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Pesticide Residue Monitoring in Marketed Fresh Vegetables and Fruits in Central Taiwan (1999-2004) and an Introduction to the HACCP System

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ABSTRACT

The objectives of this study are: (1) to collect and analyze the data of pesticide residues in vegetables and fruits in central Taiwan; (2) to compare the statistics of pesticide residues data in vegetables and fruits in 4 regions of Taiwan; and (3) to introduce the Hazard Analysis Critical Control Point (HACCP) system to industries and growers of vegetables and fruits for improving the safety of agricultural products. The 1999 samples of vegetables and fruits were collected from supermarkets and traditional markets by 6 local bureau of health in central Taiwan (1999-2004) and analyzed for the presence of 70~79 pesticide residues using multiresidue analysis methods (MRMs). The detection limits of these methods ranged from 0.03 to 0.4 ppm. For central Taiwan (1999-2004), pesticide residues were either absent or compliant with the Maximum Residue Limit (MRL) in 99.8% of the samples. Only 4 samples contained a pesticide residue that exceeded the MRL (0.2%, 4 of 1999). In whole Taiwan (1997-2003), pesticide residues were detected in 13.9% of the 9955 samples and 1.2% were violative. To decrease the health risk to human and environment from exposure to pesticides, the government samples and analyzes agricultural products for pesticide residues to enforce the limits set by Department of Health every year. Risk assessment and HACCP have some overlapping components; both HACCP and risk assessment are encompassed in risk analysis. HACCP is a preventative system and will provide a high level of food safety assurance. However, effective HACCP requires the consideration of all hazards, i.e. chemical (food additives, heavy metal contamination, pesticide residues, and animal drug residues), microbiological and physical hazards. HACCP principles have been incorporated into food safety legislation in Taiwan and worldwide.

Key words: pesticide residues, hazard analysis critical control point (HACCP), maximum residue limit (MRL), risk assessment, multi-residue method, GC, HPLC, GC/MS

INTRODUCTION

The use of pesticide has been traced by historian to 1000 B.C. Homer mentioned the use of sulfur as fumigant to avert disease and control insects. Pesticides are either chemicals or biological substances used to diminish or control pest[(1)], including insecticides, fungicides and herbicides. There are more than 30 categories of pesticides based on their chemical structure.

The behavior of pesticides in the agricultural produce is of great importance, since the disappearance, persistence, or partial transformations of such a compound determines its usefulness or its potential effects to our environment[(2,3)]. Located in the subtropical area, Taiwan is warm and humid, which leads to the growth of insects and molds. The application of pesticides is essential to effectively avoid mildew and increase agricultural production. There were more than 400 registered insecticides in Taiwan, and the Department of Health (DOH) announced the tolerance level for residues of pesticide in 20 categories including 307 insecticides in 2003[(4,5)]. Any inappropriate use of insecticides could cause residue problems. Therefore, pesticide residue is becoming a major food safety concern of consumers and government.

To decrease the health risk to human and environment from exposure to pesticides, the government analyzes agricultural product samples for pesticide residues to enforce the tolerance levels established by DOH every year. Two federal government agencies share the responsibility of pesticides. Council of Agriculture approves the use of pesticide and monitors the pesticide residues of agricultural produce in the field. However, DOH is responsible for setting "Tolerances for the Residues of Pesticides" and monitoring the pesticide residues of agricultural produce in the market. Each month, 5 samples of vegetables and fruits were collected from supermarkets and traditional markets by local public health authority throughout Taiwan during 1999 to 2004. All samples were analyzed for 70~79 pesticide residues by 4 laboratories: Division of Food Chemistry (in Taipei), Central Region Laboratory (in Taichung), Southern Region Laboratory (in Kaushing) and Eastern Region Laboratory (in Hwaling) of Bureau of Food and Drug analysis (BFDA) with GC, HPLC and GC/MS. To analyze the large numbers of samples with

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unknown pesticide treatment history, BFDA and US FDA (Food and Drug Administration, USA) use analytical methods capable of simultaneously determining a number of pesticide residues[5,6]. These multiresidue methods (MRMs) can determine about 100~400 pesticides with DOH tolerances set, and many others that have no tolerances. The most commonly used MRMs can detect many metabolites, impurities, and alternation products of pesticides. Single residue methods (SRMs) usually determine one pesticide with high sensitivity and recovery; however, SRMs are less cost effective than MRMs[4,6].

