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Assessment of Pesticide Residues in Some Vegetables Grown in Kavrepalanchok and Bhaktapur Districts

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Abstract

Pesticides have wide spread use and their toxic residues have been reported in various environmental matrices. Thus, the determination of pesticide residues in food commodities such as vegetables, cereals, fruits, and other environmental components like soil, water has become increasingly essential requirement for consumers, producers and authorities for food quality control. Therefore, monitoring of residue level and exposure assessment of organophosphate group of pesticides namely dichlorovos and methyl parathion was carried out in 30 winter and summer vegetables supplied in Kathmandu valley. It was found that the MRL value of dichlorovos exceeded in three types of vegetables whereas in case of methyl parathion the MRL value exceeded in two types of vegetables only. The estimated average daily intake (EADI) of dichlorovos exceeded the acceptable daily intake (ADI) in 18 vegetables, whereas, regarding methyl parathion the EADI exceeded the ADI in 10 types of vegetables.

Key words: dichlorovos, methyl parathion, monitoring, organophosphate pesticide, residue level

Introduction

The term pesticide is a general term that includes compounds used for a wide variety of purposes to control a range of living organisms. Basically they may be subdivided into two main groups agricultural pesticides: used in agriculture, horticulture, forestry and weed killers around water courses and non agricultural pesticides: used for wood preservation, treatment of masonry, antifouling agents and for control of insects for public hygiene (Renwick 2002). However, chemically they are classified into inorganic compounds, organic compounds and miscellaneous compounds (Manandhar 2006).

Pesticides, especially the organochlorine (OC) and organophosphate (OP) tend to persist in the environment, causing several types of damages including lowering of biodiversity, soil contamination and water contamination etc. Excessive use of persisting pesticides in the fields also have caused surface and underground water contamination are illustrated by records of some international studies (Giri 1998, Lama 2008).

Organophosphate pesticides (OP) are widely used in agriculture and animal production for the control of various insect pests mainly in developing countries. Most of the organophosphates are only slightly soluble in water and high oil- water partition coefficients and a low vapour pressure (WHO 1986). Toxic hazards of OP are therefore essentially for short term in contrast to that of persistent organochlorine (OC) pesticides and have an advantage of being more rapidly degraded in the environment. Hence, use of organophosphate group of pesticides has more widespread use than OC due to their less toxic nature. A study in Haryana, India (Kumari 2006) showed that presence of organophsphate and synthetic pyrethroides (SP) residue in vegetables and fruits was an indicative of change in usage pattern of insecticides in India where shift had taken place from organochlorine to the easily degradable groups of OP and SP insecticides in the last decade.

Pesticides have potentially adverse effects on vegetables, fruits, animal resources and human health (Perez & Rubio 1999). Because of their wide spread use, the toxic residues have been reported in various environmental matrices (Kumari *et al.* 2003, Frank *et al.* 1987). Thus, the determination of pesticide residues in food commodities and other environmental components like fruits, vegetables, total diet, soil and water have become increasingly essential for consumers, producers and authorities for food quality control (Kumari *et al.* 2006).

Presence of pesticide residues in fruits and vegetables has become a global phenomenon. Frank *et al.* (1987) have reported residues of organochlorine and organophosphate insecticides along with fungicides and herbicides in fruits from Ontario, Canada. According to Annual Bulletin of DFTQC (1995-2004) considering the leaf vegetables twenty types of vegetables were contaminated with methyl parathion (Koirala 2007). Likewise Kumari *et al.* (2002, 2003) also found the residues of organophosphate and synthetic pyrethroids in addition to organochlorine compounds in seasonal vegetables from Haryana, India. However, considering the pesticide scenario of Nepal, lack of regular monitoring scheme for MRLs on pesticides, has affected the export of tea, honey and other food commodities as well as certification of organic food materials (Lama 2008).

According to Plant Protection Directorate (PPD) 2008, the import of different types of pesticides in total was 762542.65 kg in Nepal. Of these amount organochlorines was 31560 kg and organophosphates 110653.95 kg. Moreover, the trend of import of pesticides is increasing compared to the past years. Though the organochlorine compounds are considered persistent, still their import and use can be seen. The toxicity level of pesticides that are sold in Nepalese market has been categorized according to World Health Organization (WHO) hazard levels. WHO has categorized dichlorovos as class Ib and methyl parathion as Ia. Under this category Ia is extremely hazardous and Ib is highly hazardous (Manandhar 2006). Klarman (1987) had warned the increasing trend of pesticide use in Nepal and emphasized more on the health hazards due to persistent organic pollutants (POPs) that has been banned.

