D:/Biomedica Vol.25, Jul. – Dec. 2009/Bio-23.Doc P. 171 – 174 (WC)

A QUANTITATIVE ANALYSIS FOR THE TOXIC PESTICIDE RESIDUES IN MARKETED FRUITS AND VEGETABLES IN LAHORE, PAKISTAN

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ABSTRACT
This study was an attempt to assess the health hazards faced by the consumers through possible ingestion of toxic chemicals contained in the fruits and vegetables. In this cross sectional analytical study, tomato, apple and cucumber samples were collected from four main markets of Lahore and were analysed separately for the levels of nine pesticide residues using Liquid Chromatography-Mass Spectrometry system. The levels were compared with the maximum residue limits set by the WHO for every pesticide in respective items. The results showed that most of the samples did not contain any residues of the nine selected pesticides and only two samples of tomato had detectable residues of one pesticide Imidacloprid, which were within the limits set by the WHO. So it was concluded that the analysed fruit and vegetable samples did not pose a serious threat to the health of the consumers.

Key words: *Maximum Residue Limit, Pesticide residue, LCMS*

INTRODUCTION

Fruits and vegetables are essential to a nutritious and healthy diet; however, the health benefits are compromised by consistent contamination with pesticide residues.[1] Pesticides are chemical substances used to kill animal, insect, plant and fungal pests in agricultural, domestic and institutional settings.[2] Interest on pesticide toxicity has particularly increased over the past years owing to increasing evidence of carcinogenic, mutagenic and teratogenic effects in experimental animals and exposed humans.[3] They constitute a very important group of chemical compounds that have to be controlled due to their very high toxicity and their widespread use in agricultural practice for field and post harvest protection.[4] The general population is mainly exposed to pesticides through the ingestion of contaminated foods (such as cereals, vegetables and fruits), which are directly treated with these pesticides or are grown in contaminated fields. Diet is one potentially significant source of pesticide exposure considered in aggregate and cumulative risk models.[5] The organophosphate, organochlorine and related pesticides act by binding to the enzyme acetyl cholinesterase, disrupting nerve function, resulting in paralysis and may cause death.[6] They may produce acute and chronic toxicity. The acute effects manifesting as miosis, urination, diarrhea, diaphoresis, lacrimation, excitation of CNS and salivation.[2] The chronic exposure involves neurotoxic and behavioral effects.[7] Specific effects of pesticides can include cancer, allergies and hypersensitivities, damage to the central and peripheral nervous systems, reproductive disorders and disruption of the immune system.[8] Recent studies have shown that exposures to contaminants in food may pose a public health risk.[9] Children may be more susceptible to the effects of these exposures, as they have higher rates of metabolism, less mature immune systems and different patterns of activity and behavior than adults.[10]

Pesticides can also interfere with drug metabolizing enzymes especially Cytochrome P450 leading to drug interactions. [11] About 27% of the pesticides being consumed in Pakistan are used on fruits and vegetables. Setting a balance between risk and methods to increase agricultural productivity is particularly important for developing countries. [12] The monitoring of pesticide residues in food is nowadays a priority objective in pesticide research in order to get extensive evaluation of food quality and to avoid possible risks to human health. To ensure the safety of the US food supply, the Environmental Protection Agency (EPA) sets a tolerance or maximum residue limit (MRL), which is the amount of pesticide residue that may lawfully remain in each food commodity that has been treated with a pesticide.[13]

At the international level, the Codex Alimentarius Commission of the United Nation's Food and Agriculture Organization and the World Health Organization has established maximum residue limit (MRL) for pesticides in a variety of foods.[14]

The objective of present study was to look for the safety of fruits and vegetables in terms of pesticide residues.

MATERIAL AND METHODS

It was a cross sectional analytical study carried out in the Department of Pharmacology, University of Health Sciences, Lahore, in collaboration with the Applied Chemistry Research Centre (ACRC), Pakistan Council for Scientific and Industrial Research (PCSIR), Lahore.

In this study, tomato, apple and cucumber samples weighing 1kg each were randomly collected from four main vegetable and fruit markets of Lahore including Badami Bagh, Iqbal Town, Singhpura and Kot Lakhpat. The samples of each item from each market were collected, extracted and analyzed separately. Total 12 samples were analyzed for residues of 9 pesticides.

