Validation of Multiresidue Screening Methods for the Determination of 186 Pesticides in 11 Agricultural Products Using Gas Chromatography (GC)

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Validation of multiresidue screening methods for the determination of 186 pesticides in 11 agricultural products: broccoli, asparagus, carrot, spinach, burdock, matsutake mushroom, cauliflower, orange, soybean, sesame and millet was done by gas chromatography (GC). The investigated pesticides were selected on the based of such compounds that are commonly used around the world. Although the recovery of 58 of the pesticides was low (< 50%) in some crops, the 128 pesticides that spiked in samples at 0.1 mg/kg showed satisfactory recoveries (≥ 50%) in all crops with relative standard deviation of 4–21%. These validated 128 pesticides were therefore newly acceptable for the pesticide-monitoring programme at the Quarantine Station in Japan; the quantitative limits ranged from 0.005 to 0.1 ppm by GC on a crop basis. The screening methods were applied to monitor the residue from a total of 200 pesticides including 72 previously validated in imported foods at the Station in Japan. Pesticide residue from 188 (12.4%) was found in 1516 samples. Of these, 4 (0.26%) were in violation of Japanese maximum residue limits (MRLs). No detectable residue was found in 1328 (87.6%) samples.

Key words ——— pesticide, validation, positive list system, screening method, imported food, gas chromatography

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INTRODUCTION

Japan is going to implement a so-called "positive list system" a system to prohibit in principle the distribution of foods containing a certain level of agricultural chemicals in case where no maximum residue limits (MRLs) have been set. The agricultural chemicals include pesticides, veterinary drugs and feed additives. This activity is based on the amended Japanese Food Sanitation Law published in May 2003. The system will take effect no later than May 2006. At the time of its implementation, MRLs will exist for about 750 chemicals. Pesticides are applied to agricultural crops throughout the entire world to control undesirable insects,[1,2] mites,[3,4] fungi,[5,6] weeds,[7,8] nematodes[9,10] and mollusks.[11] Pesticide-monitoring programme in imported foods are therefore important to ensure that these pesticides do not exceed the MRLs. The monitoring programme at the Quarantine Station in Japan uses the multiresidue screening methods. Thus, the validation of the screening methods is administratively indispensable to judge the applicability of these pesticides by the monitoring programmes at the Station. In the previous paper,[12] we proposed a method by gas chromatography (GC) for rapid screening of 104 pesticides in vegetables, fruits, cereals and seeds for routine purposes. In this study, another 186 pesticides to be investigated were selected on the based of their common use around the world. The aim of this work was to validate these 186 pesticides by the proposed routine screening methods using eleven common agricultural products. Pesticide-monitoring programme was also assigned at the Quarantine Station in Japan and used as the method to monitor

imported foods. The monitoring results are presented here.

MATERIALS AND METHODS

Chemicals, Reagents and Materials ——

Pesticide Standard: Certified reference pesticides were purchased from Hayashi Pure Chemical Industries (Osaka, Japan), Wako Pure Chemical Industries (Osaka, Japan) or Kanto Kagaku (Tokyo, Japan) (listed in Fig. 1). The purity of these pesticides was more than 97% by GC or HPLC. Individual stock standard solutions (generally containing 1000 μg/ml in ethyl acetate) were prepared from the reference standard materials. For the multiresidue experiments, mixed working standard solutions in acetone were prepared for each pesticide by serial dilution of the stock standard and were used as a spiking solution as well.

Chemicals: Phosphate buffer (pH 7.0, 1 mol/l); K_2HPO_4 105 g and KH_2PO_4 61 g were dissolved in distilled water and the pH was adjusted to 7 by addition of 1 mol/l HCl and NaOH solution. Mini column, strong anion exchange/primary-secondary amine combination (SAX/PSA) was obtained from Varian Inc. (CA, U.S.A., No. 1225–6061). All chemicals and solvents were a special grade for pesticide residue analysis.

Samples: broccoli, asparagus, carrot, spinach, burdock, matsutake mushroom, cauliflower, orange, soybean, sesame and millet were purchased from local retailers.

Sample Preparation ——

Extraction:

Vegetables and Fruits: Samples were homogenized, and an aliquot of 20 g was placed in a blender. Eighty ml of ethyl acetate and anhydrous sodium sulfate 50–100 g were added and the samples were blended for 3 min. The extract was decanted into a round bottom flask through a filter paper No. 5. The residue was then rehomogenized with 50 ml of ethyl acetate and filtered again and the filters were combined. These were concentrated to a few milliliters in a rotary evaporator at a water bath temperature of 40°C. After the solvent had been removed by a gentle stream of nitrogen, the residue was dissolved and adjusted to 5 ml with acetone-*n*-hexane (30 + 70) solution.

Cereals and Seeds: Samples were ground, and an aliquot of 10 g was placed in a blender. One hundred ml of 35%(v/v) water in acetonitrile was added, and the sample was blended for 3 min. The extract was decanted into a reparatory funnel through a filter paper No. 5. The residue was then washed with 30 ml of acetonitrile and filtered again and the filters were combined. Seven grams of sodium chloride and 10 ml of phosphate buffer (pH 7.0, 1 mol/l) were added and shaken for 5 min, and then allowed to stand for 15 min. The water portion was discarded. The acetonitrile layer was collected and the solvent was removed by a rotary evaporator at 40°C. Thirty ml of ethyl acetate and anhydrous sodium sulfate was added to the residue and the residue was dissolved using an ultrasonic machine, then filtered though a filter paper No. 5. The filtrates were combined. The solvent was concentrated to a few milliliters in a rotary evaporator and was removed by a gentle stream of nitrogen. Five ml of acetonitrile saturated with *n*-hexane and 1.5 ml of *n*-hexane saturated with acetonitrile were added and shaken for 5 min. After removal of the *n*-hexane, the acetonitrile layer was concentrated. The residue was dissolved and adjusted to 5 ml with acetone-*n*-hexane (30 + 70) solution. The sample was centrifuged at 3000 rpm for 5 min to separate the precipitation.