An exposure or risk assessment is necessary in order to ascertain the effects due to regular intake of pesticide residues in food. Several indices of residue levels can be used to predict pesticide residue intake and maximum residue limits (MRL) is one such index. Government of Nepal has set the maximum residue limits (MRLs) of few pesticides on certain food commodities (Lama 2008) but exposure assessment and dietary intake calculations like estimated average daily intake (EADI) has not been carried out so far. EADI of a pesticide residue in a given food is obtained by multiplying the residue level in the food by the amount of the food consumed and it should be less than its established accepted daily intake (ADI). ADI is the amount of a chemical which can be consumed every day for lifetime in the practical certainty, on the basis of all known facts, that no harm will result (PRC 2008).

However, extensive study on pesticides and their implication are lacking in Nepal. Hence, monitoring studies to know the actual status of contamination due to toxic pesticide residues in various food commodities for the formation of legal guidance and for consumer satisfaction is necessary. Therefore, the present study focuses on estimating residue level of targeted organophosphate group of pesticides namely methyl parathion and dichlorovos in thirty different vegetables of winter and summer seasons from three major vegetable supplying fields of the Kathmandu valley.

Methodology

Surveys were conducted at three locations: Panchkhal of Kavrepalanchok district; Nagadesh and Jagate of Bhaktapur district for the pesticides used in the vegetables. The survey revealed that farmers mostly used methyl parathion and dichlorovos pesticides for the vegetables pests. Two seasons' winter and summer were utilized to carry out the residual monitoring program of seasonal vegetables .Samples were taken randomly from the field before placed for the market. Three replicates of each vegetable were collected directly from the fields in Zip lock polythene bags and stored at $4^{o}C$. Sampling techniques, sample preservations and preparations were conducted following Sharma (2007). Survey revealed that mostly used pesticides in vegetables are of organophosphate group. Hence, the analysis was carried out for organophosphate group namely methyl parathion and dichlorovos following the methodology given in APHA 1989.

For the assessment, risk hazard identification was to be carried out estimating the amount and the type of pesticide in thirty different vegetables. The steps given below were followed while carrying out the exposure assessment (WHO guidelines 1997, Renwick 2002).

- **Hazard identification and characterization**

Estimation of pesticide residue by gas chromatographic (GC) method in thirty different samples of vegetables and the results were compared with Codex Ailimentarius /UK-EC Maximum Residue Limits guidelines https://secure.pesticides.gov.uk/MRLs/.

Maximum residue limit (MRL) means the maximum concentration of a residue (expressed as milligrams of residue per kilogram of food/animal feeding stuff) that is legally permitted or recognized as acceptable in or on a food or agricultural commodity or animal feedstuff (FAO 2002).

- **Estimation of human intake**
  Calculation of EADI (estimated average daily intake) was performed following Darko and Akoto (2008).The EADI was calculated by multiplying the average residual concentration (mg/kg) by the food consumption rate from GEMs Food Regional Diet (2003).
- **Risk Estimation**
  EADI was compared with acceptable daily intake (ADI) and ARfd from Inventory of IPCS and other WHO pesticide evaluations and summary of toxicological evaluations performed by the Joint Meeting on Pesticide Residues (JMPR)(http://www.who.int/ipcs/publications/jmpr/pesticide_inventory.pdf.). Hazard indices were calculated dividing EADI value with their corresponding ADI value (Darko and Akoto, 2008).

Extraction method

Approximate 100 g of each vegetable sample was weighed, washed, chopped and blended in a high speed blending machine for a min. In the blended sample 10g of Celite and 200ml of acetonytril were mixed and re-blended. The mixture was filtered through sintered glass with the help of suction pump and 25 ml acetonytril was used to rinse the sintered glass funnel. The filtrate was transferred to the 1000ml separating funnel with 100ml of petroleum ether and shaken vigorously for 2 min later 600ml of distilled water and 10ml of saturated sodium chloride were added to the separating funnel and shaken well for 2 min and left it for 30 min. The light colored aqueous layer was discarded and the solvent layer was washed with 100ml of distilled water for 3 times. The aqueous layer formed during the washing process was discarded.

The separated liquid was transferred to a 250 ml conical flask and anhydrous sodium sulphate (20-25 g) was added to absorb the moisture and shaken for 30 min. The mixture was transferred to Kunderson denis flask and heated in water bath to concentrate at 80/85^{o}C for 10 min. The concentrated extract was washed and dissolved with 20ml acetone. The extract was heated in water bath and this process was repeated for three times. The extracted liquid was then transferred to 10ml volumetric flask adding acetone and was stored at 4^{o}C and later gas chromatographic analysis was performed using GC 1000 (Chemito).