In 1991, the US National Cancer Institute implemented a national campaign, "Five a day for Better Health" which aimed at increasing consumption of fruits and vegetables in America[7]. In 2001, the US FDA pesticide residues monitoring report revealed that the violation rates for domestic vegetables and fruits respectively were 1.5% and 1.1%. Nevertheless, 6.4% of the imported vegetables and 2.8% of the imported fruits were violative[8]. The Sydney Market survey in fresh fruits and vegetables showed that pesticide residues were not detected or complied with Maximum Residue Limit (MRL) in 98.1% of samples. Only 7 samples (1.9%) contained a pesticide residue exceeding MRL[9]. According to statistical data from the BFDA during 1997 to 2003, pesticide residues were detected in 13.9% of the fruits and vegetables. Only 1.2% of samples were violative[5,10]. In addition to the annual statistic data of monitoring pesticide residues in fruits and vegetables reported by DOH[5,11], there are also several issues on establishing new analytical methods of pesticide residue in Taiwan, such as Su *et al.* (2003)[12], Chou *et al.* (2004)[13] and Tseng *et al.* (2004)[14-16].

In the US, FDA Monitoring Program was divided into 3 parts: regulatory monitoring, incidence/level monitoring and total diet study. Regulatory Monitoring placed emphasis on raw agricultural products, including the unwashed, whole (unpeeled), raw commodity as well as processed foods. A specialized type of monitoring program called "Incidence/ Level Monitoring", FDA tried to determine how often a certain pesticide is found on a particular crop[17]. The Total Diet Study (TDS), also known as the Market Basket Study, has been an important part of FDA's monitoring program for chemical contaminants in the U.S. food supply for many years. The primary purposes of TDS are to (1) estimate the dietary intakes of pesticides, industrial chemicals, toxic elements, radionuclides and essential minerals and (2) compare these intakes with established dietary intake levels. Depending on the chemical substance being evaluated, these "established" intakes include Acceptable Daily Intakes (ADIs)[18]. It is distinct from regulatory monitoring in that it determines pesticide residues in food prepared for consumption. To measure the low levels of residues found in the TDS foods, the analytical methods used are modified to permit measurement at levels 5~10 times lower than those normally used in regulatory monitoring. In general, residues present at or above a part per billion can be measured[6,17]. However, the survey of TDS was not included in this study. In US, all the pesticide residue monitoring information is also entered into a computer program specially developed to estimate health risks, called the "Dietary Exposure Evaluation Model (DEEM)". The Environmental Protection Agency (EPA) considers the exposure through food, drinking water, and home use of pesticides. EPA will set a tolerance level for food if the combined exposure from different sources is 100 to 1000 times lower than the maximum level that shows no harmful effects in experimental animals. Residue levels are usually present in very small amounts that may be expressed as parts per million (ppm), parts per billion (ppb) and parts per trillion (ppt)[17]. It is also necessary to develop a DEEM for estimating health risks in Taiwan.

Risk assessment and Hazard Analysis Critical Control Point (HACCP) are often included in the same discussions and encompassed in risk analysis in food safety literatures but their relationship are interpreted differently. Risk assessment is a logical approach which has been used successfully for managing many types of risks including radiation control, chemical contamination of the environment and foods, and water quality. HACCP is a systematic approach to the control of potential hazards in a food operation[19]. HACCP was originally developed as a microbiological safety system in the early days of the U.S. space program, as it was vital to ensure the safety of food for astronauts. At that time, most food safety and quality systems were based on end-product testing which required 100% testing to assure product safety. HACCP is a preventative system that can provide high level of food safety assurance[20]. Effective HACCP system requires the consideration of all hazards (i.e. chemical, microbiological and physical hazards)[21]. HACCP principles have been incorporated into food safety legislation for the food production, processing, distribution and retail industries in Taiwan and worldwide since 1995[22].

The objectives of this study are: (1) to collect and analyze the pesticide residues in vegetables and fruits in central Taiwan; (2) to compare the statistics of pesticide residues in vegetables and fruits in 4 regions of Taiwan (1999-2004), and (3) to introduce the HACCP system to industries and growers of vegetables and fruits for improving the safety of agricultural products. This study provides useful information for authorities to regulate pesticide residues in fruits and vegetables and to protect consumer health.

MATERIALS AND METHODS

I. *Sources of Samples*

From July 1999 through December 2004, 5 vegetable and fruit samples were collected from supermarkets and traditional markets by local Bureau of health throughout Taiwan (Miaoli County, Taichung County, Taichung City, Changhua County, Yulin County and Nantou County) every

month. All samples were analyzed for 70~79 pesticide residues by Central Region Laboratory (in Taichung) of Bureau of Food and Drug analysis (BFDA).

II. *Reagents and Materials*

Acetone, dichloromethane, petroleum-ether, *n*-hexene, acetonitile, NaOH, sodium boric acid, boric acid, *o*-phthalaldehyde, 2-mercaptoethanol, $SnCl_2$-$2H_2O$, CS_2, HCl, $NaHCO_3$, NaCl, methanol, sodium hydride and diethyl ether were purchased from E. Merck; Sep-Pak Florisil (6 mL, 500 mg) was purchased from Waters (MA, USA); 79 pesticide standards (listed on Table 1) were individually purchased from Riedeal de Haen (Germany), Chem Service (West Chester, PA, USA) or Dr. Ehrenstorfer GmbH (Germany) with a purity of 95~99.5%.