Cleanup: A mini column (SAX/PSA) conditioned with 5 ml of acetone-*n*-hexane (30 + 70) solution. Two milliliters of the extraction solution for vegetables, fruits and 4 ml of extraction solution for cereals and seeds were transferred to a mini column, respectively. Pesticides were eluted from the column with 5 ml of acetone-*n*-hexane (30 + 70) solution. After the elute was concentrated with a stream of nitrogen at a water bath temperature of 40°C, the volume was adjusted to 2 ml for all crops with acetone-*n*-hexane (30 + 70). The solution was used for GC analysis.

Recovery Test: Recovery of the 186 pesticides in broccoli, orange, asparagus, carrot, spinach, burdock, matsutake mushroom, cauliflower soybean, sesame and millet was assessed in three separate tests by fortification of each pesticide working solution to each sample. The pesticide concentration in the spiked sample was 0.1 mg/kg. The spiked sample was then prepared in the manner described in *Sample Preparation*.

Analysis of Pesticides ——

GC Analysis: The prepared sample solutions were analyzed by GC (Agilent Technologies, U.S.A.) equipped with a flame photometric detector (FPD) in P mode, nitrogen-phosphorous detector (NPD) and ^{63}Ni electron capture detector (ECD). The FPD-GC,

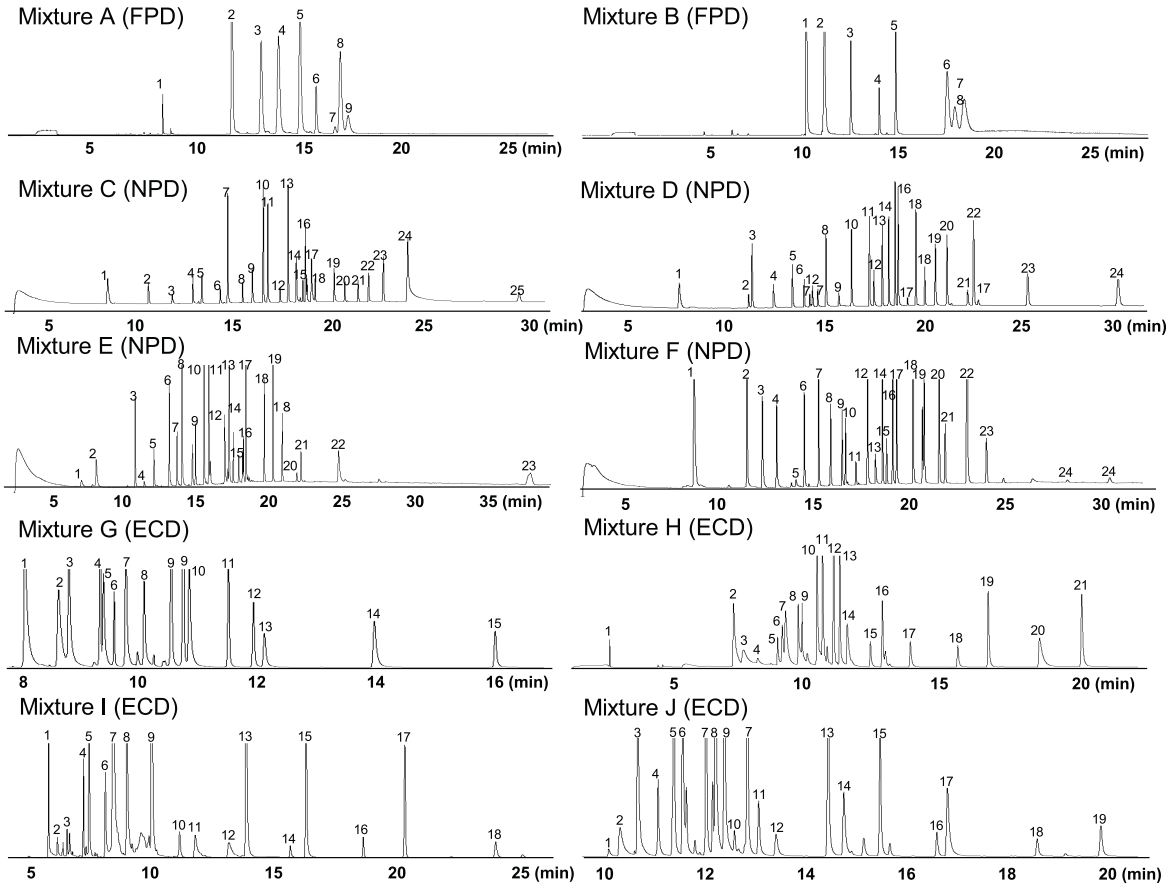

Fig. 1. Typical Gas Chromatograms of Mixed Standard Solution for 186 Pesticides

Mixture A and B, mixture C–F and mixture G–J were measured by FPD-GC, NPD-GC and ECD-GC, receptivity. Concentration of each pesticide was 1.0 μg/ml. Peaks: (Mixture A): 1, Azamethiphos; 2, Phoxim; 3, Disulfoton; 4, Bromophos; 5, Tribuphos; 6, Fenamiphos; 7, Vamidothion; 8, Pyridafenthion; 9, Azinphos methyl, (Mixture B): 1, Mevinphos; 2, Terbufos; 3, Omethoate; 4, Phosphamidon; 5, Isoprothiolane; 6, Piperophos; 7, Anilofos; 8, Pyrazophos, (Mixture C): 1, EPTC; 2, Propham; 3, Cinosulfuron; 4, Propachlor; 5, Diphenylamine; 6, Propyzamide; 7, Atrazine; 8, Bromobutide; 9, Cyromazine; 10, Ametryn; 11, Triadimefon; 12, Zoxamide; 13, Dimethametryn; 14, Paclobutrazol; 15, Iprovalicarb; 16, Fenothiocarb; 17, Uniconazole P; 18, Flamprop-methyl; 19, Fludioxonil; 20, Benalaxyl; 21, Picolinafen; 22, Pyributicarb; 23, Furathiocarb; 24, Phosmet; 25, Cafenstrole; (Mixture D): 1, Allidochlor; 2, Triflusulfuron-methyl; 3, Molinate; 4, Pencycuron; 5, Promecarb; 6, Bendiocarb; 7, Clomazone; 8, Simeconazole; 9, Terbacil; 10, Tetraconazole; 11, Penconazol; 12, Bromacil; 13, Diphenamid; 14, Hexaconazole; 15, Buprofezin; 16, Flusilazole; 17, Tepraloxydim; 18, Metominostrobin (E); 18', Metominostrobin (Z); 19, Tebuconazole; 20, Norflurazon; 21, Fenoxycarb; 22, Amitraz; 23, Fenarimol; 