Journal of Environmental Sciences (China) Vol. 4, No. 1, pp. 106–116, 1992 ISSN 1001-0742

A complete set of procedure for determination of multiresidue of pesticides in vegetables

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Abstract— A complete set of procedure for determination of multiresidue of pesticides in vegetables was presented in this paper. The complete set of procedure included three steps: bioassay, cholinesterase inhibition and GLC analysis. The samples could be identified to be contaminated with pesticides if 5% of house flies was knocked down in 50 tested house flies. Those samples contaminated with pesticides needed to be detected by AChE inhibition method. The qualitative and quantitative analyses were carried out by GLC. Recoveries ranged from 83.7% in Chinese cabbages to 105.6% in tomatoes for pyrethroids, and 84.0% in tomatoes to 102.7% in sweetbell redpeppes for organophosphorus compounds. Coefficients of variations ranged from 0.59% to organophosphorus compounds. Coefficients of variations ranged from 0.59% to 7.87% for pyrethroids, and 0.33% to 9.88% for organophosphorus compounds with vegetables. The complete set of procedure has been used successfully to analyze 7000 samples collected in Beijing. About 1% of the samples had a pesticide content exceeding the MRL.

Keywords: complete set; determination; multiresidue in vegetables.

INTRODUCTION

Numerous methods for the determination of pesticide residues have been reported in literature, including bioassay (Siv, 1970; Keith, 1984), gas liquid chromatography (GLC) (Jesper, 1976; Willem, 1976), high pressure liquid chromatography (HPLC) (Krause, 1988), and enzyme-linked immunosorbent assay (ELISA) (Van, 1989) and so on. GLC is often the method of choice for quantitative analysis of multiresidue in crops, soil and water. However, a complete set of procedure composed of bioassay, cholinesterase inhibition and GLC to determinate multiresidue in vegetables has not been presented. Contamination of vegetables with pesticides and food poisoning have made it imperative to have a reliable

method to determine low levels of multiresidue in vegetables. In China and some other developing countries, the vegetables are usually for sale on the market just after they are reaped. Therefore it is necessary to establish a rapid analysis method. The aim of this study is to present a sensitive and selective complete set of procedure for determination of multiresidues in vegetables.

EXPERIMENTAL

Materials

Cypermethrin of 99.5% and carbofuran of 99.5% purity were obtained from FMC; deltamethrin of 98% purity from Roussel-uclaf; fenvalerate of 92.4% purity from Sumitomo Chemical Co., Ltd; permethrin of 80.4% purity from Shanghai Union Chemical Plant; fenpropathrin of 98.2%, pirimicarb of 98% and pp321 of 95.3% purity from ICAMA; dichlorvos of 97.6%, phorate of 91%, and parathion of 99.2% purity from Tianjin Agrochemical Plant; dimethoate of 92.3%, trichlorphon of 81.3%, omethoate of 83.4%, parathion methyl of 99.5% purity from Hangzhou Chemical Plant; malathion of 90.1% and methamidophos of 85.7% purity from Ningpo Agrochemical Plant; monocrotophos of 88.5% purity from Nantong Agrochemical Plant; carbaryl of 98% was purchased from Institute of Shenyang Chemial Industry; aldicarb of 99.4% purity from Union Carbide Agricultural Products Company, Inc.; acetone, petroleum ether, methylene chloride were purchased from Beijing 5295 Chemical Plant. Sodium sulfate anhydrous, acetic ester were purchased from Beijing Chemical Plant. All other chemicals were of analytical-reagent grade and tested in blank procedures. For the mixture for adsorption chromatography, Florisil (Lot 14F-0476) was purchased from SIGMA Chemical Company. Celite 545 and activated carbon were purchased from Shanghai Chemical Company. The florisil was pre-treated according to the modified FMC (FMC, 1983). Disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$) and potassium phosphate monobasic (KH_2PO_4) were purchased from Beijing Chemical Company. Both chemicals were of analytical-reagent grade. For the measurement of acetylcholinesterase activity phosphate buffer solution (pH 7.5) in distilled water was prepared. The house strain of susceptible fly (Musca domestica vicina) was obtained from Taiwan strain (TS), and bred and selected in our laboratory.

