPD	(337)*	0.01	10[b]	92	4	10[c]	88	6
Butamifos	FPD	(286)*	0.01	25[d]	96	7	25[d]	95	5
Fenamiphos	FPD	(303)*	0.01	10[b]	90	4	10[c]	89	2
Isoxathion	FPD	(177)*	0.01	25[d]	96	8	25[d]	93	6
Ethion	FPD	(231)*	0.01	25[d]	97	7	25[d]	93	5
Edifenphos	FPD	(310)*	0.02	10[b]	87	6	25[d]	76	22
Triazophos	FPD	(162)*	0.01	10[b]	95	4	10[c]	95	3
Cyanofenphos	FPD	(169)*	0.01	10[b]	92	3	10[c]	92	3
EPN	FPD	(169)*	0.01	25[d]	96	9	25[d]	91	5
Piperophos	FPD	(320)*	0.01	10[b]	92	6	10[c]	93	5
Pyridaphenthion	FPD	(340)*	0.01	25[d]	93	9	25[d]	91	10
Phosalone	FPD	(182)*	0.02	25[d]	95	8	25[d]	93	8
Azinphos-methyl	FPD	----	0.02	10[b]	87	5	10[c]	84	12
Pyraclofos	FPD	(360)*	0.02	10[b]	87	4	10[c]	90	5
Pyrazophos	FPD	(221)*	0.02	10[b]	88	4	10[c]	84	15
Metolcarb	EI	108	0.02	10[b]	82	11	25[d]	81	29
Isoprocarb	EI	136	0.02	10[b]	89	12	25[d]	91	15
XMC	EI	122	0.02	10[b]	80	12	25[d]	87	20
Xylylcarb	EI	122	0.02	10[b]	86	10	10[c]	83	19
Tecnazene	EI	203	0.01	10[b]	77	9	10[d]	82	8
Fenobucarb	EI	121	0.02	10[b]	87	8	25[d]	89	14
Propachlor	EI	120	0.02	10[b]	87	8	10[d]	83	5
Propoxur	EI	110	0.02	10[b]	86	9	25[d]	83	19
Chlorpropham	EI	127	0.02	10[b]	87	13	25[d]	90	12
Bendiocarb	EI	151	0.03	10[b]	82	20	20[c]	79	30

Table 2. (Continued)

Spiking level (μg/g)				0.05			0.1		
Compound	Detector	Monitor ion (*m/z*)	LOD (μg/g)	n	Mean recovery (%)	RSD (%)	n	Mean recovery (%)	RSD (%)
Trifluralin	EI	264	0.02	10[b]	86	10	25[d]	85	13
Benfluralin	EI	292	0.01	10[b]	86	7	10[d]	84	10
Dicloran	EI	176	0.01	10[b]	81	6	10[d]	83	11
Simazine	EI	201	0.02	10[b]	86	9	10[d]	87	8
Carbofuran	EI	164	0.03	10[b]	84	17	20[c]	89	24
Atrazine	EI	200	0.02	10[b]	88	9	10[d]	85	8
Clomazone	EI	204	0.02	10[b]	85	10	10[d]	84	6
Quintozene	EI	237	0.02	10[b]	88	9	10[d]	82	9
Propyzamide	EI	173	0.02	10[b]	88	9	10[d]	85	7
Pyrimethanil	EI	198	0.02	10[b]	85	10	10[d]	83	6
Tri-allat	EI	268	0.02	10[b]	79	14	10[d]	77	8
Benoxacor	EI	120	0.02	10[b]	91	9	10[d]	88	8
Pirimicarb	EI	166	0.01	10[b]	93	5	25[d]	88	10
Ethiofencarb	EI	168	0.02	10[b]	78	17	20[c]	67	24
Benfuresate	EI	163	0.01	10[b]	87	6	25[d]	87	8
Propanil	EI	161	0.02	10[b]	83	9	10[d]	82	6
Bromobutide	EI	232	0.02	10[b]	86	9	10[d]	83	7
Dimethenamid	EI	154	0.01	10[b]	88	7	25[d]	89	12
Metribuzin	EI	198	0.02	10[b]	82	12	25[d]	80	16
Acetochlor	EI	223	0.01	10[b]	87	7	10[d]	81	8
Vinclozolin	EI	285	0.02	10[b]	94	9	10[d]	82	6
Simetryn	EI	213	0.02	10[b]	85	9	10[d]	84	8
Carbaryl	EI	144	0.04	10[b]	82	26	20[c]	69	50
Alachlor	EI	188	0.02	10[b]	89	12	25[d]	88	12
Ametryn	EI	227	0.03	10[b]	75	25	10[d]	58	44
Prometryn	EI	241	0.02	10[b]	87	8	10[d]	85	4
Metalaxyl	EI	160	0.02	10[b]	92	9	10[d]	84	12
Ethofumesate	EI	207	0.02	10[b]	89	12	10[d]	80	14
Esprocarb	EI	222	0.02	10[b]	81	14	25[d]	83	13
Bromacil	EI	205	0.04	10[b]	77	31	10[d]	85	21
Probenazole	EI	130	0.03	9[b]	57	26	10[d]	71	26
Thiobencarb	EI	257	0.03	10[b]	75	19	10[d]	82	17
Diethofencarb	EI	225	0.04	10[b]	76	34	25[d]	84	18
Metolachlor	EI	162	0.02	10[b]	84	9	25[d]	86	8
Fenpropimorf	EI	128	0.02	10[b]	87	10	10[d]	82	7
Cyanazine	EI	225	0.02	10[b]	79	15	10[d]	68	41
Triadimefon	EI	208	0.02	10[b]	94	9	25[d]	82	11
Chlorthal-dimethyl	EI	301	0.02	10[b]	87	9	10[d]	81	3
Nitrothal-isopropyl	EI	236	0.02	10[b]	80	9	10[d]	77	8
Tetraconazole	EI	336	0.02	10[b]	89	10	20[c]	84	15
Fthalide	EI	243	0.02	10[b]	86	11	10[d]	80	12