24, Fenbuconazole, (Mixture E): 1, Diuron; 2, Prosulfuron; 3, Trifluralin; 4, Captan; 5, Chlorpropham; 6, Spiroxamine; 7, Ethoxyquin; 8, Simazine; 9, Dimethenamid; 10, Prometryn; 11, Terbutryn; 12, Triadimenol; 13, Cyanazine; 14, Pretilachlor; 15, Ethychlozate; 16, Imazamethabenz-methyl ester; 17, Flutriafol; 18, Pyriminobac-methyl (Z); 19, Carbosulfan; 18', Pyriminobac-methyl (E); 20, Nicosulfuron; 21, Cyhalofop-butyl; 22, Bitertanol; 23, Difenoconazole, (Mixture F): 1, Propamocarb; 2, Benfuracarb; 3, XMC (Macbal); 4, Propoxur; 5, Phenmedipham; 6, Tri-allate; 7, Fenpropimorph; 8, Alachlor; 9, Metalaxyl; 10, Diethofencarb; 11, Methiocarb; 12, Cyprodinil; 13, Quinoclamine (CAN); 14, Dimepiperate; 15, Flutolani; 16, Napropamide; 17, Bupirimate; 18, Azaconazole; 19, Propiconazole; 20, Halosulfuron methyl; 21, Etoxazole; 22, Hexazinone; 23, Pyriproxyfen; 24, Cycloxydim, (Mixture G): 1 Tecnazene; 2, Dicloran; 3, Quintozene; 4, Dithiopyr; 5, Fenchlorphos; 6, Thiazopyr; 7, Chlorthal-Dimethyl; 8, Fipronil; 9, trans-Chlordane; 10, sis-Chlordane; 11, Oxyfluorfen; 12, Fluacrypyrim; 13, Trifloxystrobin; 14, Pyraflufen-ethyl; 15, Clomeprop; 16, Butafenacil, (Mixture H): 1, Fluroxypyrl 2, Hexaflumuron; 3, Triflumuron; 4, Fluoroimide; 5, Flufenoxuron; 6, Nitenpyram; 7, Chlorothalonil; 8, Chlorfluazuron; 9, Metolachlor; 10, Dicofol; 11, Diclocymet; 12, Oxadiazon; 13, Cyflufenamid; 14, Metamitron; 15, Clodinafop-propargyl; 16, Diclofop-methyl; 17, Cloquintocet-mexyl; 18, Iodosulfuron-methyl; 19, Etobenzanide; 20, Thiacloprid; 21, Azoxystrobin, (Mixture I): 1, Ethalfluralin; 2, Furilazole; 3, Benoxacor; 4, Acetochlor; 5, Dichlofluanid; 6, Isoxaflutole; 7, Fthalide; 8, Hexythiazox; 9, Fenoxanil; 10, Fluazinam; 11, Chloridazon; 12, Acetamiprid; 13, Tetradifon; 14, Pyraclostrobin; 15, Fluquinconazole; 16, Pyrimidifen; 17, Indoxacarb; 18, Fluthiacet-methyl, (Mixture J): 1, Dimethipin; 2, Pyrazosulfuron-ethy; 3, Vinclozolin; 4, Nitrothal-isopropyl; 5, Allethrin; 6, Triflumizole; 7, α-Endosulfan; 8, Thifluzamide; 9, Diclobutrazol; 10, Chlorbenzilate; 7', β-Endosulfan; 11, Carfentrazone-ethyl; 12, Quinoxyfen; 13, Bromopropylate; 14, Rimsulfuron; 15, Lactofen; 16, Pyridaben; 17, Prochloraz; 18, Flumioxazin; 19, Flumiclorac-pentyl.

NPD-GC and ECD-GC were used in capillary columns: DB-210 (30 m $\times$ 0.25 μm film thickness), DB-17 (30 m $\times$ 0.25 μm film thickness), and DB-5 (30 m $\times$ 0.25 μm film thickness), all from Agilent Technologies (California, U.S.A.). General operating conditions were as follows: inlet temperature 250°C, detector temperature 280°C, column temperature for the DB-210 column-initially 60°C for 2 min, pro-