III. *Methods*

The multiresidue analytical methods are based on the DOH announced methods[23-25]. Those analytical methods including their detection limits are illustrated in Table 1.

(I) *Sample Preparation*[23-24]

1. *Extraction*

Fresh vegetable and fruit samples were thoroughly shredded and homogenized and 50 g of which were extracted with 100 mL of acetone for 3 min. The extraction solution was then filtered under suction. The residues and container were washed with another 50 mL of acetone, which was then filtered to 250 mL. Fifty milliliter of above filtrate was extracted with 50 mL of petroleum ether and 50 mL of dichloromethane twice. The combined organic phase was filtrated through a funnel containing anhydrous sodium sulfate and evaporated to dryness at 35~40°C using a rotary evaporator. The residue was dissolve in 5 mL of acetone for detection of GC-FPD. Two milliliter of acetone residue was evaporated to dryness with N_2 and dissolved in 2 mL of acetonitrile and filtered through a 0.45 μm nylon membrane prior to HPLC analysis. One milliliter of acetone residue was evaporated to dryness with N_2 and dissolved in 1 mL of *n*-hexene for clean-up.

2. *Solid Phase Extraction (SPE) for Sample Cleanup*

The above concentrate (1 mL) was loaded into a Sep-Pak Florisil cartridge, which was rinsed with *n*-hexene prior to applying samples. The concentrate in cartridge was then eluted with 15 mL of *n*-hexene/dichlormethane (1/2), the eluent was collected for GC-ECD analysis.

(II) *GC-FPD Analysis*

Gas chromatography (Varian 3400, Varian Technologies, Walnut Creek, CA, USA), equipped with a Flame photometric detector, was used for analysis. The column was a J&W DB-608 (30 m × 0.53 mm, 0.83 μm film thickness) or a J&W DB-1 (30 m × 0.53 mm, 0.83 μm film thickness). Carrier gas and make-up gas was nitrogen at flow rate of 8.0 and 30 mL/min, respectively. The temperature of oven, injector and detector was set at 250°C, 280°C and 300°C, respectively. The oven temperature was programmed as follows: 180°C for 1 min, 15°C/min up to 250°C, held for 3 min and non-programmed 220°C for 10 min.

(III) *GC-ECD Analysis*

Gas chromatography (Varian 3300, Varian Technologies, Walnut Creek, CA, USA), equipped with an Electron capture detector, was used for analysis. The column was a J&W DB-608 (30 m × 0.53 mm, 0.83 μm film thickness) or a J&W DB-1701 (30 m × 0.53 mm, 0.83 μm film thickness). Carrier gas and make-up gas was nitrogen at flow rate of 8.0 and 30 mL/min, respectively. The temperature of oven, injector and detector was set at 250°C, 280°C and 300°C, respectively. The oven temperature was programmed as follows: 180°C for 1 min, 15°C/min up to 250°C, held for 18 min.

(IV) *Test of Dithiocarbamates*[25]

Sample was homogenized with a blender. Two grams of samples or 2 mL of CS_2 (Merck, Germany) standard solution were added with 9 mL of reactive solution (1.5% $SnCl_2$-$2H_2O$ in 5M HCl) in 22-mL vial. Having been closed tightly, the vial was shaken thoroughly for 3 min and placed in 80°C water bath for 2 hr. Gas of head space (500 μL) was injected to GC- FPD (Shimadzu 14B equipped with a GS-Q column).

(V) *HPLC- Fluorescence Analysis and HPLC- UV Analysis*

HPLC- Fluorescence Analysis: A Shimadzu (Shimadzu corporation, Kyoto, Japan) instrument includes Shimadzu LC-10AT pump system with a Shimadzu RF-10AXL detector and a *TSK-GEL ODS-80TM* column. Acetonitrile/water (50/50, v/v) was used as the mobile phase.

HPLC- UV Analysis: Another Shimadzu LC-10AD pump system with a Shimadzu SPD-M10A photodiode array UV detector (280 nm) and a Polaria 5 μ C18 column. The mobile phase was 5% acetic acid/acetonitrile (90/10, v/v).

