Health Risk Assessment of Pesticide Residues via Dietary Intake of Market Vegetables from Nagpur District, India

M. CHAVAN[1+], J. L. TARAR[2] AND N. THACKER[3]

The study was carried out to assess the health risk of pesticide residues via dietary intake of vegetables collected from the agro-based market of Nagpur District, Maharashtra. The analysis was carried out as per the standard method of ICAR followed by Gas Chromatographic technique with electron capture detector (GC-ECD). It was used to identify organochlorine pesticides (OCPs) (e.g. aldrin, HCH, endosulphan, endosulphan sulfate, DDT, DDE, DDD, dicofol), in common vegetables of Nagpur district (cauliflower, brinjal, chili, carrot). Pesticide residues were compared with MRL established by Ministry of Health & Family Welfare and Codex Alimentarius Commission. It was found that pesticide residues detected in all vegetable samples were within the prescribed limits, whereas the highest health indices were found for aldrin (1.540), endosulphan (2.190) and dicofol (3.657) in brinjal, alone. Therefore, the main health risk may be posed by these recorded compounds, while the remaining pesticide residues present no risk in the other vegetables analyzed.

Key words: *Vegetables, health risk, pesticide residues, Nagpur district*

Introduction

Fruits and vegetables are important components of the human diet since they provide essential nutrients that are required for most of the reactions occurring in the body. A high intake of fruits and vegetables (five or more servings per day) has been encouraged not only to prevent consequences due to vitamin deficiency but also to reduce the incidence of major diseases such as cancer, cardiovascular diseases and obesity[1].

Food is required for survival but its contamination by chemical toxicants is a worldwide public health concern. Contamination may occur through environmental pollution of the air, water and soil, such as the case with toxic metals, PCBs and dioxins, or through the intentional use of various chemicals, such as pesticides, animal rugs and other agrochemicals. The presence of pesticide residues is a concern for consumers because of their toxic effects such as interfering with the reproductive systems and foetal development as well as their capacity to cause cancer and asthma[1]. Some of the pesticides are persistent and therefore remain in the body causing long term exposure.

Pesticides fate after application to fruits and vegetables

After pesticides are applied to the crops, they may interact with the plant surfaces, be exposed to the environmental factors such as wind and sun and may be washed off during rainfall. The pesticide may be absorbed by the plant surface (waxy cuticle and root surfaces) and enter the plant transport system (systemic) or stay on the surface of the plant (contact). While still on the surface of the crop, the pesticide can undergo volatilization, photolysis

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chemical and microbial degradation. All these processes can reduce the original pesticides concentration but can also introduce some metabolites in the crops.

Viewing the concern of the Government for the human exposure to these compounds in the diet and ensuing potential risk to health, the present study was carried out to assess the health risk of pesticide residues via dietary intake of vegetables collected from the agro based region of Nagpur District, from where the analysed soil, ground and surface water samples were detected with higher levels of pesticide residues[2] (OCPs) i.e. 276.67 ug/kg, 10.064 ug/L, 4.782 ug/L, respectively.

Materials and methods

Sample collection and preservation

Samples of four vegetables viz. cauliflower, chili, brinjal, carrot, that are available all year round, were purchased from the local markets of Nagpur city, seasonally. These vegetable samples were collected as a representative sample of Umred region, from a vendor selling the vegetables of the same region. As per the collected information, the vegetables were sprinkled with water after plucking and even while transportation, to retain its freshness, for a longer duration. The details of different vegetable samples during the experiment are as follows:

Vegetable samples used for the research work:

Sample code	Common name	Scientific name	Family	Edible part
Veg I	Brinjal	*Solanum melongena L.*	Solanaceae	Fruit
Veg II	Cauliflower	*Brassica oleracea*	Brassicaceae	Floret
Veg III	Chili	*Capsicum annum*	Solanaceae	Fruit
Veg IV	Carrot	*Daucus carota*	Apiaceae	Taproot

Samples were taken among commodities considering high consumption rate and relatively cheap to buy. Four different batches of samples were taken for analysis. The average sample size was 1 kg. each, which was collected in separate sterile polythene bags, sealed, labeled with unique sample identity, placed in ice chest box and transported to laboratory. Samples were stored at 4°C until analysis was performed (within 24 hr.). In the laboratory, samples were chopped and ground in an electric blender to obtain a homogeneous composite. Then 100 g homogenized samples from mother vegetable were taken for further analysis.

Analytical methods

Chemicals and reagents

All pesticide analytical standards were procured from Dr. Ehrenstorfer Gmbh, Germany. The solvents used for the extraction were obtained from Merck (HPLC grade for Chromatography). Individual pesticide stock standard solutions were prepared by exact weighing of high-purity substances in 10 mL volumetric flasks and filled up with an appropriate solvent like acetone and n-hexane. All stock standard solutions were stored in a deep freezer protected from light at -20°C. An intermediate and working standard of suitable concentration was made from the stock as and when required.

Extraction (multi-residues method)

Hundred grams (100 g) chopped or blended homogenized vegetable sample was mixed with 200 mL acetone for 2 min in high-speed blender. The slurry was filtered through Buchner funnel fitted with a filter paper. (Filtration should be completed in <1 min.) From the extract, an aliquot of 80 mL was transferred to 1 liter separatory funnel and extracted with 200 mL solvent mixture (hexane: dichloromethane = 1:1, v/v), by vigorous shaking for 1 min. The lower aqueous phase was then transferred to another 1 L separatory funnel. The organic phase of the first separatory funnel was dried by passing through approximately 1.5"sodium sulphate supported on pre-washed cotton in 4" funnel. 10 mL of saturated sodium chloride was added to the separatory funnel containing aqueous phase. The extraction was repeated twice with 100 mL dichloromethane and the organic layer was dried on the same sodium sulphate that was

used for drying organic extract of the first separatory funnel, which was then rinsed with 50 mL dichloromethane.