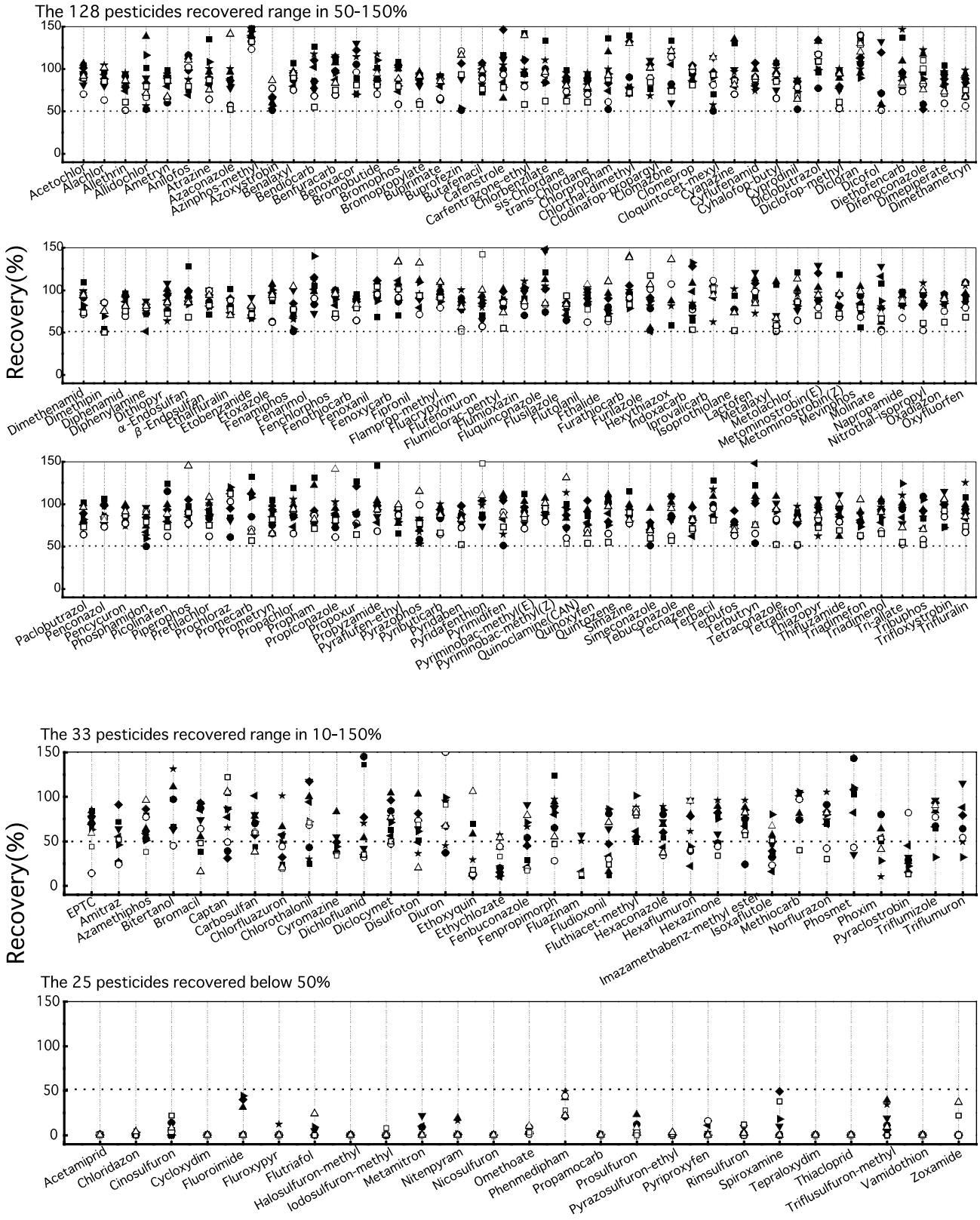

Fig. 2. Recovery of 186 Pesticides Obtained for Eleven Agricultural Products Using the Proposed Screening Methods for Vegetable-Fruits and Cereals-Seeds

Each pesticide was spiked at 0.1 mg/kg level as well as analysis of one blank extract. Values are average of three replicate determinations. ● Orange, ▲ Asparagus, ■ Broccoli, ▼ Carrot, ◆ Spinach, ◄ Burdock, ► Matsutake mushroom, ★ Cauliflower, ○ Soybean, □ Sesame, △ Millet.

Table 1. Limit of Quantitation (LOQ) of GC and GC/MS and Conformation Ions of GC/MS for Pesticide-Monitoring Programme by the Proposed Screening Methods