(VI) *Gas Chromatography and Mass Spectrophotometer (GC/MS)*

A Shimadzu GCMS-QP2010 and a J&W DB-5 analytical column (30 m × 0.25 mm) were used. A Shimadzu GCMS solution software was used as the data analysis system. The oven temperature was programmed at 80°C for 5 min and then raised on to 280°C at 10°C/min. The injection port and MSD interface temperature were

Table 1. The determination methods and detection limit of the 79 pesticides

Analytical methods	International pesticide names (detection limit in ppm)
GC-FPD-P	Acephate (0.03), bromophos-methyl (0.06), chlorpyrifos (0.03), carbophenothion (0.03), diazinon (0.03), dichlorvos (0.04), dimethoate (0.03), disulfoton (0.03), EPN (0.03), ethion (0.03), fenitrothion (0.03), fenthion (0.03), fonofos (0.03), ethyl-parathion (0.03), ethoprophos (0.03), isoxathion (0.03), mevinphos (0.03), demeton-s-methyl (0.03), mephosfolan (0.03), methyl-parathion (0.03), methidathion (0.03), pirimiphos (0.03), malathion (0.03), methmidophos (0.02), monocrotophos (0.03), omethoate (0.08), phenthoate (0.03), prothiofos (0.03), phosalone (0.05), pyrachlofos (0.1), phorate (0.03), phosmet (0.03), quinalphos (0.03), cyanofenphos (0.03), triazophos (0.03), trichlorfon (0.03)
GC-ECD	Alphacypermethrin (0.2), bifenthrin (0.1), bromopropylate (0.06), captan (0.05), captafol (0.03), cyfluthrin (0.2), chinomethionat (0.03), cypermethrin (0.2), chlorothalonil (0.03), cyhalothrin (0.05), deltamethrin (0.1), dicofol (0.1), esfenvalerate (0.1), endosulfan (0.05), fenpropathrin (0.1), flucythrinate (0.2), fluvalinate (0.2), fenvalerate (0.05), iprodione (0.1), permethrin (0.2), procymidone (0.05), profenophos (0.05), pyridaphenthion (0.2), terbufos (0.01), triadimefon (0.03), vinclozolin (0.05)
HPLC- fluorescence	Aldicarb (0.1), aldicarb sulfone (0.1), aldicarb sulfoxide (0.2), carbaryl (0.1), carbofuran (0.1), 3-keto carbofuran (0.2), 3-OH carbofuran (0.1), fenobucarb (0.08), isoprocarb (0.1), methiocarb (0.1), metolcarb (0.1), methomyl (0.1), thiodicarb (0.1)
HPLC-UV	Carbendazim (0.4), formetanate (0.4), thiabendazole (0.4)
GC-FPD-S	Dithiocarbamates (0.1)

Table 2. Pesticide residue in fruits and vegetables in central Taiwan (1999-2004)

	1999-2001	2002-2004
No residue detected		
Fruit	96.7%	88.5%
Vegetable	97.1%	82.4%
Within tolerance		
Fruit	3.3%	11.5%
Vegetable	2.8%	17.3%
Violative		
Fruit	0%	0%
Vegetable	0.1%	0.3%
Total of samples	1023	976
(fruit/vegetable)	182/841	226/750

Table 3. Pesticide residue in fruits and vegetables in Taiwan (1999-2003)

	1999-2001	2002-2003
No residue detected		
Fruit	91.2%	85.7%
Vegetable	88.1%	81.3%
Within tolerance		
Fruit	7.0%	13.6%
Vegetable	11.1%	18.1%
Violative		
Fruit	1.8%	0.7%
Vegetable	0.8%	0.6%
Total of samples	3418	2580
(fruit/vegetable)	727/2691	561/2019

250°C and 280°C. The injection volume was 1 μL using helium as carrier gas at 8 psi. The ionization energy was set at 70 eV.

RESULTS AND DISCUSSION

I. *The Multiresidue Analytical Methods and the Analysis of 70~79 Pesticides Residue*

From July 1999 to December 2004, a total of 1999 samples including 408 fruits and 1591 vegetables were collected from supermarkets and traditional markets by local public health authorities in central Taiwan. The unwashed, whole, unpeeled, raw samples were analyzed for 70~79 pesticide residues by the Central Region Laboratory. The multiresidue analytical methods including their detection limits of 79 pesticides residue in vegetables and fruits are illustrated in Table 1. Seventy pesticide residues were tested in 1999-2000. However, 79 pesticide residues were monitored in studies during 2001-2004. The 9 additional pesticides were dichlorvos, omethoate, trichlorfon, bromopropylate, dithiocarbamates, carbendazim, formetanate, thiabendazole, and fenbucab. Lower detection limits of analytes for trace analysis, increasing automation to reduce analytical costs and improve precision, smaller sample sizes, and use of coupled systems allowing cleanup, separation, detection, and conformation have been observed in recent pesticide residue analytical chemistry studies[16,26,27].