Reagents and Apparatus

HPLC-grade methanol and organic trace analysis grade ethyl acetate were purchased from Merck (Germany). Anhydrous sodium sulfate was purchased from Pancreac (Spain). Standard solutions of selected pesticides including:-

Diazinon, Carbaryl, Chlorpyrifos, Imidacloprid, Endosulfan, Cypermethrin, Cyfluthrin, Deltamethrin, Metalaxyl (All from Riedel de Haen, Germany).

Sample Preparation

Each sample (1kg) was chopped and homogenized for 3 minutes at high speed using a food chopper.

Extraction Procedure

A 50 g amount of chopped sample was placed in a 250 ml glass beaker and mixed thoroughly with 100 ml of ethyl acetate and 50g of anhydrous sodium sulfate, using a food chopper for 2 min. The homogenate was allowed to settle and the supernatant was passed through a filter paper into a 500 ml rotary-evaporation flask. The solid residue was again homogenized with 100 ml ethyl acetate, filtered through the anhydrous sodium sulfate and collected with the first extraction fraction. Twice 25 ml ethyl acetate was used to rinse the glass beaker and the rinsing was passed through the filter and collected. A rotary evaporator set at 40°C and 250 mbar was used to evaporate the extract to less than 5 ml, and then, the extract was passed to a graduated conical tube (15 ml) and evaporated to dryness under a stream of nitrogen at 50°C. The sample was reconstituted in 10ml of methanol. A volume of 20 µl of the final extract was injected into the LC-MS system.[15]

Analytical Procedure

Multiresidue method was selected for the detection of different pesticide residues in different samples. A Thermofinnigan (LCQ Advantage Max, USA) LC-MS system (LC Finnigan Surveyer) equipped with a binary solvent pump and an auto sampler was used. The system consisted of standard atmospheric pressure ionization (API) source configured as atmospheric chemical ionization (APCI). The analytical column was ODS.[3]

Optimization of operating conditions

The operating conditions of the APCI interface in positive ionization mode were optimized. Following parameters were set:-

Source heater temperature 300°C, Sheath gas flow rate 40, Aux/sweep gas flow rate 10

Discharge current 5(µA), Capillary Temperature 175°C, Capillary voltage 40V

Tube lens offset (V) 35.

Main ions for the selected pesticides were selected by flow injection analysis (FIA) with the syringe pump of the individual solutions of the pesticides (10 mg/ml). The lowest reporting levels were 0.02 and above. Selected ion monitoring (SIM) mode was used for the quantitative analysis of the samples. The residues of different pesticide were identified on the basis of their selected ions, quantified on the basis of their respective peaks and reported on the basis of their sample weight and are expressed as mg/Kg.[16]

Table 1: *Selected ions of the analyzed pesticides*

Pesticide	Molecular mass	Selected ions (m/z)
Diazinon	304	304, 179
Carbaryl	201	201
Chlorpyrifos	349	349, 314, 197
Imidacloprid	256	256, 175
Endosulfan	406.9	406
Cypermethrin	415	415209, 181
Cyfluthrin	433	433, 206, 127
Deltamethrin	503	503, 253, 115
Metalaxyl	279.34	279

RESULTS

NDL: No detectable Level

The results showed that 83% of the fruit and vegetable samples did not have detectable levels of any pesticide residue. Only 17% of all samples were positive for pesticide residues. 25% of the vegetable samples were positive and 75% were negative for residues. 100% of the fruit samples were negative for residues. 50% of the tomato samples analyzed

had detectable residue levels while 100% of the apple and cucumber samples had no detectable level of residues. 100% of the samples positive had residue levels below the maximum residue limit (MRL) set by WHO.