No.	Pesticide[a)]	LOQ (ppm)		GC/MS confirmation ions, *m*/*z*			No.	Pesticide[a)]	LOQ (ppm)		GC/MS confirmation ions, *m*/*z*		
		GC	GC/MS						GC	GC/MS			
1	Acetochlor	0.1	0.2	223	174	162	51	Etoxazole	0.1	0.1	359	330	300
2	Alachlor	0.01	0.01	269	188	160	52	Fenamiphos	0.01	0.01	303	288	260
3	Allethrin	0.05	0.05	168	136	123	53	Fenarimol	0.1	0.1	330	251	219
4	Allidochlor	0.1	0.1	173	138	132	54	Fenchlorphos	0.01	0.01	287	167	109
5	Ametryn	0.03	0.03	227	212	185	55	Fenothiocarb	0.1	0.1	253	160	72
6	Anilofos	0.01	0.01	334	228	226	56	Fenoxanil	0.05	0.05	328	293	189
7	Atrazine	0.03	0.03	215	200	173	57	Fenoxycarb	0.1	0.1	301	255	186
8	Azaconazole	0.1	0.1	217	173	145	58	Fipronil	0.01	0.01	367	351	213
9	Azinphos-methyl	0.05	0.05	160	132	125	59	Flamprop-methyl	0.1	0.1	335	276	230
10	Azoxystrobin	0.01	0.01	403	388	344	60	Fluacrypyrim	0.01	0.01	426	320	204
11	Benalaxyl	0.1	0.1	325	266	206	61	Flufenoxuron	0.02	0.02	331	268	227
12	Bendiocarb	0.02	0.02	223	166	151	62	Flumiclorac-pentyl	0.1	0.1	423	353	318
13	Benfluralin	0.1	0.1	292	276	264	63	Flumioxazin	0.02	0.02	354	287	259
14	Benoxazol	0.1	0.2	259	176	120	64	Fluquinconazole	0.02	0.02	375	340	298
15	Bromobutide	0.1	0.2	296	232	119	65	Flusilazole	0.01	0.01	315	233	206
16	Bromophos	0.01	0.01	331	213	125	66	Flutolanil	0.1	0.2	323	281	173
17	Bromopropylate	0.02	0.02	341	183	157	67	Fthalide	0.1	0.1	272	243	215
18	Bupirimate	0.05	0.05	316	273	208	68	Furathiocarb	0.05	0.05	382	325	194
19	Buprofezin	0.05	0.05	305	249	172	69	Furilazole	0.1	0.2	262	220	181
20	Butafenacil	0.03	0.03	331	180	124	70	Hexythiazox	0.1	0.1	227	184	156
21	Cafenstrole	0.1	0.1	188	119	100	71	Indoxacarb	0.1	0.2	366	264	218
22	Carfentrazone-ethyl	0.05	0.05	411	376	340	72	Iprovalicarb	0.1	0.2	260	158	134
23	Chlorbenzilate	0.1	0.1	251	139	111	73	Isoprothiolane	0.02	0.02	290	231	204
24	Chlordane	0.02	0.02	410	373	272	74	Lactofen	0.05	0.05	461	344	223
25	Chlorpropham	0.05	0.05	213	171	127	75	Metalaxyl	0.1	0.1	249	234	220
26	Chlorthal-dimethyl	0.02	0.02	322	301	221	76	Metolachlor	0.02	0.02	238	162	146
27	Clodinafop-propargyl	0.1	0.1	349	266	238	77	Metominostrobin	0.1	0.2	284	238	196
28	Clomazone	0.1	0.4	204	138	125	78	Mevinphos	0.05	0.05	192	164	127
29	Clomeprop	0.05	0.05	323	288	203	79	Molinate	0.05	0.05	187	126	98
30	Cloquintocet-mexyl	0.1	0.1	220	192	163	80	Napropamide	0.05	0.05	271	171	128
31	Cyanazine	0.02	0.02	240	225	198	81	Nitrothal-isopropyl	0.05	0.05	254	212	136
32	Cyflufenamid	0.1	0.1	412	321	294	82	Oxadiazon	0.02	0.02	344	302	258
33	Cyhalofop-butyl	0.1	0.1	357	256	229	83	Oxyfluorfen	0.05	0.05	361	317	300
34	Cyprodinil	0.02	0.02	225	186	128	84	Paclobutrazol	0.01	0.01	236	167	125
35	Diclobutrazol	0.05	0.05	270	201	159	85	Penconazol	0.05	0.05	248	213	159
36	Diclofop-methyl	0.05	0.05	340	281	253	86	Pencycuron	0.1	0.1	209	180	125
37	Dicloran	0.1	0.1	210	206	176	87	Phosphamidon	0.01	0.01	264	193	127
38	Dicofol	0.1	0.2	250	215	139	88	Picolinafen	0.1	0.1	376	266	238
39	Diethofencarb	0.1	0.1	267	225	196	89	Piperophos	0.03	0.03	320	140	122
40	Difenoconazole	0.02	0.02	325	323	152	90	Pretilachlor	0.1	0.1	262	238	225
41	Dimepiperate	0.1	0.1	263	145	119	91	Prochloraz	0.05	0.05	308	266	180
42	Dimethametryn	0.1	0.1	255	240	212	92	Promecarb	0.1	0.1	207	150	135
43	Dimethenamid	0.01	0.01	230	203	154	93	Prometryn	0.05	0.05	241	226	184
44	Dimethipin	0.05	0.05	210	124	118	94	Propachlor	0.1	0.5	211	196	176
45	Diphenamid	0.05	0.05	239	167	152	95	Propham	0.1	0.1	179	137	120
46	Diphenylamine	0.05	0.05	169	141	83	96	Propiconazole	0.05	0.05	259	191	173
47	Dithiopyr	0.05	0.05	354	306	286	97	Propoxur	0.1	0.1	152	110	81
48	Endosulfan	0.05	0.05	339	277	265	98	Propyzamide	0.1	0.2	255	240	173
49	Ethalfluralin	0.1	0.1	333	316	292	99	Pyraflufen-ethyl	0.05	0.05	412	349	339
50	Etobenzanide	0.1	0.1	339	304	179	100	Pyrazophos	0.01	0.01	373	265	232

a) Validated pesticides in this study. *b*) Validated pesticides in our previous study.