PROCEDURES

Bioassay

Vegetables were homogenized and an aliquot (5g fresh weight) was placed in a 250 ml glass jar to which 50 house flies (TS) anaesthetized by CO_2 was also placed for 3 hours at 25°C. The sample was identified to be contaminated with pesticides if the ratio of knockdown

house flies was more than 5 percent. Three-day-old female flies were used all experiments.

Determination of acetylcholinesterase (AChE) inhibition

Acetylcholinesterase preparation

Batches of house flies died at -20℃ were homogenized in phosphate buffer solution (0.1 mol/L, pH 7.5) for 30 sec., and centrifugalized at 3500 g for 5 min. The supernatant was filtrated through the double gauze, and kept in freezer at −20℃, but it, enzyme solution diluted as necessary, was used for measuring kinetic parameters of AChE.

Extraction

One gram macerated vegetable was placed in a test tube with 0.5ml of methanol, and vibrated on the mixer for 30 sec. The extract was drained into another test tube for assaying.

Assaying

In three centrifugal tubers, 0.18 ml of phosphate buffer, 0.2 ml of AChE solution (or phosphate buffer for control) and 20 μl of sample extract (or methanol for normal and control) were added. After mixing and standing for 10−20 min. at 30℃, 0.1 ml of acetylthiocholine iodine (ATCh) (10 mmol/L) was added to start the reaction to test residual activity of AChE. After incubation for 15 min at 30℃, 3.6 ml of DTNB-ethanol-buffer solution was added and then the absorbance (Abs) was determined by UV-120-20 spectral photometer at 412 nm. The inhibition % was calculated below:

$$\text{Inhibition}\% = \frac{\text{Abs(normal)} - \text{Abs(sample)}}{\text{Abs (normal)}} \times 100\%.$$

All the samples contaminated with pesticides needed to be detected by AChE inhibition method and divided into two groups according to levels of inhibition AChE: A sample with no or weak inhibition effect can be identified to be contaminated with pyrethroids, and a sample with strong inhibition effect can be considered to be contaminated with organophosphorus or / and carbamates.

Gas chromatography

Extraction

Vegetable sample was macerated and an aliquot (50g fresh weight) was transferred into one quart laboratory blender. Added 100−160 ml of acetone was added and blended for 2 min. Extraction and clean-up procedures were shown in Fig. 1.

Gas chromatographic conditions

1. For pyrethroids

A Varian model 3700 gas chromatography with ^{63}Ni electron capture detector (ECD) was used. Borosilicate glass column (1m x 3mm I.D.) coated with 5% (w/w) OV-101 on

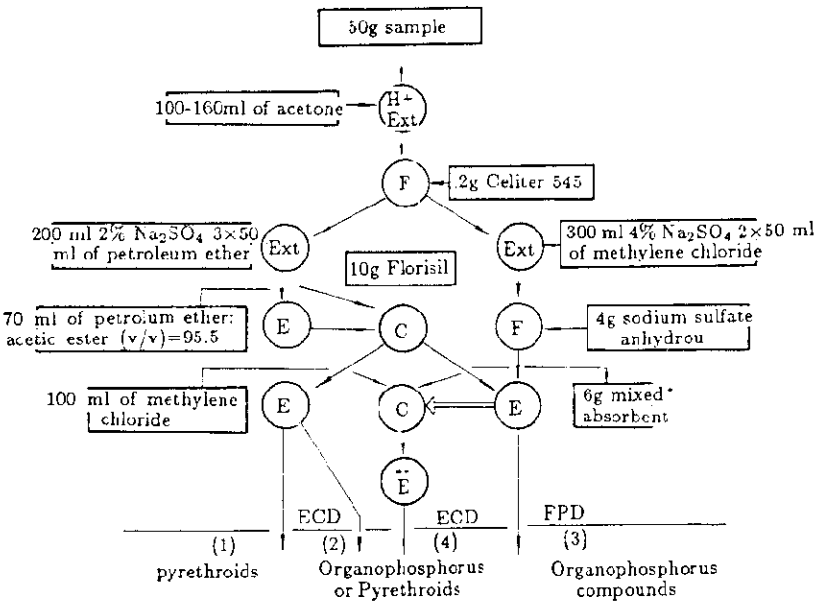


Fig. 1 Scheme of extracted and cleaned up program

H: Homogenization; Ext: Extraction; F: Filtration;

C: column chromatography; E: Evaporation;

⇒: Clean up once again. *: activated carbon: Florisil : celite 545 = 1:2:4;

**: Extracts in petroleum ether before injection;

(1), (2), (3), (4): Gas chromatographic conditions.