Results and Discussion

The present study revealed that dichlorovos (ppm) residue was present in cauliflower (0.0031), cabbage (0.0046), broad bean (0.0038), elephant ear plant (0.004), garlic green (0.01880, lettuce (0.012), spinach (0.008), spring onion (0.0050), turnip (0.03) and zucchini (0.0028). Similarly, methyl parathion (ppm) residue was present in cauliflower (0.0038), broad bean (0.0054), chilly (0.025) and elephant ear plant (0.113). The residue concentration was the mean of three samples of each vegetable. In rest of the vegetables the level of dichlorovos residue was below 0.002 and methyl parathion residue was below 0.001.

According to EC/UK guidelines (https://secure.pesticides.gov.uk/MRLs/), the MRL value for dichlorovos is 0.01 and for methyl parathion is 0.02. The MRL value of dichlorovos was exceeded in three vegetables, garlic green, lettuce and turnip, whereas in case of methyl parathion the MRL value was exceeded in two vegetables, chilly and elephant ear plant. MRLs are primarily trading standards, but they also help to ensure that residue levels do not pose unacceptable risks for consumers.

The estimated average daily intake (EADI) of dichlorovos exceeded the acceptable daily intake (ADI) in 18 vegetables, whereas for methyl parathion EADI exceeded ADI in 10 types of vegetables (Table 1). Concerning the elephant ear plant, EADI has not been calculated as the consumption pattern has not been shown in the GEMs regional diet plan. Therefore, only the residue level has been shown in Fig 1 and 2 and the residue levels in both the pesticides exceeded the ADI level. In ten types of vegetables, cabbage, cauliflower, cucumber, egg plant, garden cress, pepper chilly, potato (fresh and stored), spinach and tomato the EADI values exceeded the ADI values. Considering both type of pesticides, the EADI has not exceeded the ADI in twelve types of vegetables such as common bean, rajma bean, broad bean, cowpea, carrot, coriander, chilly, lettuce, mustard green, pakchoi, raddish and turnip.

Table 1. EADI and ADI value for dichlorovos and methyl parathion

SN	Vegetables	Dichlorovos		Methyl parathion	
		EADI	ADI**	EADI	ADI**
1	Common bean	0.0016	0.004	0.0008	0.003
2	Rajma bean	0.0016	0.004	0.0008	0.003
3	Broad bean	0.0038	0.004	0.00054	0.003
4	Cow pea	0.0016	0.004	0.0008	0.003
5	Bitter Gourd	**0.0044**	**0.004**	0.0022	0.003
6	Bottle Gourd	**0.0044**	**0.004**	0.0022	0.003
7	Cabbage	**0.04462**	**0.004**	**0.0097**	**0.003**
8	Cauliflower	**0.00465**	**0.004**	**0.0057**	**0.003**
9	Carrot	0.005	0.004	0.0025	0.003
10	Coriander	0.0002	0.004	0.0001	0.003
11	Cucumber	**0.009**	**0.004**	**0.0045**	**0.003**
12	Chilly	0.0002	0.004	0.0025	0.003
13	Egg plant)	**0.006**	**0.004**	**0.009**	**0.003**
14	*Elephant ear plant	**Residue 0.004**	**0.004**	**Residue 0.113**	**0.003**
15	Garden cress	**0.0194**	**0.004**	**0.0097**	**0.003**
16	Garlic green	**0.04136**	**0.004**	0.0022	0.003
17	Lettuce	0.0012	0.004	0.0001	0.003
18	Mustard Green	0.0002	0.004	0.0001	0.003
20	Pepper chili	**0.0042**	**0.004**	0.0021	0.003
21	Potato	**0.0384**	**0.004**	**0.0192**	**0.003**
22	Potato (stored)	**0.0384**	**0.004**	**0.0192**	**0.003**
23	Pumpkin	**0.0044**	**0.004**	0.0022	0.003
24	Radish	0.0002	0.004	0.0001	0.003
25	Spinach	**0.0776**	**0.004**	**0.0097**	**0.003**
26	Spring onion	**0.01**	**0.004**	0.002	0.003
27	Sponge gourd	**0.0044**	**0.004**	0.0022	0.003
28	Tomato	**0.0114**	**0.004**	**0.0057**	**0.003**
29	Turnip	0.003	0.004	0.0001	0.003
30	Zucchini	**0.00616**	**0.004**	0.0022	0.003

**ADI(Acceptable daily Intake has been taken from Inventory of IPCS and other WHO pesticide evaluations and summary of toxicological evaluations performed by the Joint Meeting on Pesticide Residues (JMPR). http://www.who.int/ipcs/publications/jmpr/pesticide_inventory.pdf