Table 1. Continued

No.	Pesticide[a)]	LOQ (ppm)		GC/MS confirmation ions, *m*/*z*			No.	Pesticide[b)]	LOQ (ppm)		GC/MS confirmation ions, *m*/*z*		
		GC	GC/MS						GC	GC/MS			
101	Pyributicarb	0.1	0.1	181	165	108	129	Acrinathrin	0.1	0.1	289	208	181
102	Pyridaben	0.05	0.05	364	309	147	130	Bifenox	0.05	0.05	341	310	189
103	Pyridafenthion	0.01	0.01	340	199	188	131	Bifenthrin	0.05	0.05	181	166	152
104	Pyrimidifen	0.1	0.1	220	184	161	132	Buthchlor	0.1	0.1	311	237	176
105	Pyriminobac-methyl	0.1	0.1	330	302	256	133	Butamifos	0.05	0.05	286	232	200
106	Quinoclamine(CAN)	0.1	0.1	207	172	144	134	Cadusafos	0.01	0.01	270	214	159
107	Quinoxyfen	0.1	0.1	307	272	237	135	Carbofuran	0.1	0.1	221	164	149
108	Quintozene (PCNB)	0.1	0.1	295	265	249	136	Chlorfenvinphos	0.02	0.02	323	295	267
109	Simazine (CAT)	0.1	0.1	201	186	128	137	Chlorphenapyr	0.05	0.05	408	363	328
110	Simeconazole	0.05	0.05	278	211	195	138	Chlorpyrifos	0.01	0.01	314	286	258
111	Tebuconazole	0.05	0.05	250	163	125	139	Chlorpyrifos-methyl	0.01	0.01	323	286	125
112	Tecnazene	0.1	0.1	261	215	203	140	Cyanophos	0.02	0.02	243	180	125
113	Terbacil	0.04	0.04	216	161	117	141	Cyfluthrin	0.05	0.05	226	206	163
114	Terbufos	0.005	0.005	288	231	186	142	Cyhalothrin	0.04	0.04	449	208	197
115	Terbutryn	0.02	0.02	241	226	185	143	Cypermethrin	0.05	0.05	209	181	163
116	Tetraconazole	0.05	0.05	336	267	171	144	Deltamethrin	0.01	0.01	253	209	181
117	Tetradifon	0.01	0.01	359	229	159	145	Diazinon	0.01	0.01	304	199	179
118	Thiazopyr	0.1	0.1	396	381	327	146	Dichlofenthion	0.01	0.01	279	251	223
119	Thifluzamide	0.1	0.1	447	429	194	147	Dichlorvos(DDVP)	0.02	0.02	220	185	145
120	Triadimefon	0.1	0.2	208	181	128	148	Dimethoate	0.1	0.1	229	143	125
121	Triadimenol	0.1	0.2	168	128	112	149	Dimethylvinphos	0.04	0.04	295	204	170
122	Tri-allate	0.1	0.1	268	143	128	150	Edifenphos	0.02	0.02	310	218	201
123	Tribuphos	0.05	0.05	314	258	202	151	EPN	0.02	0.02	323	185	157
124	Trifloxystrobin	0.02	0.02	222	206	187	152	Esprocarb	0.1	0.1	265	222	162
125	Trifluralin	0.05	0.05	335	306	264	153	Ethion	0.01	0.01	384	231	153
126	Uniconazole P	0.1	0.1	234	165	131	154	Ethoprophos	0.005	0.005	242	200	158
127	Vinclozolin	0.05	0.05	285	212	198	155	Etrimfos	0.01	0.01	292	277	181
128	XMC (Macbal)	0.1	0.1	179	122	107	156	Fenitrothion	0.01	0.01	277	260	125
							157	Fenobucarb	0.1	0.1	207	150	121
							158	Fenopropathrin	0.01	0.01	349	265	181
							159	Fensulfothion	0.02	0.02	308	293	141
							160	Fenthion	0.01	0.01	278	169	153
							161	Fenvalerate	0.05	0.05	419	225	181
							162	Flucythrinate	0.05	0.05	451	225	199
							163	Fluvalinate	0.01	0.01	502	250	208
							164	Fonofos	0.01	0.01	246	137	109

grammed to 250°C at 15°C/min, finally 250°Cfor 5 min; for the DB-17 column-initially 100°C for 2 min, heated to 270°C at 10°C/min, finally 250°C for 6 min; for the DB-5 column-initially 150°C for 2 min, programmed to 260°C at 40°C/min, finally 260°C for 6 min; carrier gas (helium) flow 2 ml/min, sample injection volume 2 μl; injection mode, splitless.

GC/MS Analysis: A mass chromatogram was obtained on a GC/MS (Agilent Technologies, U.S.A.) equipped with a quadrupole mass spectrometer. The GC/MS was used in the above capillary column DB-5. General operating conditions were as follows: inlet temperature 250°C; transfer line temperature 280°C, ionization temperature, 150°C, column temperature initially 60°C for 1 min, programmed to 280°C at 10°C/min, finally 280 for 7 min; mass spectrometer setting; electro ionization potential 70 eV, sample injection volume 2 μl; injection mode, splitless.

Table 1. Continued

No.	Pesticide[b)]	LOQ (ppm)		GC/MS confirmation ions, *m/z*		
		GC	GC/MS			
165	Fosthiazate	0.02	0.02	283	227	195
166	Halfenprox	0.05	0.05	478	263	183
167	Iprobenfos	0.01	0.01	288	246	204
168	Isazophos	0.01	0.01	285	257	161
169	Isofenphos	0.01	0.01	255	213	185
170	Isoprocarb	0.1	0.1	193	136	121
171	Kresoxim-methyl	0.1	0.1	313	206	131
172	Malathion	0.01	0.01	285	173	158
173	Mepronil	0.1	0.25	269	210	119
174	Methacrifos	0.01	0.01	240	208	180
175	Methamidophos	0.05	0.05	141	126	94
176	Methidathion	0.01	0.01	302	145	125
177	Myclobutanil	0.1	0.1	288	179	150
178	Parathion	0.01	0.01	291	186	139
179	Parathion-methyl	0.01	0.01	263	125	109
180	Pendimethalin	0.05	0.05	281	252	162
181	Permethrin	0.05	0.05	390	183	163
182	Phenthoate	0.01	0.01	274	246	157
183	Phorate	0.01	0.01	260	231	121
184	Phosalone	0.02	0.02	367	182	121
185	Pirimiphos-methyl	0.01	0.01	305	290	276
186	Procymidone	0.1	0.2	283	255	212
187	Profenofos	0.02	0.02	399	374	208
188	Propaphos	0.01	0.01	304	262	220
189	Prothiofos	0.01	0.01	309	267	239
190	Pyraclofos	0.05	0.05	360	194	138
191	Pyrimethanil	0.05	0.05	198	184	158
192	Quinalphos	0.01	0.01	298	241	146
193	Salithion	0.01	0.01	216	201	183
194	Tebufenpyrad	0.05	0.05	333	318	276
195	Tefluthrin	0.1	0.1	197	177	141
196	Tetrachlorvinphos	0.01	0.01	329	240	109
197	Thenylchlor	0.1	0.1	323	288	127
198	Thiobencarb	0.05	0.05	257	125	100
199	Tolclofos-methyl	0.02	0.02	265	250	125
200	Triazophos	0.02	0.02	313	285	257

RESULTS AND DISCUSSION

Pesticide Determinations by GC

Optimal conditions for the determinations of organophosphorus, organonitrogen, organochlorine and pyrethroid pesticides were investigated. Figure 1 shows the typical gas chromatograms of standards for the 186 pesticides. Organophosphorus pesticides, organochlorine and pyrethroid pesticides, and organonitrogen pesticides were determined by FPD-GC, ECD-GC, and NPD-GC, respectively. These pesticides were detected within 30 min except for difenoconazol. Pesticides were divided into ten groups for their complete separation in the test recovery. Adequate separation of all pesticides was achieved with good sharp peaks.