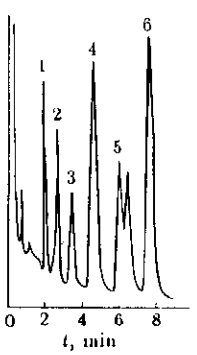


Fig.2 Chromatogram of (1) fenpropathrin; (2) pp321; (3) permethrin; (4) cypermethrin; (5) fenvalerate; (6) deltmethrin on 5% OV-101 column

chromosorb WHP, 80−100 mesh, 80−100 mesh, and conditioned 60 to 72 hours was used. Temperatures: injection block 300 ℃, oven 250 ℃, detector 350℃, carrier gas (nitrogen 99.999% purity) 60ml/min. 1μl of the mixtures of the pyrethroids in petroleum ether (b. p. 60−90 ℃) was injected through a septum on to the column. The effects were illustrated in Fig. 2 and Fig. 3.

2. For identification of pyrethroids

GLC idem (1): Borosilicate glass column (2m×2mm I. D.) coated with 1.5% (w/w) OV-17, 2% (w/w) QF-1 on chromosorb WHP, 80−100 mesh, and conditioned 48 to 60 hours

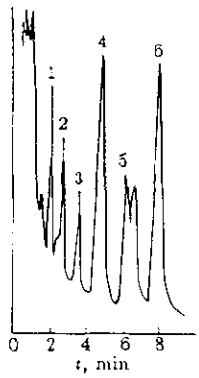


Fig. 3 Chromatogram of a cucumber sample, 50g: (1), (4), (5), (6) 0.05 ppm; (2) 0.01 ppm; (3) 0.10 ppm; injection volume, 1μl on 5% OV-101 column

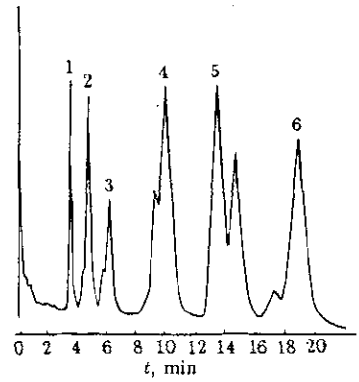


Fig. 4 Chromatogram of 1. fenpropathrin; 2. pp321; 3. permethrin; 4. cypermehtrin; 5. fenvalerate; 6. deltamethrin on 1.5% OV-17 2% QF-1 column

was used. Temperatures: injection block 260°C, oven 240°C, detector 330°C, carrier gas (nitrogen 99.999% purity) 50 ml/min. The gas chromatogram of injection volume, 1 μl was shown in Fig 4.

3. For organophosphorus compounds

A Varian Model 3700 gas chromatography with a flame photometric detector (FPD) was used. Borosilicate glass column (2m x 2mm I. D.) coated with 5% (w/w) OV-101 on chromosorb WHP, 100−120 mesh was used. Temperatures: injection block 210°C; detector 230°C. The following temperature programme was used: 1 min at 160°C, increased at 20 ℃ /min to 220 ℃, the last temperature being held for 9 min. Gas flows: carrier gas

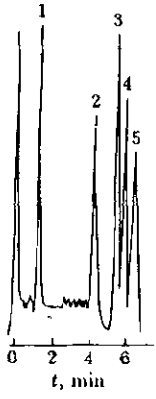
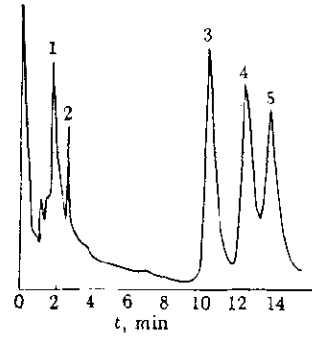


Fig. 5 Chromatogram of (1) dichlorvos; (2) dimethoate (3) parathion methyl; (4) malathion; (5) parathion on 5% OV-101 column



Fig, o Chromatogram of (1) dichlorves; (2) dimethoate; (3) parathion methyl; (4) malathion; (5) parathion on 1.5% OV-17 2% QF-1 column

(nitrogen 99.999% purity) 33 ml/min; detector: hydrogen 143 ml/min, air[1]* 80ml/min and air[2]* 171ml/min. 1μl of mixtures of the organophosphorus compounds in methylene chloride was injected through a septum on to the column. As an illustration of the possibilities of the proposed methods has chromatogram of a mixture of several components was shown in Fig. 5.