*Amount of consumption has not been shown in GEMs Food Regional Diet chart for elephant ear plant, hence residue level alone has been given

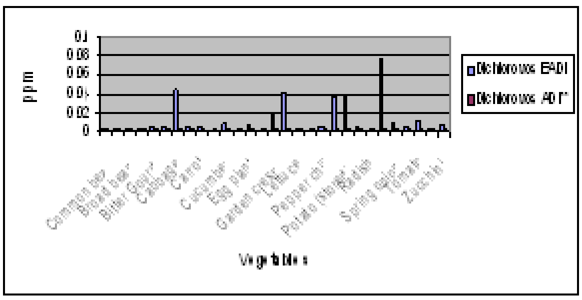

Fig .1. Comparison of EADI and ADI for dichlorovos

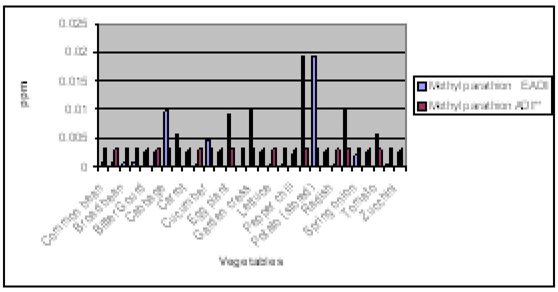

Fig. 2. Comparison of EADI and ADI for methyl parathion

According to WHO (1997) short term intake of levels of pesticides above the ADI for a day or several days should not represent a human health hazard. However, ADI derived from short or long term studies was probably not appropriate toxicological benchmark for assessing risks posed by short term exposure to acutely toxic residues. The acute reference dose (acute RfD) was developed to assess acute hazards using the same basic principles and methods to derive the ADI.

Joint meeting on pesticide residues (JMPR) specifies the subgroups of population (children, old adults, pregnant women, etc) that are at risk on the basis of the acute toxicity endpoints used to establish the acute RfD. The acute RfDs for dichlorovos has not been assigned and for methyl parathion the value of acute RfD is 0.03 mg/kg Body weight. Therefore, in all the analyzed vegetable samples except elephant ear plant, the level of EADI did not exceed the acute Rfds. Hence, it can be said that though the EADI value exceeds the ADI value the short term intake of these vegetables may not cause immediate negative health impact. According to WHO (1997) the short term intakes above the acute RfD are of more concerned because the toxicity might be observed after one or only few doses but in the present study the EADI values have not crossed the limit of acute RfDs.

Table 2. Hazard indices values of dichlorovos and methyl parathion

S.N.	Type of vegetable	Hazard indices (dichlorovos)	Hazard indices (methyl parathion)
1	Common bean	0.4	0.266
2	Rajma bean	0.4	0.266
3	Broad bean	0.95	0.18
4	Cowpea	0.4	0.266
5	Bitter Gourd	**1.1**	0.733
6	Bottle Gourd	**1.1**	0.733
7	Cabbage	**11.155**	**3.23**
8	Cauliflower	**1.1625**	**1.9**
9	Carrot	**1.25**	0.833
10	Coriander	0.05	0.033
11	Cucumber	**2.25**	**1.5**
12	Chilly	0.05	0.833
13	Egg plant	**1.5**	**3**
14	*Elephant ear plant		
15	Garden cress	**4.85**	3.23
16	Garlic green	**10.34**	0.733
17	Lettuce	0.3	0.033
18	Mustard Green	0.05	0.033
19	Pakchoi	0.05	0.033
20	Pepper Chili	**1.05**	0.7
21	Potato	**9.6**	**6.4**
22	Potato (stored)	**9.6**	**6.4**
23	Pumpkin	**1.1**	0.733
24	Radish	0.05	0.033
25	Spinach	**19.4**	**3.233**
26	Spring onion	**2.5**	0.666
27	Sponge gourd	**1.1**	0.733
28	Tomato	**2.85**	**1.9**
29	Turnip	0.75	0.033
30	Zucchini	**1.54**	0.733

Hazard indices values of 18 vegetables (Table 2) regarding dichlorovos cross the limit of hazard index, whereas for methyl parathion 8 vegetables exceeded the limit. According to Darko (2008) in Ghana, Africa, tomato and egg plant of Kumasi market had hazard index value more than one in some organophosphate pesticides considered for monitoring. Therefore, lifetime consumption of those plants that crosses the limit pose some health risks due to residues present in them. In case of elephant ear plant the hazard index has not been calculated as the EADI value was not calculated since the consumption pattern of elephant ear plant was not available.