Recovery Studies

Vegetables-fruits and cereals-seeds methods were used to validate the 186 pesticides in the eleven agricultural products, as well as analysis of one blank extract. Recovery results are indicated in Fig. 2. As shown in Fig. 2, the recoveries of 128 pesticides were satisfactory (50–150%). Three repetitive determinations of recovery achieved good reproducibility for these pesticides (RSDs: 4–21%). All organochlorine and pyrethroid compounds in the 128 pesticides were recovered at greater than 70%. The organonitrogen pesticides belonging to acetamide, acid amide, azine, benzofuran, diazine, dicarboxyimide, diphenyl ether, naphthoquinone and dinitroaniline classes also gave good recoveries (> 70%). On the other hand, the recoveries of 33 pesticides were within 10–150% and 25 pesticides were below 50% in all samples. Lower recoveries (< 50%) are thought to be due to the unique traits of these particular pesticides. Amitraz is likely to be hydrolyzed[13,14)] in a sample at the extraction step. *N*-trihalomethylthio fungicides, such as captan and dichlofluanid may be degraded by a chemical reaction with thiol groups (-SH) of crop compounds during the extraction step.[15)] Phoxim and carbosulfan may be decomposed by the interaction of food components at the inlet liner of GC.[16,17)] The polar pesticides ethychlozate, phenmedipham, acetamiprid, chloridazon, cinosulfuron, fluoroimide, flutriafol, halosulfuron methyl, indosulfuron methyl, metamitron, nitenpyram, nicosulfuron, omethoate, phoxim, propamocarb, pyrazosulfuron-ethyl, pyriproxyfen, rimsulfuron, thiacloprid, triflusulfuron-methyl and vamidothion might not be eluted sufficiently with 30% acetone/*n*-hexane from a SAX-PSA cartridge. Overlapping peaks interfering with target pesticides also cause poor recovery. Cycloxydim, fluroxypyr, prosulfuron, spiroxamine and zoxamide were significantly overlapped with a sample matrix of almost all agricultural products.

These results clearly demonstrate that the 128 pesticides recovered in amounts of from 50 to 150% are acceptable for screening purposes.

Regulatory Sample Analysis in Quarantine Station

The proposed methods were used for pesticide

Table 2. Results of Monitoring of Imported Foods by Pesticide-Monitoring Programme Using Proposed Methods at the Quarantine Station in Japan during April 1 to 30, 2005

Commodity group		Positive					
		Pesticide	Commodity	Country	No.	Detection Ratio[a)] (%)	Concentration (ppm)
Vegetables		Chlorpyrifos	Welsh onion	Taiwan	1	0.13	0.02[b)]
			Cabbage	China	2	0.26	0.01–0.03
			Giant corn	Peru	1	0.13	0.02
			Red pepper	Thailand	1	0.13	0.19
			Okra	Thailand	1	0.13	0.69[b)]
			Frozen qing gin cai	China	1	0.13	0.01
			Frozen pimento	China	1	0.13	0.01
			Broccoli	U.S.A	1	0.13	0.07
		Chlorphenapyr	Paprika	Korea	5	0.65	0.10–0.17
			Frozen qing gin cai	China	1	0.13	0.09
		Cyfluthrin	Broccoli	U.S.A	1	0.13	0.05
			Onion	China	1	0.13	0.08
			Sweet basil	Laos	2	0.26	0.24–0.31
		Cypermethrin	Frozen green soybeans	China	1	0.13	0.07
			Okra	Philippines	1	0.13	0.14
			Perilla	China	2	0.26	0.16–0.30
			Froen red pepper	Thailand	1	0.13	0.22
			Frozen green soybeans	China	1	0.13	0.12
			Okra	Philippines	6	0.78	0.05–0.15
			Frozen qing gin cai	China	1	0.13	0.08
			Frozen pimento	China	2	0.26	0.06
			Broccoli	U.S.A	1	0.13	0.05
			Water mimosa	Thailand	1	0.13	0.79
		Deltamethrin	Okra	Philippines	1	0.13	0.01
		Difenoconazole	Celeriac	Netherlands	2	0.26	0.03–0.05
		Ethion	Welsh onion	Taiwan	1	0.13	0.3
			Red pepper	Thailand	1	0.13	0.1
		Fenopropathrin	Frozen qing gin cai	China	1	0.13	0.04
		Fenvalerate	Frozen pimiento	China	1	0.13	0.82[b)]
		Methamidophos	Frozen red pepper	Thailand	1	0.13	0.1
			Okra	Philippines	1	0.13	0.29
			Cabbage	China	1	0.13	0.07
			Welsh onion	China	1	0.13	0.33
		Methidathion	Young pepper	Thailand	2	0.26	0.02–0.1
		Parathion-methyl	Pandanus palmleaves	Thailand	1	0.13	0.06
		Permethrin	Broccoli	U.S.A	1	0.13	0.05
			Okra	Thailand	1	0.13	0.05
		Pirimiphos-methyl	Popcorn	U.S.A	1	0.13	0.02
		Profenofos	Frozen red pepper	Thailand	1	0.13	0.62
		Pyridaben	Paprika	Korea	1	0.13	0.07
Total	765				55	7.2	

a) Number of positive samples/number of measured samples. *b*) Violation of Japanese maximum residue limits (MRLs).

monitoring in real imported foods at the Quarantine Station for the period April 1 to 30, 2005. During this period, 1516 samples were screened for a total of 200 pesticides including 72 which had been previously validated. Table 1 lists the pesticides, limits of quantitation and GC/MS conformation ions used in the monitoring. The GC/MS [selected ion monitoring (SIM)] techniques have also been used to iden-