II. *Monitoring Pesticide Residues in Vegetables and Fruits in Central Taiwan*

GC remains the most widely used method for pesticide residue analysis, e.g., the classic Luke *et al.* method[28] and its modification[29]. GC/MS is a legally accepted confirmation technique. Advances were reported in residue analysis by GC/MS with full ion scanning and SIM. The majority of pesticide residue determination is still performed on RP-LC system with a UV (fixed- or variable-wavelength or PDA) or fluorescence detector, but MS is being reported increasingly for detection[27]. The 1999-2004 survey of pesticide residues in central region of Taiwan is illustrated in Table 2. The 1023 samples (182 fruits and 841 vegetables) were collected during 1999 and 2001; however, 976 samples

(226 fruits and 750 vegetables) were collected between 2002 and 2004. Pesticide residues were not detected in 96.7% of fruits and in 97.1% of vegetables. Within MRL in 3.3% of fruits and in 2.8% of vegetables, only 0.1% of vegetables contained pesticide residue that exceeded the MRL between 1999 and 2001. Monitoring results also showed that between 2002 and 2004 no residue was found in 88.5% of fruits and in 82.4% of vegetables. Within MRL in 11.5% of fruits and in 17.3% of vegetables, only 0.3% of vegetables contained pesticide residue that exceeded the MRL. Detection by GC and HPLC, the survey results of pesticide residues from 1999 to 2003 in Taiwan are illustrated in Table 3 (data from annual scientific reports of BFDA, DOH[5,30]). A total of 3418 samples including 727 fruits and 2691 vegetables were collected during 1999 and 2001. On the other hand, 2580 samples including 561 fruits and 2019 vegetables were collected between 2002 and 2003. Pesticide residues were not detected in 91.2% of fruits and in 88.1% of vegetables. Within MRL in 7.0% of fruits and in 11.1% of vegetables, only 1.8% of fruits and 0.8% of vegetables contained pesticide residue that exceeded the MRL during 1999 and 2001. Monitoring results also showed that during 2002 and 2003 no residue was found in 85.7% of fruits and in 81.3% of vegetables. Within MRL in 13.6% of fruits and in 18.1% of vegetables, only 0.7% of fruits and 0.6% of vegetables contained pesticide residue that exceed the MRL.

Table 4. The pesticide residue of vegetables and fruits exceeding 1/2 MRL (maximum residue limit) in central Taiwan, 1999-2004

Years	Vegetables or fruits	Pesticide residue (MRL in ppm)	
1999-2000	Water spinach	3-OH Carbofuran	0.74 (1.0)
	Chinese chive	Ethion	0.69 (0)*
	Carrot	3-OH Carbofuran	0.29 (0.5)
	Water spinach	3-OH Carbofuran	0.51 (1.0)
	Garden lettuce	Thiocarb	0.54 (1.0)
2001	Grape	Omethate	0.18 (0.2)
2002	Chinese mustard	Turbufos	1.99 (0.05)**
	Cabbage	Chlorpyrifos	0.44 (0.5)
	Chinese cabbage	Carbofuran	1.84 (0.5)**
2003	Celery	Chlorpyrifos	0.90 (1.0)
	Chinese mustard	Profenofos	0.69 (1.0)
	Celery	Dithiocarbametes	2.73 (4.0)
	Water spinach	Carbaryl	0.78 (1.0)
	Radish	Dithiocarbamates	0.28 (0.5)
	Ching Geeng	Chlorpyrifos	0.70 (1.0)
	Chinese mustard	Carbofuran	0.51 (1.0)
	Wax apples	Dithiocarbamates	2.90 (5.0)
2004	Cabbage	Methamidofos	0.39 (0.5)
	Chinese mustard	Dithiocarbamates	2.18 (4.0)
	Celery	Chlorpyrifos	0.62 (1.0)
	Bitter melen	Dithiocarbamates	1.36 (2.5)
	Chinese cabbage	Dithiocarbamates	1.43 (2.5)
	Ching Geeng	Prophenofos	0.57 (1.0)
	Green pepper	Dithiocarbamates	1.32 (2.5)
	Chinese mustard	Terbufos	2.23 (0.05)**
	Chinese mustard	Prophenofos	0.64 (1.0)
	Radish	Dithiocarbamates	0.3 (0.5)

*Ethion not permitted for use in Chinese chive.
**Pesticide residue exceeding MRL.

III. *Violative Pesticide Residues of Vegetables and Fruits in Central Taiwan (1999-2004)*

The pesticide residue levels on vegetables and fruits which exceeded MRL or were higher than 1/2 MRL in central region of Taiwan (1999-2004) are shown in Table 4. These figures still conformed to the "Tolerance for the residues of pesticides" regulation, but still possess higher risk than those were lower 1/2 MRL. In America, the Pesticide Data Program (PDP) was created in 1991 by USDA to provide guideline on pesticide use and residue detection to EPA, FDA and consumers. EPA also used this information in estimating health risk from a pesticide[17].