Table 2. (Continued)

Spiking level (μg/g)				0.05			0.1		
Compound	Detector	Monitor ion (m/z)	LOD (μg/g)	n	Mean recovery (%)	RSD (%)	n	Mean recovery (%)	RSD (%)
Diphenamid	EI	167	0.02	10^{b}	86	11	10^{a}	85	15
Dimethametryn	EI	212	0.02	10^{a}	91	9	10^{a}	85	10
Pendimethalin	EI	252	0.02	10^{b}	83	10	25^{b}	84	6
Penconazole	EI	248	0.02	10^{b}	87	11	25^{b}	88	10
Pyrifenox	EI	262	0.02	10^{b}	85	11	25^{b}	75	22
Triadimenol	EI	168	0.03	5^{c}	83	23	20^{c}	93	52
Triflumizole	EI	218	0.03	10^{a}	87	22	10^{a}	IF	----
Chinomethionate	EI	206	0.02	10^{b}	26	45	20^{c}	38	48
Pacrobutrazol	EI	236	0.04	10^{b}	74	27	25^{b}	87	17
α-Endosulfan	EI	241	0.03	5^{c}	93	18	10^{a}	IF	----
Butachlor	EI	160	0.02	10^{a}	90	11	5^{c}	72	12
Flutriafol	EI	123	0.02	5^{c}	88	13	10^{a}	IF	----
Napropamide	EI	128	0.02	10^{b}	95	13	10^{a}	IF	----
Flutolanil	EI	173	0.02	10^{b}	92	10	25^{b}	89	21
Hexaconazole	EI	214	0.02	10^{a}	88	13	20^{c}	88	20
Isoprothiolane	EI	204	0.03	10^{b}	81	19	10^{a}	89	12
Metominostrobin E	EI	191	0.02	10^{b}	95	13	10^{a}	86	14
Uniconazole	EI	234	0.02	10^{b}	95	10	20^{c}	91	18
Pretilachlor	EI	238	0.02	10^{a}	94	9	25^{b}	90	11
Fludioxonil	EI	248	0.02	10^{b}	84	8	25^{b}	85	13
Oxadiazon	EI	175	0.02	10^{b}	84	12	10^{a}	81	8
Flamprop-methyl	EI	230	0.01	10^{b}	93	7	10^{a}	85	7
Myclobutanil	EI	179	0.02	10^{b}	88	12	25^{b}	78	28
Oxyfluorfen	EI	252	0.02	10^{a}	87	11	10^{a}	86	8
Buprofezin	EI	175	0.02	10^{b}	91	14	10^{a}	80	33
Flusilazole	EI	233	0.02	10^{b}	90	11	25^{b}	82	14
Bupirimate	EI	193	0.02	10^{b}	93	10	10^{a}	86	10
Kresoxim-methyl	EI	116	0.02	10^{a}	92	9	25^{b}	88	14
Metominostrobin Z	EI	191	0.02	10^{b}	90	14	10^{a}	81	10
Cyproconazole	EI	222	0.02	10^{b}	87	12	20^{c}	88	13
β-Endosulfan	EI	241	0.04	10^{b}	88	29	5^{b}	56	24
Oxadixyl	EI	132	0.03	10^{b}	87	19	10^{a}	94	12
Mepronil	EI	269	0.04	10^{b}	92	24	25^{b}	80	24
Fluacrypyrim	EI	204	0.02	10^{b}	93	9	10^{a}	81	6
Carfentrazone-ethyl	EI	312	0.02	10^{b}	94	11	10^{a}	89	16
Diofenolan	EI	186	0.02	10^{b}	92	13	10^{a}	87	17
Benalaxyl	EI	148	0.02	10^{b}	90	8	10^{a}	87	12
Quinoxyfen	EI	237	0.02	10^{b}	84	9	10^{a}	83	10
Norflurazon	EI	303	0.02	10^{a}	93	13	10^{a}	87	9
Lenacil	EI	153	0.02	10^{b}	91	9	25^{b}	84	17
Trifloxystrobin	EI	116	0.03	10^{b}	93	15	10^{a}	85	11