Table 2. Continued

Commodity group		Positive					
		Pesticide	Commodity	Country	No.	Detection Ratio[a)] (%)	Concentration (ppm)
Tea		Chlorpyrifos	Tea	England	1	2.6	0.01
			Tea	France	1	2.6	0.05
		Cypermethrin	Oolong tea	China	1	2.6	0.5
		DDT	Tea	England	3	7.7	0.02–0.04
			Tea	France	1	2.6	0.05
		Endosulfan	Tea	England	2	5.1	0.06–0.07
		Ethion	Tea	France	1	2.6	0.02
			Tea	Italy	1	2.6	0.01
			Tea	England	5	12.8	0.02–0.07
		Fenvalerate	Oolong tea	China	1	2.6	0.8
Total	39				17	43.6	
Fruits		Azoxystrobin	Banana	Philippines	2	0.63	0.02–0.06
			Mango	Philippines	1	0.32	0.01
		Bifenthrin	Frozen raspberry	U.S.A	2	0.63	0.08–0.17
		Chlorpyrifos	Orange	U.S.A	29	9.2	0.01–0.33
			Banana	Philippines	5	1.6	0.01–0.02
			Grape	Chile	10	3.2	0.01–0.43
			Lemon	U.S.A	12	3.8	0.02–0.13
			Minneola	U.S.A	1	0.32	0.22
			Tangerine	U.S.A	2	0.63	0.01–0.21
			Grapefruit	U.S.A	1	0.32	0.01
			Persimmon	New Zealand	1	0.32	0.02[b)]
		Chlorphenapyr	Leaf of radish	China	1	0.32	0.11
		Cyfluthrin	Lemon	U.S.A	1	0.32	0.09
		Cypermethrin	Avocado	Mexico	2	0.63	0.07–0.11
		Cyprodinil	Grape	Chile	7	2.2	0.13–0.32
			Frozen blueberry	U.S.A	1	0.32	0.02
			Frozen raspberry	U.S.A	2	0.63	0.03–0.04
		Deltamethrin	Orange	U.S.A	2	0.63	0.02
			Banana	Philippines	1	0.32	0.01
		Edifenphos	Orange	U.S.A	1	0.32	0.16
		Ethion	Avocado	Mexico	1	0.32	0.02
		Fenopropathrin	Orange	U.S.A	2	0.63	0.01–0.06
		Fenvalerate	Frozen raspberry	U.S.A	1	0.32	0.09
		Malathion	Grape	U.S.A	1	0.32	0.02
			Orange	U.S.A	1	0.32	0.12
			Frozen blueberry	U.S.A	1	0.32	0.11
			Frozen raspberry	U.S.A	1	0.32	0.02
		Methidathion	Orange	U.S.A	1	0.32	0.22
Total	315				93	29.5	
Cereals		Chlorpyrifos-methyl	Wheat	U.S.A	4	5.9	0.02–0.09
			Wheat flour	Korea	1	1.5	0.06
		Cyfluthrin	Soybean	U.S.A	1	1.5	0.06
		Fenvalerate	Millet	China	1	1.5	0.12
		Malathion	Wheat	Canada	1	1.5	0.02
				U.S.A	4	5.9	0.08–0.78
			Wheat flour	Korea	1	1.5	0.03
Total	68				13	19.1	

Table 2. Continued

Commodity group	Positive					
	Pesticide	Commodity	Country	No.	Detection Ratio[a)] (%)	Concentration (ppm)
Seeds, beans	Amethryn	Bean	U.S.A	1	0.30	0.08
	Chlorpyrifos	Cacao bean	Ghana	2	0.61	0.06–0.15
	Cyfluthrin	Coffee	Guatemala	1	0.30	0.06
	Dichlorvos(DDVP)	Walnut	China	1	0.30	0.04
	Malathion	Cacao bean	Ecuador	1	0.30	0.15
		Green bean	China	3	0.91	0.02–0.04
	Methamidophos	Frozen kidney bean	China	1	0.30	0.05
Total 329				10	3.0	
Grand total 1516				188	12.4	(Positive sample)
				4	0.26	(Violation sample)

tify the pesticides detected by GC analysis. Since Sphon[18)] reported that GC/MS must operate at least 3 diagnostic ions for identification to be confirmed with the SIM mode, three characteristic masses were chosen for each pesticide. In case the positive list system is going to be implemented, additional methods development is thought to be necessary to detect pesticides at the detection limits of 0.01 ppm or MRLs level.

The results of monitoring are shown in Table 2. A total of 188 residues were detected in 1516 samples, corresponding to a detection rate of 12.4%. The pesticides implicated were azoxystrobin, amethryn, bifenthrin, chlorphenapyr, chlorpyrifos, chlorpyrifos-methyl, cyfluthrin, cypermethrin, cyprodinil, DDT, deltamethrin, dichlorvos (DDVP), difenoconazole, edifenphos, endosulfan, ethion, fenopropathrin, fenvalerate, malathion, methamidophos, methidathion, parathion-methyl, permethrin, pirimiphos-methyl, profenophos and pyridaben. High residues were found in tea samples extracted directly with ethyl acetate when the pesticides were analyzed by the proposed method. However, after the pesticides were analyzed by a method using a hot water infusion, the residue level was decreased to less than detection limits (data not shown). Four samples (0.26%) were in violation: chlorpyrifos in welsh onion, okra and persimmon, and fenvalerate in frozen pimiento. No detectable residues were found in 1328 (87.6%) of the samples.

In conclusion, 128 pesticides were newly validated by the proposed multiresidue screening vegetables-fruits and cereals-seeds techniques. The validation study provides a judgment that a total of 200 pesticides including 72 previously validated by the proposed methods are available to the pesticide-monitoring programme in imported foods at the Quarantine Station in Japan.

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