Two water spinach and 1 carrot sample were detected to contain 3-OH carbofuran residue within the MRL but above 1/2 MRL, i.e. 0.74 ppm, 0.51 ppm and 0.29 ppm, respectively. A garden lettuce sample was detected to contain thiocarb 0.54 ppm. In addition, a violative Chinese chive sample contained ethion residue (0.69 ppm) that was not permitted to apply on small leave vegetables between 1999 and 2000. In 2001, only 1 fruit (grape) was detected to contain omethate 0.18 ppm above 1/2 MRL. Two vegetables were detected violative in 2002, one was Chinese mustard containing 1.99 ppm of terbufos which exceeded the 0.05 ppm MRL and the other one was Chinese cabbage containing 1.84 ppm of carbofuran exceeding the MRL (0.5 ppm). A cabbage contained 0.44 ppm of chloropyrifos which as within the MRL but above 1/2 MRL.

In 2003, 2 celeries, 2 Chinese mustards, 1 water spinach, 1 radish, 1 Ching Geeng, and 1 wax apple contained pesticide residues which were over 1/2 MRL. At last, 3 Chinese mustards were detected to contain 0.64 ppm of prophenofos, 2.18 ppm of dithiocarbamates and 2.23 ppm of terbufos that exceeded 1/2 MRL. Four dithiocarbamates residues were found in 1.36 ppm of bitter melen, 1.43 ppm of Chinese cabbage, 1.32 ppm of Green pepper and 0.3 ppm of radish which were over 1/2 MRL. One cabbage had 0.39 ppm of methamidofos; 1 celery had 0.62 ppm of chloropyrifos and 1 Ching Geeng had 0.57 ppm of prophenofos within the MRL but over 1/2 MRL in 2004.

IV. *Frequency of Pesticide Residues Detected from the Marketed Vegetables and Fruits in Central Taiwan (1999-2004)*

A total of 212 samples including 26 fruits and 186 vegetables were detected to contain 26 residues among 79 residues. In this study, we compared pesticides detection frequency within fruits and vegetables. Statistical data on the frequency of pesticide residues detected from marketed fruits and vegetables is illustrated in Table 5.

Chlorothalonil residue was the most often detected from 41 agricultural produces. The second was prophenofos residue detected in 36 samples, followed by 34 samples of dithiocarbamates residue, 25 samples of methomyl residue, 13 samples of chlorpyrifos residue,

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Table 5. The frequency of pesticide residue detected from the marketed vegetables and fruits in central Taiwan

Pesticide residues* F/V **	1999-2000 F/V	2001 F/V	2002 F/V	2003 F/V	2004 F/V	Total
Acephate	0/1	0/1	0/3			0/5
Bifenthrin		0/1			0/1	0/2
Bromoprophylate				0/1	1/0	1/1
3-OH Carbofuran	0/7	1/2			0/1	1/10
Carbofuran	0/2	0/2	0/1	0/1	0/2	0/8
Carbaryl				1/1		1/1
Carbendazim			0/1	0/1		
Chlorothalonil	0/3	1/6	0/8	1/7	2/13	4/37
Chlorpyrifos			0/3	0/7	1/2	1/12
Cypermethrin	0/1				0/1	
Dithiocarbamates			0/1	2/10	4/17	6/28
Deltamethrin					0/1	0/1
Ethion	0/1			1/0	1/0	2/1
Fenpropathrin			0/2		0/2	
Iprodione			0/1	1/0	1/1	
Isoxathion					1/0	1/0
Malathion		0/1				0/1
Methomyl	1/4	1/1	2/5	1/3	1/6	6/19
Methamidophos	0/1	0/4			0/1	0/6
Profenophos	0/1	0/2	0/2	0/15	0/16	0/36
Procymidone			0/1		2/0	2/1
Thiodicarb	0/2				0/1	0/3
Vinclozolin	0/1	0/1	0/3			0/5
Omethoate		0/1				0/1
Terbufos			0/1		0/1	0/2
Phosmet				0/1		0/1
Total detected	1/24	3/22	2/28	6/49	14/63	26/186

*More than one residue can be detected in a samples simultaneously.
**F/V: no. of fruit samples / no. of vegetable samples.

11 samples of 3-OH carbofuran residue and 8 samples of carbofuran residue were found. Six samples of fruits were detected to contain methomyl or dithiocarbamates residues, which were the highest detection frequency of pesticide residues in fruit. Bifenthrin, bromopropylate, carbaryl, fenpropathrin, iprodione and terbufos had been detected twice from 1999 to 2003. Carbendazim, cypermethrin, deltamethrin, isoxathion, malathion, omethoate, and phosmet residue were detected only once. More than one residue was occasionally detected in fruits and vegetables. In 2003, 1 guava exported to Canada was detected to contain bromopropylate residue illegally. As the result, 5 samples of guava were collected by local public health authority in Taiwan for analyzing bromopropylate residue, and 1 guava was detected from 47 samples[5].

