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Pesticide Residues in Vegetables and Fruits Monitored in São Paulo City, Brazil, 1994–2001

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Brazil is the 8th in pesticide world consumption, using 3.2 kg of active substance per hectare and more than 278 active substances are registered for agricultural use (Sindag 2004). The Federal Government established directions to regulate the production, the commerce, the surveillance and the use of pesticides. The Health Ministry of Brazil, through the National Sanitary Agency is responsible for establishing the Maximum Residue Limits (MRLs) for pesticides in food. About 2300 MRLs have been established in more than a hundred commodities after field studies realized in the country and conduced according to good agricultural practices (Brasil Ministério da Saúde 2000).

As a result of the great sensibility of pest attack and the necessity to launch them quickly in the market, the fruits and vegetables need special attention regarding pesticide use. The determination of pesticide residues is very important in order to guarantee food quality and to evaluate alimentary risk.

In Brazil some monitoring studies have been reported in vegetables and fruits (Ungaro *et al.* 1983 and 1987; Zandoná *et al.* 1993; Gebara *et al.* 1999 and 2001; Oviedo *et al.* 2002). Pesticide Residues Laboratory of "Instituto Biologico" has been developing a monitoring work on pesticide residues in fruits, and vegetables traded in Sao Paulo city since 1978. This laboratory has been accredited by the Agricultural Ministry of Brazil for monitoring pesticide residues in food. The results have been used by the agricultural government extension service to improve pesticide use and application practices by farmers to minimize risks to consumers.

This study reports on the results from 1994 to 2001 period of an on-going fruit and vegetable monitoring program to determine if pesticides were present in samples collected at Commercialization Centre of Sao Paulo City, and if the residue results violated the MLRs permitted by the Brazilian Legislation.

MATERIALS AND METHODS

The present survey includes 2223 samples (700 vegetables and 1523 fruits) collected at Sao Paulo General Storage and Wholesale (CEAGESP), from January 1994 through December 2001. The most representative vegetables and fruits (table 1) were

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included in the study. All samples were taken in accordance with the guidelines of the *Codex Alimentarius* 1993.

The samples were analyzed for 100 different pesticide residues (insecticides and some fungicides) according to gas chromatographic multiresidue method (DFG, method S19). A 50g of macerated sample was blended with acetone. Water is added beforehand in an amount that takes full account of the natural water content of the sample so that during extraction the acetone: water ratio remains constant at 2:1 (v/v). The extract was partitioned with dichloromethane in the presence of sodium chloride, resulting in separation of excess water. After evaporation the organic phase is cleaned up by gel permeation chromatography (BIO BEADS® S-X_3 polystyrene gel), using cyclohexane and ethyl acetate (v/v) as eluant. A supplemental cleanup was followed by silica gel deactivated 1.5%. The silica gel column was eluted with: hexane + toluene (65:35 v/v), toluene (100), toluene + acetone (95:5 v/v), toluene + acetone (80:20 v/v) and acetone (100). Each eluate (10mL) was collected and then injected (1μL) into a gas chromatograph for qualitative and quantitative analysis. The solvents used were pesticide residue analysis grade.

For identification and quantification of the organochlorine pyrethroid and some fungicides an ECD was used, and the chromatographic conditions were: capillary column 30m x 0,32mm i.d. and 0.25μm film thickness (SPB™ 608); temperature program: 90°C to 210°C (30°C/minute), hold 2 minutes, 250°C (10°C/minute), hold 14 minutes; *splitless* injection system at 220°C, hold 2 minutes; detector temperature 300°C; gas flow rates: carrier gas (nitrogen) 1mL/minute on column and 29mL/minute make up. Peak identities were confirmed on alternative capillary columns with different phases and polarities (PTE™5 and SPB™1).

A NPD was used for determination of organophosphorus and carbamates compounds and the chromatographic conditions were: capillary column 30m x 0.32mm i.d and 0.25μm film thickness (PTE™5); temperature program: 80°C to 200°C (30°C/minute), hold 2 minutes, 230°C (15°C/minute), hold 6 minutes, 250°C (5°C/minute), hold 2 minutes; *splitless* injection system at 200°C, hold 2 minutes; detector temperature: 300°C; gas flow rates: carrier gas (nitrogen) 1mL/minute and 29mL/minute make up, hydrogen: 4,5mL/minute, air 175mL/minute. For confirmation were used capillary columns 30m x 0.32mm i.d and 0.25μm film thickness (SPB™20 and HP™1).

Acephate, monocrotophos and methamidophos were analyzed by using FPD detection with megabore column 30m x 0.53mm i.d and 0.25μm film thickness (DB ™ 1701); temperature program: 200°C/10minutes, on column injection system at 220°C, hold 2 minutes, detector temperature: 220°C; gas flow rates: carrier gas (nitrogen) 5mL/minute and 25mL/minute make up, hydrogen 140mL/minute, air 1: 80mL/minute and air 2: 175mL/minute. For confirmation was used megabore column 30m x 0.53mm i.d and 0.25μm film thickness (DB™5).