Due to high demand for the vegetables and low perception of the toxic effects of pesticide residues in vegetables, farmers do not wait long enough for the residues to wash off before harvesting. According to Koirala (2007), in Nepal, commoditywise detection of pesticide residues showed the highest level of contamination in root vegetables followed by leafy vegetables and hence there is a threat of pesticide residues in foods and may endanger to public health. However, the potential hazards to consumers cannot be considered very high because most of these vegetables undergo processing like trimming, washing, peeling and cooking by which substantial amounts (80-90%) of pesticides other than organochlorines get degraded (Kumari 2003). Thus, the routine monitoring of the pesticides in food items is required to prevent, control and reduce the indiscriminate use of pesticides and to minimize health risks.

References

APHA. 1989. *Standard methods for the examination of water and wastewater*. 20th edition. American Public Health Association, Washington, DC.

Darko, G. and O. Akoto. 2008. Dietary intake of organophosphorus pesticide residues through vegetables from Kumasi, Ghana. *Food and Chemical Toxicolog,y* **46**: 3703-3706.

FAO. 2002. *International code of conduct on the distribution and use of pesticides* (Revised Version), Rome.

Frank, R., H.E. Braun and B.D. Ripley. 1987. Residues of insecticides, fungicides and herbicides in fruit produced in Ontario, Canada, 1980-1984. *Bull. Environ. Contam. Toxicol.* **39**: 272-279.

GEMS/ Food Regional diets. 2003. *Food safety issues, regional per capita consumption of raw and semi-processed agricultural commodities.* Prepared by the Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme (GEMS/Food), Food Safety Department, World Health Organization.

Giri, N.P. 1998. *Pesticides pollution in vegetable crop in Kathmandu valley*. M.Sc. thesis. Department of Zoology, Tribhuvan University, Nepal.

Internet visit .2009. http://www.who.int/ipcs/publications/jmpr/pesticide_inventory.pdf.

Internet visit .2009. https://secure.pesticides.gov.uk

Klarman, W.L.1987. *Pesticide use in Nepal*. Winrock International Institute for Agricultural Development, USAID, Nepal. Agricultural Research and Production Project and Agricultural Inputs Corporation. Department of Agriculture. His Majesty Government of Nepal.

Koirala P., D.B. Khadka., S. Dhakal and J.P.Lama. 2007. Common tea pests and pesticide use in Nepal. *Journal of Food Science and Technology Nepal* **3,** ISSN**:** 1816-0727.

Kumari, B., V.K. Madan and T.S. Kathpal. 2006. Monitoring of pesticide residues in fruits. *Environmental Monitoring and Assessment* **123:** 407-412.

Kumari, B., V.K. Madan., R. Kumar and T.S. Kathpal.2002. Monitoring of seasonal vegetables for pesticide residues. *Environmental Monitoring and Assessment* **74:** 263-270.

Kumari, B., R. Singh., V.K. Madan., R. Kumar., T.S. Kathpal and J. Singh 2003. Magnitude of pesticidal contamination in winter vegetables from Hisar, Haryana. *Environment Monitoring and Assessment* **87**: 311-318.

Lama, J.P. 2008. Standard setting on pesticide residues to ensure food safety. *The Journal of Agriculture and Environment* **9:** 46-53.

Manadhar, D. N. 2006. *Pesticides in Nepal*. The Rising Sun Printer, Kathmandu, Nepal.

Perez, B. D. and S. Rubio. 1999. *Environmental analytical chemistry*, (Vol XXXII Comprehensive Analytical Chemistry), Elseveir, Amsterdam.

Pesticide Residues Committee (PRC).2008. *Pesticide residues monitoring report*. Third Quarterly Report (March) .

Plant Protection Directorate.2008 (2064/65). *National trend in the amount import and composition of pesticide use in Nepal*. Unpublished report.

Renwick, A.G. 2002. Pesticide residue analysis and its relationship to hazard characterization(ADI/ARfD) and intake estimations(NEDI/NESTI). *Pest Management Science* **58:**1073-1082 (online 2002), DOI: 10.1002/ps.544.

Sharma, K.K. 2007. *Pesticide residue analysis manual*. ICAR, Directorate of Information and Publications of Agriculture, New Delhi.

WHO.1986. *Organophosphate insecticides: A general introduction*, vol 63. World Health Organization, New York.

WHO.1997. *Guidelines for predicting dietary intake of pesticide residues (revised) in collaboration with the Codex Committee on pesticide residues*. Program of Food safety and Food Aid.