Table 2. (Continued)

Spiking level (μg/g)				0.05			0.1		
Compound	Detector	Monitor ion (*m/z*)	LOD (μg/g)	n	Mean recovery (%)	RSD (%)	n	Mean recovery (%)	RSD (%)
Hexazinone	EI	171	0.02	10[a]	85	11	10[a]	75	20
Tebuconazole	EI	250	0.02	10[a]	86	12	25[a]	86	17
Diclofop-methyl	EI	340	0.02	10[a]	96	9	10[a]	88	16
Thenylchlor	EI	288	0.02	10[a]	87	15	25[a]	87	15
Propargite	EI	135	0.02	5[a]	85	12	5[a]	82	48
Diflufenican	EI	266	0.02	10[a]	89	13	25[a]	87	12
Pyributicarb	EI	165	0.02	10[a]	90	13	10[a]	84	21
Iprodione	EI	314	0.05	8[a]	90	32	19[a]	82	23
Brompropylate	EI	341	0.04	9[a]	102	25	10[a]	IF	—
Bifenthrin	EI	181	0.02	10[a]	88	9	25[a]	87	10
Picolinafen	EI	376	0.02	10[a]	93	11	10[a]	92	11
Methoxychlor	EI	227	0.02	10[a]	91	10	10[a]	86	14
Fenpropathrin	EI	265	0.02	5[a]	79	9	20[a]	96	10
Tebufenpyrad	EI	333	0.04	10[a]	59	45	25[a]	91	13
Phenothrin	EI	183	0.02	10[a]	85	15	10[a]	87	32
Tetradifon	EI	356	0.03	5[a]	118	39	5[a]	88	10
Furametpyr	EI	298	0.02	10[a]	93	12	25[a]	88	17
Pyriproxyfen	EI	136	0.03	10[a]	96	16	10[a]	83	26
Cyhalofop-butyl	EI	357	0.03	10[a]	105	32	10[a]	87	9
Mefenacet	EI	192	0.03	10[a]	86	22	24[a]	94	24
Fenarimol	EI	139	0.02	10[a]	90	8	24[a]	85	24
Bitertanol	EI	170	0.03	10[a]	90	18	25[a]	87	35
Pyridaben	EI	147	0.04	10[a]	73	28	10[a]	IF	—
Fenbuconazole	EI	129	0.04	9[a]	99	26	10[a]	IF	—
Pyrimidifen	EI	184	0.03	5[a]	65	26	24[a]	89	16

[a] Recovery data for orange, paprika, lettuce and tomato. [b] Recovery data for lettuce and tomato. [c] Recovery data for tomato. [d] Recovery data for apple, banana, grapefruit, broccoli and spinach. [e] Recovery data for apple, banana, grapefruit and broccoli. [f] Recovery data for apple, banana, grapefruit and spinach. [g] Recovery data for broccoli and spinach. [h] Recovery data for broccoli. [i] Recovery data for spinach. ND: Not detected. IF: Interfered.