The residue concentrations were calculated by the calibration curve, generated from peak area obtained by the results of standard injections. The detection limit was 0.001 mg/kg for organochorine, 0.01 – 0.05 mg/kg for organophosphorus,

carbamates, pyrethroids and fungicides studied. The standards for the pesticide studied were obtained from Dr. Ehrenstorfer GmbH.

The Analytical method used was validated and tested in spiked sample analysis from Analytical Quality Assurance Study on Pesticide Residues organized by Pesticide Service Project – Deutsche Gesellschaft für Zusammenarbeit (GTZ), Germany.

Internal laboratory checks included analysis of blanks, fortified samples and internal duplicates. Recovery studies covered the range from the limit of detection to concentration greater than the MRL were conducted. Recoveries generally ranged from 70 to 120%. Limits of quantitation were set at 0,01mg/kg for the most of pesticides analyzed. In every analytical series, blank and spiked samples containing different types of pesticides were analyzed routinely to monitor method performance.

RESULTS AND DISCUSSION

Table 1 shows the number of samples analyzed for each commodity, as well as the percent of sample that contained a detectable pesticide residue. Overall, 67.4% of the samples had no detectable pesticide residue; whereas 32.6% of the samples contained 1 or more pesticide residues. More fruit samples (34.2%) than vegetable samples (29.0%) had a detectable residue.

The pesticides detected in this study are listed in table 2, including their frequency of detection and the range of residues. Agricultural commodities in which the pesticides were detected are also presented. Residues from banned pesticides were found in fruit samples such as: dieldrin in 2 melon samples with a mean concentration of 0.01mg/kg and HCH in strawberry sample with a concentration of 0.09mg/kg. These pesticides have been banned in Brazil since 1985 and their presence may be attributed to the persistence in soil.

The most of the detected residues were present in a low concentration with 53.6% of the positive vegetable and 37.1% of the positive fruit samples having a pesticide quantity residue that was lower than 0.10mg/kg. The most frequently found pesticides were: captan, chlorothalonil, endosulfan and procymidone. Endosulfan has no regulation for use in fruits and vegetables in according with Brazilian Legislation (Brasil Ministério da Saúde 2000). Its use is permitted in cocoa, coffee, cotton, sugar cane and soy crops.

Multiple residues were detected on 5.8% of the vegetable and 11.4% of the fruit samples. The vegetable samples, 0.7% had 4 residues; green bean and tomato had the highest incidence of multiple residues. The 1.1% of fruit samples contained 4 different pesticides; peach and strawberry had the highest incidence of multiple residues.

Violations of the MRL were observed only in 3.0% of the vegetable and 0.9% of the fruit samples. Overall, most of the residues were below the MRLs, about 44.4% of the detectable residues were minor than 10% of the MRL and 16.4% were minor than 50% of the MRL. In addition, 10.1% of the vegetable and 14.6% of the fruit samples contained pesticide that there were no specified MRLs for the particular

Table 1 Results of vegetables and fruits analyzed in Sao Paulo city, Brazil, 1994-2001.

Commodity	N° of samples analyzed	Percent positive[a]	Sample violations	
VEGETABLES			> MRL[b]	No MRL[c]
Beet	9	0	0	0
Carrot	114	6.9	0	3.5
Cucumber	19	21.1	0	15.8
Green bean	58	29.3	0	29.3
Lettuce	30	23.3	0	16.7
Onion	22	45.5	0	45.5
Summer squash	21	4.8	0	4.8
Sweet pepper	74	43.2	16.2	14.9
Tomato	339	35.4	2.7	4.4
Spice	14	35.7	0	35.7
FRUIT				
Apple	78	19.2	0	1.3
Grape	193	18.7	1.6	6.2
Guava	111	27.0	0	16.2
Mango	15	0	0	0
Melon	40	42.5	0	30.0
Orange	44	4.5	0	0
Papapaya	152	9.9	0.7	5.3
Peach	186	41.9	2.2	19.9
Persemmon	61	1.6	0	1.6
Pineaple	28	0	0	0
Strawberry	523	61.9	0.9	25.6
Tangerine	63	3.2	0	0
Watermelon	29	3.4	0	0
Total	1523	34.2	0.9	14.6

a. Percent of samples containing 1 or more pesticide residues
b. MRL Brazilian Maximum Residue Levels
c. Samples with both >MRL and no MRL (pesticide not authorized for use on the commodity) were counted as samples with no MRL.

Table 2 Pesticides detected in fruit and vegetable samples, 1994-2001, Sao Paulo, Brazil.