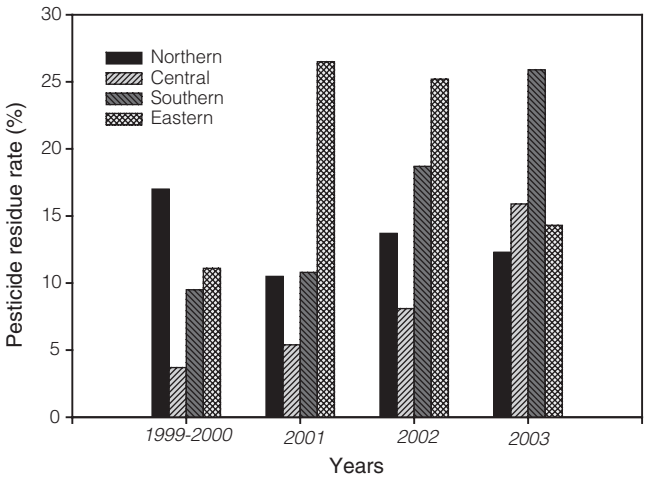

Figure 1. The detection rates of pesticide residue in fruits and vegetables in Taiwan.

V. *Comparison of Pesticide Residue Detection Rates in Four Areas of Taiwan (1999-2003)*

Located in the subtropical region, Taiwan has warm and humid weather which leads to the growth of insects and molds. To effectively avoid mildew, prevent diseases and increase agricultural production, it is difficult to avoid using pesticide. Comparison of pesticide residues in 4 areas of Taiwan (1999-2003) is showed in Figure 1. The detection rates of pesticide residue (above Minimal Detection Limit) decreased to 17%, 10.5%, 13.7%, 12.3% in northern area; the detection rates of pesticide residue were increased to 3.7%, 5.4%, 8.1%, 15.9% in central area; the detection rates of pesticide residue increased to 9.5%, 10.8%, 18.7%, 25.9% in southern area. The detection rates of pesticide residue were ranging from 11.1%, 26.5%, 25.2% to 14.3% in eastern area. The detection rates of central, southern, eastern areas were increasing except for northern area which was decreasing. Pesticide treatment history in vegetables

and fruits, improved detection methods, detection condition and additional pesticides detected might affect the detection rates. However, the detection rates of pesticide residue among 4 areas in Taiwan were between 12.3% and 25.9% in 2003.

According to statistical data from the BFDA during 1997-2003[5,30], 13.9% (1384/9955) of fruits and vegetables were found to have pesticide residues within 'the tolerance level of pesticide residues". Nevertheless, 1.2% of samples contained pesticide residue that exceeded MRL. Pesticide residues were detected in 42.3% of agricultural products, and 1.1% of the samples were violative in America (1997-2001)[5,6]. Comparison of violative pesticide residues in 4 areas of Taiwan (1999-2003) is shown in Figure 2. It can be seen that violation rates of pesticide residue were decreasing from 3.3% to 0.8% in northern area. The violation rates of pesticide residue were ranging from 0% to 0.3% in central area, 0.5% to 0.9% in southern area, and 0% to 0.6% in eastern area.

VI. *Analysis of a Violative Case: Terbufos Residue Exceeded Tolerance Level in Chinese Mustard*

One Chinese mustard collected by Taichung City health authority was detected to have 2.23 ppm of terbufos residue which exceeded MRL (0.05 ppm) in 2004. According to DOH announcements, the tolerance level for the residue of terbufos in leaflet vegetables and cabbages is 0.05 ppm, and 0.01 ppm for sugarcane, citrus and melons. Terbufos (Phosphorodithioic acid S-[[(1,1-dimethylethyl)thio]methyl]O,O-diethyl ester) is a organophosphate insecticide. Commercial product is clear, colorless to pale yellow liquid, with boiling point at 69°C and melting point at -29.2°C. Oral LD_{50} in quail is 15 mg/kg[31]. After different extraction procedures, GC-FPD was used to detect terbufos in this survey. Terbufos, which was separated by DB-608 column at 180°C and detected by FPD, showed a sharp and symmetrical peak at the retention time of about 3.7 min. Terbufos standard solution was dissolved in acetone, and made the concentration range of 0.5~4.0 μg/mL. The linear regression equation is: Y= 64698x + 7265 with coefficient correlation (r^2) of 0.9993. The peak retention time of sample solution and standard solution was compared and the concentration of terbufos was estimated by the equation: terbufos in sample (μg/mL) = C × V/M, where C was terbufos concentration estimated from the standard curve; V was the final volume (mL) in volumetric bottle; and M was the original sample weight (g). Figure 3 shows the total ion chromatogram and mass spectrum of terbufos (100 μg/mL) and Chinese mustard with 2.23 μg/mL terbufos, respectively. The retention time was 17.808 min. GC/MS condition in this detection is as follows: oven temperature programmed at 80°C for 5 min followed by rising up to 280°C at 10°C/min. The injection port and MSD interface temperature was 250°C and 280°C, respectively.