* Also monitored by EI mode GC/MS.

identification. A few organophosphorous pesticides were measured with GC/MS because of interference in broccoli. Captan was not recovered from spinach and broccoli. Captafol was not recovered from spinach, it was poorly recovered in broccoli, and its recovery was fractured in other crops. Carbaryl, endosulfan, pyridaben, fenbuconazole and probenazole showed low recoveries and/or high RSDs in tested crops. The method was considered a screening procedure for these compounds. The limits of detection (LODs) were defined as 3 times the standard deviation of 5–25 replicate analyses of samples fortified at 0.05 or 0.1 μg/g with EI mode GC/MS. The LODs of the pesticides detected with GC/FPD and NCI mode GC/MS were calculated based on the noise levels on the chromatograms of the blank sample solution and the respective standard peaks, since serious interfering peaks were not observed. In this work, the minimum LOD was defined as 0.01 μg/g to take account of instrumental dispersion.

The proposed method shows good sensitivity and recovery and allows for rapid analysis. A single chemist can prepare 6 homogenized samples within 4 hr. The method requires only a small volume of solvent per sample and needs no special equipment. It covers a wide range of pesticides, is applicable to various fruits and vegetables, and is ideally suited for use in a regulatory laboratory. Further research will focus on the expansion of this method to other pesticides.

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Systematic analysis and overall toxicity evaluation of dioxins and hexachlorobenzene in human milk

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Abstract

A systematic method for analyzing dioxins (PCDDs, PCDFs and dioxin-like PCBs), hexachlorobenzene (HCB), heptachlor epoxide and β-hexachlorocyclohexane (HCH) in human milk was developed to determine the residual amount of HCB in human milk and to evaluate the overall toxicity of both dioxins and HCB in human milk. The fractionation behavior of HCB on chromatography with silica gel, alumina, and activated carbon/silica gel, and the concentrated sulfuric acid decomposition method, which is widely used as a dioxin cleanup method, were studied in order to make the preprocessing operation for HCB measurement compatible with that for conventional dioxin measurement. HCB was found to be eluted in the 2% dichloromethane (DCM)/hexane 60 ml fraction from an alumina column. Heptachlor epoxide and a part of β-HCH were eluted in the 10% DCM/hexane 50 ml fraction from a silica gel column, while the remaining β-HCH was eluted in the 25% DCM/hexane 60 ml fraction from an activated carbon/silica gel column. Moreover, HCB showed significant correlation with dioxin congeners having high toxicity equivalence factors (TEFs). The results suggest that the exposure route to HCB and its accumulation behavior in the human body are similar to those of the dioxins.

Keywords: Methodology; Persistent organic pollutant; Biological sample; Risk assessment; Correlation analysis

1. Introduction

Hexachlorobenzene (HCB), an organochlorine pesticide (OCP), was used as a fungicide for seeds and as a wood preservative. In addition, HCB exists as an impurity in such organochlorine chemicals as pentachlorophenol, pentachloronitrobenzene and tetrachlorothiophene, and as a by-product in the manufacture of chlorinated organic solvents such as trichloroethylene, tetrachloroethylene and carbon tetrachloride (Sakai et al., 2001). HCB is also generated by garbage incineration and metal refinement. Similar to dioxins (PCDDs, PCDFs and dioxin-like PCBs), HCB is listed as an intentional and unintentional Persistent Organic Pollutants (POPs) in the "Stockholm Convention" adopted in 2001. As

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regards the main toxic effects of HCB on the living body, its carcinogenicity (Cabral et al., 1977), teratogenicity (Khera, 1974) and endocrine-disrupting effects (Foster et al., 1995) have been shown in animal experiments. On the other hand, porphyria cutanea tarda (Peters et al., 1982; Jarrell et al., 1998) and immune diseases (Queiroz et al., 1998) have been detected in humans, although its carcinogenicity in humans remains to be proven. The past studies so far have indicated that HCB binds to the aryl hydrocarbon (Ah) receptor (Hahn et al., 1989; Van Birgelen, 1998), resulting in dioxin-like effects and bioaccumulation. Accordingly, the overall toxicity evaluation of dioxins and HCB in human milk should be re-examined as the toxicity equivalency factor (TEF) set by WHO is only for PCDDs, PCDFs, non-ortho PCBs, mono-ortho PCBs and does not include HCB. Many studies of dioxins or OCP pollution in human milk have been conducted. However, only a few of them have analyzed both dioxins and HCB in the same sample (Polder et al., 1998). This may be due to the limited availability of the samples, as repeated sampling of large amounts of human milk is difficult. Moreover, only a few studies are available regarding the overall toxicity evaluation of dioxins and HCB in human milk.