4. For organophosphorus compounds

GLC and column idem (2): Temperatures: injection block 260 ℃, detector 330 ℃. The following temperature programme was used: 1 min at 140 ℃, increased at 15 ℃ /min to 180 ℃, the last temperature being held for 15 min, carrier gas (nitrogen 99.999% puritry) 50 ml/min. A typical chromatogram of organophosphorus compounds appeared in Fig. 6.

The procedures for multiresidue analysis were shown in Fig. 7.

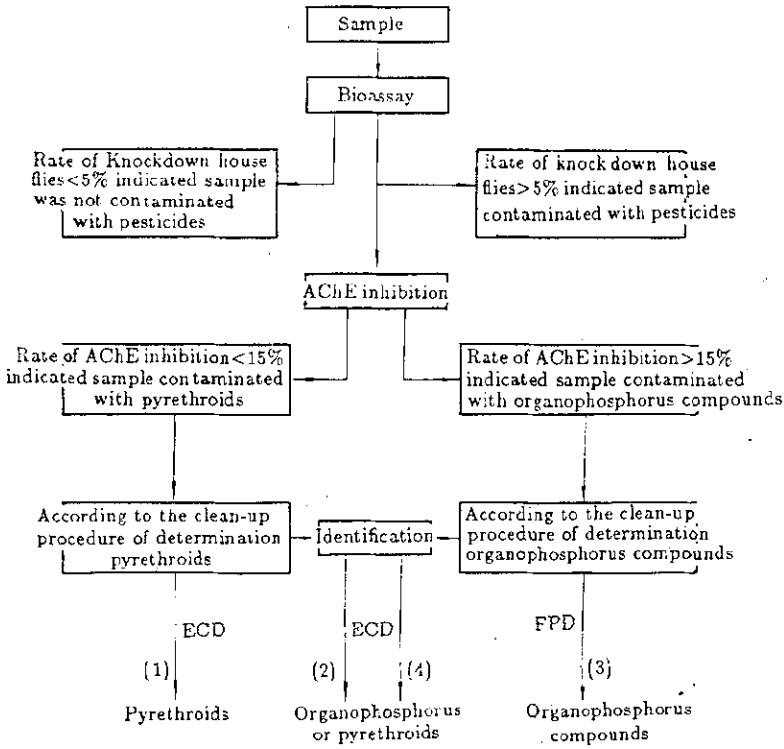


Fig. 7 Flow chart of the working procedure
(1), (2), (3), (4): Gas chromatographic conditions

RESULTS

Bioassay

Aliquots of standard solutions of the pesticides were added to chopped vegetables. The samples were analyzed by bioassay according to the steps described above. LC_{50} and LC_{95} values for the TS are given in Table 1, which shows the toxicities of the pesticides on the basis of 3-hour-test with the TS at 25°C. LC_{50} values were in order of aldicarb > carbofurnan > phoxin > dimethoate > malathion > methamidophos > parathion > dichlorvos > deltamethrin. However, the TS was unusually sensitive to high temperatures. For this reason, the results with this strain were given only at 25°C. If there was only one kind of pesticide in a sample, the method could be used for determination of its approximate concentration. In Table 1, most of the sensitivities detected from bioassay are higher than MRL (Li, 1981; JMPR, 1984; 1988; Pan, 1984).