Table 2: *MRLs of analyzed pesticides by the WHO in selected fruits and vegetables*

Pesticide	MRL in tomato[17] (mg/kg)	MRL in apple [17] (mg/kg)	MRL in cucumber [17] (mg/kg)
Diazinon	0.5	NA	0.1
Carbaryl	5	5	NA
Chlorpyrifos	0.5	NA	NA
Imidacloprid	0.5	0.5	1
Endosulfan	0.5	NA	1
Cypermethrin	0.5	NA	0.2
Cyfluthrin	0.5	0.5	NA
Deltamethrin	0.3	0.2	NA
Metalaxyl	0.5	NA	0.5

MRL: Maximum residue limit, WHO: World Health Organization, NA: Not available

DISCUSSION

Apple and cucumber mostly and tomato usually are used uncooked. Pesticides are the part of majority of chemicals applied on them. The present investigation determined the pesticide residues in tomato, apple and cucumber samples collected from different markets of Lahore city and compared them with the limits set by the WHO. It was found that most of the samples did not contain any detectable level of residues of any of the nine pesticides analyzed. Two samples of tomato did have residues of Imidacloprid but they were within the MRL. So it was found that most of the fruit and vegetable samples did not pose a serious threat to the health of consumers.

Table 3: *Pesticide residues in fruit and vegetable samples in markets of Lahore in 2008.*

Samples	No. analyzed	Residues +ve	NDL	% +ve	<MRL	> MRL	%> MRL
Tomato	4	2	2	50	2	0	0
Apple	4	0	4	0	0	0	0
Cucumber	4	0	4	0	0	0	0

The results of the present investigation further support the findings of the study carried out in Multan in 2002, in which the residues of cypermethrin, methamedophos, monocrotophos, cyfluthrin, dialdrin and methyl –parathion in different varieties of mangoes were analyzed. None of the samples had residues in excess of the MRLs.[12]

Similarly, in an investigation carried out in 1998, by monitoring insecticide residues in vegetables and fruits at the market level in Mauritius, it was concluded that most of the vegetable and fruit samples analyzed did not contain residues of the monitored insecticides above the MRL, although, some insecticide residues were detected in few samples only.[18] Fenske RA et al (2002) made an assessment of organophosphorous pesticide expoures in the diets of preschool children in Washington State. Their work supported the theory that due to dietary differences, young children may experience higher levels of pesticide exposures through their diets than older children and adults. According to their study, none of the OP pesticide detections were above the legal tolerances.[5] In 2006, Bai Y et al concluded that the OP pesticide residues were present in fruits and vegetables in Shaanxi area of China.[6] Hura C et al (1999) in their study the Eastern Romania area concluded that organochlorine pesticides were found in all analyzed samples.[19]

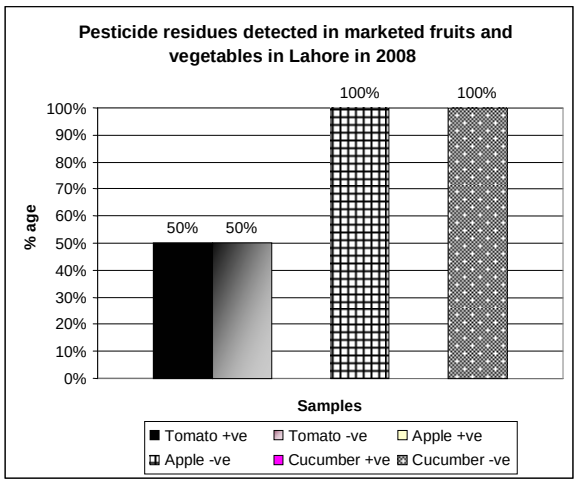

Figure 1:

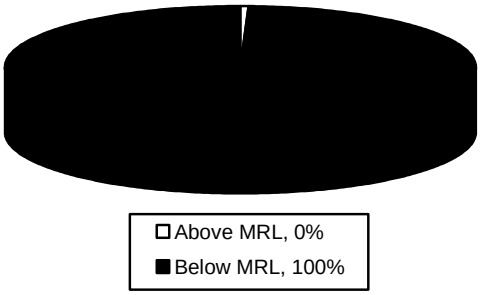

Figure 2: *Pesticide residues in fruits and vegetables compared with WHO allowed levels.*

The studies disclose that even a low level exposure to pesticide residues puts consumers especially children on risk in a cumulative manner. So an analysis showing the residues in undetectable or safe range does not essentially mean that it is absolutely safe and free of any untoward effects. Many studies carried out in different parts of the world show varying results. This variation may be due to difference in the agricultural practices in applying the pesticides. The lack of data is still the major factor and there is a dire need for further studies and investigations.