The objective of the present study was to develop a systematic method for analyzing dioxins and HCB, and to obtain additional information of the overall toxicity evaluation of dioxins and HCB in human milk. The correlation between HCB residual level and each dioxin congeners in human milk was also examined.

2. Materials and methods

2.1. Materials and chemicals

All of the dioxin standards such as PCDDs, PCDFs and non-ortho PCBs were from Wellington Laboratories and were diluted with decane to appropriate concentrations. The OCPs were α-hexachlorocyclohexane (HCH), β-HCH, γ-HCH, δ-HCH, *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDE, *p,p'*-DDE, heptachlor and heptachlor epoxide, all of which were from Wako Pure Chemical Industries (Osaka, Japan) and were diluted with hexane to the appropriate concentrations. Most of the organic solvents, such as hexane, acetone, dichloromethane (DCM), toluene, diethyl ether and ethanol, were of dioxin analysis quality and were from Kanto Kagaku (Tokyo, Japan) or Wako Pure Chemical Industries (Osaka, Japan). All other chemicals were of PCB analysis quality grade for PCB measurement or special quality grade and used without further purification.

2.2. Measurement of dioxins and HCB

Human milk was sampled from 100 Japanese primiparae whose mean age was 28.5 years old. The standard sampling timing for the human milk was set at 30 days after birth. Approximately 50 g of the milk sample was used for the analysis. The sample pretreatment for dioxin measurement was carried out in accordance with the manual compiled by the Ministry of Health, Labour and Welfare, Japan. Briefly, a stable isotope of each congener of PCDD/Fs and non-ortho PCBs was added as a surrogate after fat was extracted from the milk sample. The fat was then subjected to washing with concentrated sulfuric acid and then to chromatography with silica gel (1.5 g of silica gel packed in a glass column of 30 cm × 1.0 cm i.d.; eluted with 120 ml of hexane, followed by 60 ml of 10% DCM/hexane); alumina (6.5 g of basic alumina packed in a glass column of 30 cm × 1.5 cm i.d.; eluted with 60 ml of 2% DCM/hexane, followed by 100 ml of 60% DCM/hexane); and activated carbon/silica gel (0.5 g of activated carbon/silica gel packed in a glass column of 25 cm × 0.8 cm i.d.; eluted with 60 ml of 25% DCM/hexane, followed by toluene) as the cleanup operation, followed by GC/MS measurement of the dioxins.

For the measurement of HCB, the 2% DCM/hexane fraction that was eluted from the alumina column was evaporated to near dryness in vacuo, and the residue was dissolved with 1 ml of hexane and subjected to GC-ECD (electron capture detection). For the measurement other OCPs such as heptachlor epoxide and part of β-HCH, the 10% DCM/hexane fraction that was eluted from the silica gel column was subjected to the same procedure as above. For the measurement of the remaining β-HCH, the 25% DCM/hexane fraction that was eluted from the activated carbon/silica gel column was also subjected to the same procedure as above.

2.3. GC/MS measurement

The PCDD/Fs were subjected to HRGC/HRMS using a JEOL JMS-700 mass spectrometer equipped with a capillary DB-17HT column (30 m × 0.25 mm i.d., 0.15 μm film thickness) with helium as the carrier gas at a linear velocity of 35 cm/s in the splitless injection mode (1 μl). The GC program was as follows: 150 °C (1 min) to 220 °C (0 min) at 20 °C/min and subsequently at 4 °C/min to 280 °C, then maintained for 16.5 min at 280 °C. The injector temperature was 280 °C and the GC/MS interface temperature was held at 280 °C. The MS was operated in the selected ion monitoring mode with a mass resolution of 10000, and the electron impact ionization energy was 38 eV with an ion source temperature of 260 °C. The PCDD/Fs and non-ortho PCBs were quantified using a molecular ion (M), an M + 2