Table 1 The limits of pesticides determined by the bioassay compared with MRL in vegetables

Pesticides	Sample	LC_{50}, ppm	LC_{95}, ppm	Regression equation	Concentration of 5% knocked down, ppm	MRL*, ppm
aldicarb	Chinese cabbages	7.52	31.81	$y=2.70+2.63x$	1.50	not used
carbofurnan	Chinese cabbages	5.94	29.20	$y=3.16+2.37x$	1.25	not used
phoxim	Cucumbers	5.91	34.4	$y=3.34+2.15x$	1.00	0.05 –0.2
dimethoate	Cucumbers	3.45	25.7	$y=3.99+1.89x$	0.50	1.0 –2.0
malathion	Chinese cabbages	2.21	8.38	$y=4.02+2.84x$	1.00	1.45 –8.0
methamidophos	Chinese cabbages	1.72	18.13	$y=4.62+1.61x$	0.50	0.01 –1.0
parathion	Cucumbers	1.12	6.35	$y=4.89+2.15x$	0.20	0.05 –1.0
dichlorvos	Cucumbers	0.47	8.95	$y=5.42+1.29x$	0.10	0.1 –2.0
deltamethrin	Chinese cabbages	0.024	0.14	$y=8.52+2.18x$	0.01	0.01 –0.2
deltamethrin	Cucumbers	0.023	0.24	$y=7.67+1.63x$	0.01	0.01 –0.2

* included: white cabbage, cauliflower, cucumber, tomato, sweetbell redpepper, eggplant and bean.

AChE inhibition

Fig. 8 summarizes the courses of inhibited AChE by 13 pesticides, and shows the sensitivities of AChE to different target pesticides. The sensitivity of AChE to dichlorvos, carbaryl

and trichlorphon was much more than to other pesticides based on percentage of inhibition-log dosage of pesticides lines. It was suggested that AChE inhibition method could be used as a screening test to sort out which of these samples were contaminated with organophosphorus or / and carbamates, and pyrethroids before GLC determination. If percentage of inhibition was more than 15 percent, the samples might be considered as positive response. The result indicated presence of organophosphorus or / and carbamate in the sampled vegetable. In a negative response, the sampled vegetable could be considered to be contaminated with pyrethoids.

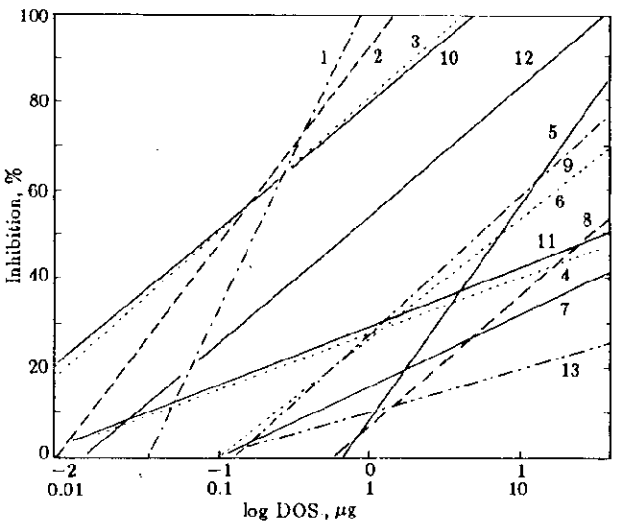


Fig. 8 Courses of inhibited AChE by 13 pesticides pesticide line b values of dosage-inhibition, %

(1) dichlorvos	0.0151	(2) carbaryl	0.0227	(3) trichlorphon	0.0323
(4) monocrotophos	0.0800	(5) malathion	0.0213	(6) omethoate	0.0379
(7) pirimicarb	0.0654	(8) dimethoate	0.0351	(9) parathion	0.0327
(10) methamidophos	0.1024	(11) carbofuran	0.0358	(12) aldicarb	0.0753
(13) phorate	0.0453				

GAS chromatography

Recovery experiments should be carried out by adding known amount of pesticides to untreated (control) samples before extraction. At least one untreated (control) and one fortified (spike) sample must be analyzed. The fortification concentration should be in the range of the residue levels expected in treated samples. If no measurable residues were expected in the sameples, the fortification concentration should be at or near limit of determination. The recovery values from fortified samples were summarized in Table 2, which shows recoveries

of organophosphorus compounds ranged from 84.0% in tomatoes to 102.7% in sweetable redpeppers, and of pyrethroids ranged from 83.7% in Chinese cabbages to 105.6% in tomatoes. Coefficients of variations ranged from 0.59% to 7.87% for pyrethroids, and 0.33% to 9.88% for organophosphorus compounds. A minimum concentration of organophosphorus and pyrethroids ranged from 0.005 to 0.013 ppm could be measured accurately. The levels of 0.005 and 0.013 ppm were the minimum amounts of the identified pesticides which could be determined with good accuracy and precision. Fig. 3 displayed a representative chromatogram from the procedure described above.