CONCLUSIONS

To decrease the health risk to human and environment from exposure to pesticides, the Taiwanese government

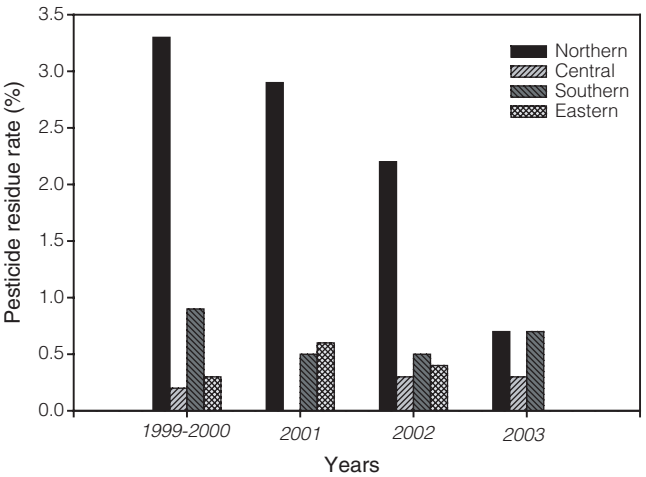

Figure 2. The violation rates of pesticide residue in fruits and vegetables in Taiwan.

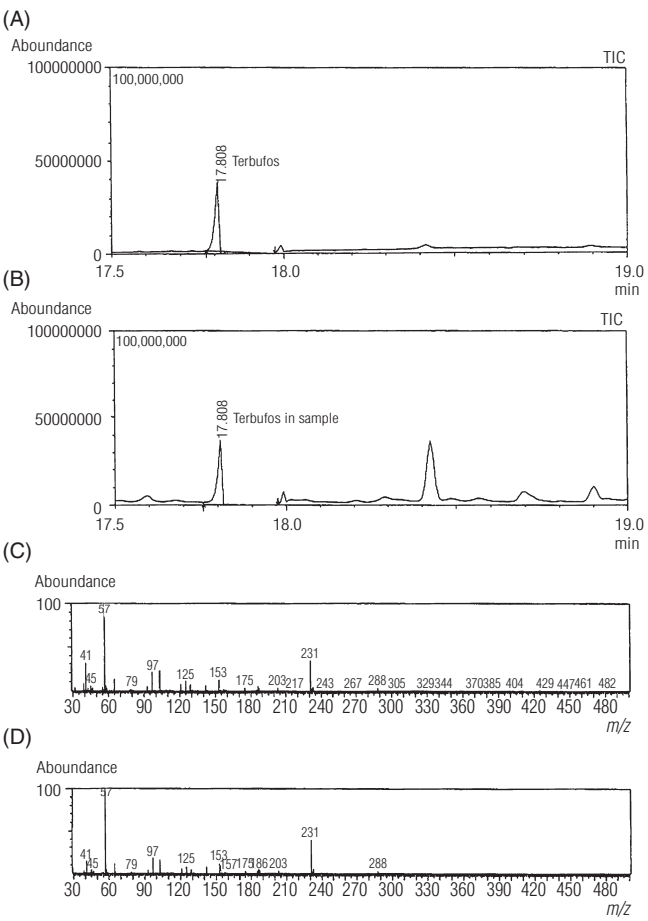

Figure 3. (A) Total ion chromatography of 100 ppm terbufos; retention time was 17.808 min. (B) Total ion chromatography of Chinese mustard with terbufos; retention time was also 17.808 min. (C) Total scanning of sample Chinese mustard with terbufos. (D) Total scanning of standard terbufos.

samples and analyzed agricultural products and analyzes them for pesticide residues to enforce the tolerances set by DOH every year. In the US, FDA Monitoring Program was divided into 3 parts: regulatory monitoring, incidence/level monitoring and total diet study (TDS). Regulatory Monitoring focuses on the raw agricultural product, which is analyzed as the unwashed, whole (unpeeled), raw commodity. However, this study is following the US FDA Monitoring Program and Incidence/Level Monitoring. The TDS is an annual program that determines levels of various pesticide residues, contaminants, and nutrients in foods and estimates intakes of these substances in representative diets of specific age-sex groups in the US. Risk assessment is a logical approach which has been used successfully for managing many types of risks including radiation control, chemical contamination of the environment and foods, and water quality. HACCP and risk assessment have some overlapping components and both risk assessment and HACCP are encompassed in risk analysis. To provide low pesticide residue level vegetables and fruits, more efforts from government and agriculture grower are needed. Although the government and agriculture grower have made tremendous progress in reducing pesticide residues in vegetables and fruits, there is still room for improvement. Anyway, we need further data of pesticide residues and TDS to prove if the mandatory implementation of HACCP systems in all "from farm to table" processing establishments could further improve the quality and safety of all agricultural products.

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