So further work on a broader scale is needed and mutual and coordinated efforts by the experts from environmental, chemical and health sciences are needed to be initiated.

ACKNOWLEDGMENTS

Authors are thankful to the Ethical Committee and the Vice Chancellor of UHS for their support.

REFERENCES

1. Guan H. Tan and Ngat N. Tang. Determination of Organophorous Pesticide Residues in selected fruits by Gas Chromaography- Mass Spectrometry. Malaysian Journal of Chemistry 2005; 7: 49-56.
2. Sanborn MD, Cole D, Abelsohn A, Weir E. Identifying and managing adverse environmental health effects:4 Pesticides. CMAJ 2002; 166: 1431-6.
3. Hrelia P, Maffei F, Fimognari C, Vigagni F, Cantelli-Forti G. Cytogenic effects of Metalaxyl on human and animal chromosomes. Mutation Research 1996; 369: 81-6.
4. Ortelli D, Edder P, Corvi C. Multiresidue analysis of 74 pesticides in fruits and vegetables by liquid chromatography-electrospray-tandem mass spectrometry. Analytica Chimica Acta 2004; 520: 33-4.
5. Fenske RA, Kedan G, Lu C, Fisker-Andersen JA and Curl CL. Assessment of Organophosphorous pesticide exposures in the diets of preschool children in Washington State. Journal of Exposure Analysis and Environmental Epidemiology 2002; 12: 21-8.
6. Bai Y, Zhou L, Wang J. Organophosphorous pesticide residues in market foods in Shaanxi area, China. Food Chemistry 2006; 98: 240-2.
7. Pesticides and Human Health, Environmental Health Committee Newsletter for Family Physicians, the Ontario College of Family Physicians. [Cited on 2007 July 24] Available at http://chebuctons.ca/Environment/RATE/Pesticides_and_human_healt.html
8. Robert A. Van Steenwyk, Frank G. Zalom. Food Quality Protection Act launches search for pest management alternatives. California Agriculture 2005; 59: 7-11.
9. Rene vanderhoff G and Zoonen PV. Trace analysis of pesticides by gas chromatography. J. Chromatogr. A 1999; 843: 301-22.
10. Morasso G et al. Pesticides as food contaminants: a pilot project for correct public information. Trends in Food Science and Technology 2001; 11: 379-82.
11. Hodgson E and Patrica E. Pesticides: An important but underused model for the environmental health sciences 1996; 104: 97-106.
12. Hussain S, Masud T and Ahad K. Determination of pesticide residues in selected varieties of mango. Pakistan Journal of Nutrition 2002; 1: 41-2.
13. Aroud M, Douki W, Rhim A, Najjar M F and Gazzah N. Multiresidue analysis of pesticides in fruits and vegetables by gas chromatography-mass spectrometry. Journal of Environmental Science and Health 2007; Part B 42: 179-87.
14. Ahmed FE. Analyses of pesticides and their metabolites in foods and drinks. Trends in analytical chemistry 2001; 20: 49.
15. Parveen Z and Masud SZ. Studies on pesticide residues in human blood. Pak. J. Sci. Ind. Res 2001; 44: 137-41.
16. Blasco C, Font G, Pico Y. Comparison of micro extraction procedures to determine pesticides in oranges by liquid chromatography-mass spectrometry. Journal of Chromatography A 2002; 970: 201-12.
17. Official standards, Pesticide MRLs [Cited on September 21 2007] Available at http://www.codexalimentarius.net/mrls/pestdes/jsp/pest_q-e.jsp
18. Choy LHLF and Seeneevassen S. Monitoring insecticide residues in vegetables and fruits at the market level. AMAS. Food and Agricultural Research Council, Reduit, Mauritius 1998; 98: 95-102.
19. Hura C et al. Risk assessment of pollution with pesticides in food in the Eastern Romania area (1996-1997). Toxicology Letters 1999; 107: 103-7.