Table 2 Recoveries of organophosphorus compounds and pyrethroids with vegetables

Pesticide	Added, ppm	Cucumbers		Tomatoes		Chinese cabbages		Sweetbell redpeppes	
		Recovery, %	C. V. %	Recovery, %	C. V. %	Recovery, %	C. V. %	Recovery, %	C. V. %
fenpropathrin	0.5	96.4±2.1	2.18	99.3±2.62	2.64	93.7±4.10	4.37	87.0±2.14	2.47
	0.05	95.6±2.04	2.14	95.1±4.80	5.04	89.6±4.01	4.47	96.6±1.58	1.63
pp321	0.1	98.4±2.31	2.35	98.5±3.62	3.68	83.7±2.89	3.45	87.5±1.87	2.14
	0.01	97.6±3.02	3.09	88.7±1.19	1.34	90.0±4.28	4.75	89.5±2.21	2.47
permethrin	1.0	93.0±1.83	1.97	93.5±1.30	1.39	90.9±2.15	2.36	90.7±7.14	7.87
	0.1	97.0±0.57	0.59	102.8±3.46	3.36	92.3±6.65	7.21	89.7±5.15	5.74
cypermethrin	0.5	97.5±0.61	0.62	99.5±3.70	3.71	87.5±1.79	2.05	91.0±6.10	6.71
	0.05	102.8±5.38	5.23	104.9±1.03	0.98	91.7±3.92	4.28	100.9±2.88	2.86
fenvalerate	0.5	99.5±3.08	3.10	97.9±2.32	2.37	95.7±3.73	3.89	99.1±2.09	2.11
	0.05	96.7±5.75	5.95	105.1±5.64	5.37	91.1±4.68	5.14	101.1±6.27	6.20
deltamethrin	0.5	98.4±1.76	1.79	98.1±1.37	1.40	91.4±1.85	2.03	86.6±4.26	4.91
	0.05	100.6±4.74	4.71	105.6±2.57	2.44	91.0±5.56	6.11	98.3±1.94	1.98
dichlorvos	1.0	95.8±2.5	2.61	91.2±4.31	4.73	92.8±3.04	3.28	97.7±1.16	1.19
	0.1	92.2±9.08	9.85	91.7±2.92	3.26	90.9±7.96	8.76	89.5±3.06	3.42
	0.05	96.8±0.32	0.33	84.0±2.55	3.04	87.1±6.75	7.75	85.2±4.74	5.57
dimethoate	1.0	99.9±2.86	2.87	95.0±5.37	5.65	99.0±1.85	1.87	94.5±3.56	3.77
	0.1	99.2±4.02	4.05	96.5±2.25	2.33	99.5±5.63	5.66	97.5±2.20	2.26
	0.05	96.1±5.93	6.17	100.3±6.69	6.62	96.6±2.25	2.23	96.7±1.39	1.44
parathion methyl	0.4	99.4±1.96	1.98	95.4±1.49	1.56	101.2±1.09	1.08	95.8±2.66	2.78
	0.04	98.2±1.70	1.74	94.9±4.67	4.92	98.2±1.45	1.48	95.9±2.22	2.31
	0.02	87.1±6.90	7.92	97.4±8.59	8.87	98.4±1.55	1.58	94.2±3.73	3.96
malathion	1.0	94.3±4.65	4.94	93.2±2.47	2.65	91.7±1.03	1.12	88.4±2.05	2.32
	0.1	89.4±8.84	9.88	88.3±2.20	2.49	99.2±1.53	1.55	90.5±4.04	4.47
	0.05	90.9±2.05	2.26	97.4±1.15	1.14	94.7±5.50	5.80	88.4±6.27	7.10
parathion	1.0	97.9±1.91	1.95	93.2±2.47	2.65	97.2±1.22	1.25	95.8±1.81	1.89
	0.1	93.9±3.38	3.49	87.5±6.62	7.56	99.8±2.65	2.66	100.3±0.96	0.96
	0.05	93.5±2.54	2.70	102.7±6.81	6.63	94.4±2.53	2.68	93.9±3.22	3.22