Pesticide detected	Residues (mg/kg)		Total detection	Total violation	Commodity
	range	Mean			
Acephate	0.05-2.58	0.49	11	10	Melon, strawberry, tomato
Azinphos-ethyl	0.05-1.00	0.52	2	1	Cucumber, green bean
Captan	0.01-14.64	1.74	270	4	Carrot, cucumber, grape, guava, melon, papaya, peach, strawberry, tomato
Chlorothalonil	0.01-9.24	0.51	195	61	Apple, cucumber, grape, green bean, guava, lettuce, melon, onion, papaya, peach, spice, strawberry, sweet pepper, tangerine, tomato, watermelon
Chlorpyrifos	0.01-0.49	0.29	14	4	Apple, carrot, grape, orange, strawberry, sweet pepper, tomato
Deltamethrin	0.78	0.78	1	1	Spice
Diazinon	0.02-0.70	0.14	20	1	Cucumber, grape, green bean, guava, onion, papaya, peach, strawberry, spice, sweet pepper, tangerine, tomato
Dieldrin	0.01-0.01	0.01	2	2	Melon
Dicofol	0.05-0.17	0.13	5	3	Apple, onion, strawberry
Dimethoate	0.03-3.42	1.05	53	51	Apple, guava, peach, strawberry
Disulfoton	0.10-0.12	0.11	3	1	Green bean, strawberry
Endosulfan	0.01-1.56	0.11	111	111	Apple, carrot, cucumber, grape, green bean, guava, melon, onion, papaya, peach, summer squash, strawberry, sweet pepper, tomato
Ethion	0.05-0.18	0.11	2	2	Orange, tomato
Fenitrothion	0.02-10.94	0.12	10	2	Apple, peach, tomato
Fenpropathrin	0.01	0.01	1	1	Grape
Fensulfothion	0.10-7.26	3.68	2	2	Peach
Folpet	0.02-7.53	0.82	77	2	Apple, grape, green bean, peach, spice, strawberry
HCH	0.09	0.09	1	1	Strawberry
Iprodione	0.05-11.97	1.50	19	6	Apple, carrot, grape, lettuce, peach, strawberry
Malathion	0.10-8.69	0.24	12	4	Guava, green bean, strawberry, tomato
Methamidophos	0.10-6.96	0.57	19	14	Carrot, green bean, peach, persimmon, strawberry, sweet pepper, tomato
Monocrotophos	0.27-0.87	0.51	5	3	Peach, persimmon, strawberry, sweet pepper
Omethoate	0.10-1.30	0.64	7	7	Peach, strawberry
Parathion-ethyl	0.05-0.10	0.09	4	4	Cucumber, green bean, sweet pepper, spice
Parathion-methyl	0.10-0.41	0.15	26	1	Guava, lettuce, peach, strawberry
Permethrin	0.07-0.14	0.10	3	0	Tomato
Pirimiphos-methyl	0.05-0.11	O0.06	7	5	Green bean, guava, strawberry, spice, sweet pepper
Procymidone	0.25-7.88	0.98	91	7	Carrot, grape, lettuce, melon, peach, strawberry, sweet pepper, tomato
Quintozene	0.01-1.26	0.31	15	15	Green bean, onion, papaya, spice, strawberry
Vinclozolin	0.05-0.21	0.08	5	5	Cucumber, onion, sweet pepper, tomato

pesticide – commodity combination and the Brazilian Legislation does not have fixed lower value for MRL in such cases. The largest percent of violations have occurred in onion (45.5%) and spice (35.7%) for vegetables, melon (30.0%) and strawberry (26.5%) for fruits.

The results of this study compared with other surveys (Juhler *et al.* 1999; Ripley *et al.* 2000; Akyama *et al.* 2002; Singh and Gupta 2002), show a higher number of samples with violative residue (above MRLs and no MRL specified). It must be pointed out that MRLs vary considerably throughout the world, for example in Brazil endosulfan has no MRLs for vegetables and fruits whereas the *Codex Alimentarius* 1998, establishes MRLs varying of 0.10 to 2.00mg/kg. In Brazil, the number of pesticides registered, mainly, in vegetables is small. In Brazil the number of pesticides available, especially for vegetables, is small. As the farmers have not enough range of options, at the end they use inappropriate ones. In the cases where the levels were over the Brazilian MLRs, it appears that noncompliance with the pre-harvest interval for the different pesticides in each crop was the cause.

This information is very important to give an orientation to farmers about phytossanitary management for crops. When a violation occurred, agronomists of the Agricultural Department of Sao Paulo State visited the rural property and advised on pesticide use in accordance with registered use and good agricultural practices. Pesticide residue monitoring programs should be implemented to assure the minimum allowable residue levels in vegetables and fruits.

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