DISCUSSION

The established set of methods have been used successfully to analyze vegetables samples. About 7000 samples of vegetables collected in Beijing were analyzed for content of pesticides with the bioassay. Most of the samples were not contaminated with pesticides. Only 138 samples (5% of knocked down house flies) were contaminated with pesticides, and were determined by AChE inhibition method. The results were arbitrarily divided into three groups: with no or weak AChE inhibition, 0 to 15 percent, with medium inhibition, 16 to 30 percent; with strong inhibition, >30 percent. About 32 percent of the samples had no or weak AChE inhibition. 48 percent had medium, and 20 percent has strong inhibition. The samples with no or weak inhibition could be considered to be contaminated with pyrethroids, and the others could be believed to be contaminated with organophosphorus compounds or / and carbamate. The strong inhibition was found more frequently in the cucumbers than in others.

All the samples with no or weak inhibition were analyzed by GLC with ECD. In 44 samples with no or weak inhibition, 3 different kinds of pyrethroids were found, indentified and quantitated. Results showed that residues of 0.01 to 0.1, 0.1 to 0.2 and > 0.2 ppm were found in 70.5, 20.5 and 9 percent, respectively. The vegetables with residues for deltamethrin, fenvalerate and cypemethrin were found in 59.1, 22.7 and 18.2 percent, respectively. The levels from 0.01 to 0.2 ppm are MRL for pyrethroids residues in most of the vegetables (Li, 1981; JMPR, 1984; 1988; Pan, 1984). The 6 samples (in 44 samples) have been found to have a pesticide content exceeding the MRL.

The samples with medium and strong inhibition were determined by GLC with FPD. In 94 tested samples, 4 different organophosphorus compounds were identified and quantitated, among them dichlorvos was in 50 percent, dimethoate in 20 percent, malathion in 17 percent, parathion in 10 percent. But the kind of pesticides existed in 3% of the samples with medium and strong inhibition was not identified yet. Cucumbers, tomatoes and sweetbell redpeppers were the vegetables in which residues were found most frequently. In 53 cucumber samples with medium and strong inhibition, dichlorvos residues were found in 55 percent, dimethoate in 25 percent, both malathion and parathion was in 15 percent. Sometimes there were malathion and decametrin in a same sample. If there were several pesticides (organophosphorus and pyrethroids) in a same sample it might be necessary to be analyzed by GLC with FPD or / and ECD. In samples contaminated with organophosphorus compounds, 49 samples were found to have a pesticide content exceeding the MRL. It should be noted that the identified measures were used only in cases of some peaks were difficult to distinguish on one column or one detector. The double identification was unnecessary in general.

In addition, the production of the chlorinated hydrocarbon compounds, such as DDT and BHC were stopped 7 years ago, and most of carbamate were prohibited from applying on the vegetables in China. Therefore, we did not study the conditions to determine these residues by GLC.

REFERENCES

FMC., Category A, 1983, Oct. 4
Jeferey, G., Pesticide Biochemistry and Physiology, 1984, 21: 53
Jesper Kjolholt, Journal of Chromatography, 1976, 325: 231
JMPR, ANNEX, 1984, I: 79
JMPR, ANNEX, 1988, II: 87
Keith, D, Wing, Pesticide Biochemistry and Physiology, 1984, 21: 22
Krause, R. T. and Wang Yi, Journal of Chromatography, 1988, 459: 151
Li Guoqin, Agricultural Sciences (Taiwan), 1981, 29 (7—8): 211
Pan Dajun, Journal of Pesticide Control (in Chinese), 1984, 1: 21
Roman, M. Sawicki and Johannes Keiding, Pesticide Sciences, 1981, 12: 587
Siv Renvall and Malin Akerblom, Residue Reviews, 1970, 34: 1
Van Emon, J. M., Analytical methods for pesticides and plant growth regulators, New York: Academic Press, 1989: 17: 217
Willem, K., Journal of Chromatography, 1976, 117: 201

(Received December 4, 1990)