The extract was transferred to round bottom flask and evaporated in a rotary evaporator at 35-40°C under mild pressure, which helped to remove liquid solvents without excessive heating that ultimately retained even the volatile fraction of organic pesticides from the solution. The concentration step was repeated in the presence of hexane to remove all traces of dichloromethane, and then repeated again to produce final extract in acetone solution. The volume was adjusted to 7 mL with acetone.

Clean-up and concentration

The samples were cleaned up using the procedure described elsewhere[3]. Florisil column chromatography was used to clean up the extract. The florisil (mesh size 60-100) was activated at 200°C for 6 h and deactivated with 2% distilled water. After pouring 50 mL hexane through the column, it was filled with 4 g of activated florisil, followed by 2 g of sodium sulphate. One mL of extract was diluted to 10 mL with 10% acetone in hexane and transferred to florisil column. Elution was done with a 50 mL solvent mixture of 50% dichloromethane, 1.5% acetonitrile, 48.5% hexane (v/v/v). Elute was concentrated in a rotary vacuum evaporator to 1 mL and transferred to a vial to be determined by GC on ECD.

Calculations

The equivalent sample weight in final solution was calculated by the following formula:

$$\frac{mg\ sample\ weight\ equivalent}{\mu l\ of\ final\ extract} = 100 \times \frac{80}{200+W-10} \times \frac{1}{mL\ final\ volume\ (i.e.\ 7\ mL)}$$

Where 100 g sample analyzed, 80 mL filtered extract taken for hydromatrix partition, W-amount of water present in 100 g sample, 200 mL acetone used for blending, 10-adjustment for water/acetone volume concentration.

Water content in the vegetable sample analyzed as per Pesticide Residue Analysis Manual is mentioned below:

Commodity	Water content (%)
Cauliflower	92.26
Chili	87.74
Brinjal	91.93
Carrot	87.79

Further concentration of pesticide residue in vegetable sample was detected on Gas Chromatograph.

Instrumentation

The pesticide residues were analyzed by gas chromatograph equipped with [63]Ni electron capture detector (SHIMADZU GC-2010). The column specifications and operating conditions are listed
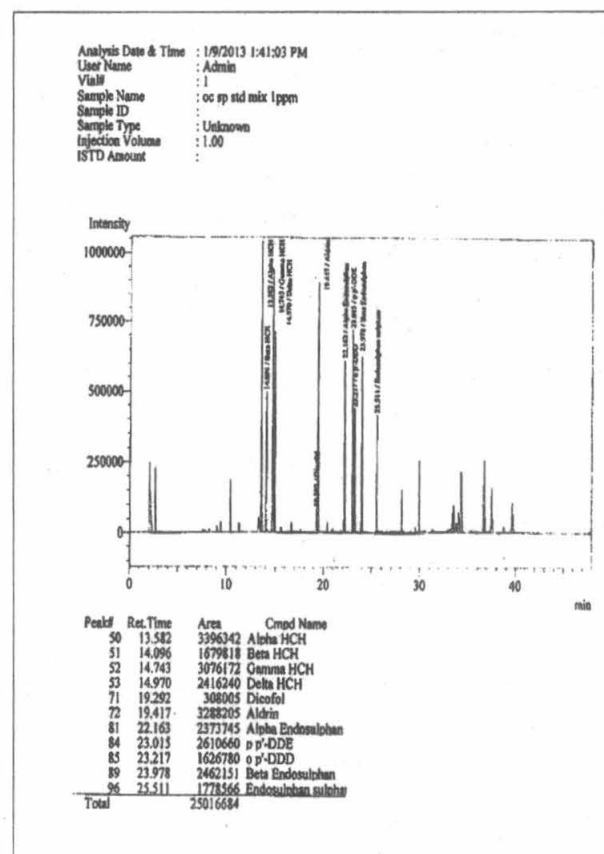

Fig. 1.: Chromatogram for standard mixture OCPs of 1 ppm for vegetable sample

Table 1 and the chromatogram of mixture of 11 analytical standards of 1 ppm is presented in Fig. 1. Equal volumes of differently concentrated (1.0, 0.5, 0.1, 0.05 and 0.01 mg/kg) standard solutions of pure analytical standards were injected into GC column and the calibration curve for each standard OCP was determined. For this purpose, the detection limit was estimated up to that concentration where a signal to noise ratio is 1:3 was observed.

Table 1.: GC conditions for OCPs analysis (apparatus manual)

Item	Condition
GC	Make – SHIMADZU Model –GC-2010 (Auto Sampler)
Detector	Electron Capture Detector (ECD)
Column	DB-5 - 30 m l., 0.25mm ID & film - 0.25µm thickness
Column temp. (^{0}C)	100
Oven (^{0}C)	200-250
Detector (^{0}C)	300 – 350
Carrier gas flow rate (mL/min)	30
Pressure (kpa)	110

Quality control analysis

The quantitation of the OCPs was performed via calibration curves with spiked vegetable samples (Cauliflower, chili, brinjal, carrot) whereby the area under each peak was referred to the area under the peak of the internal standard. Samples of untreated control sample of the matrix of interest were fortified at the level of calibration curve concentration and the samples were extracted, cleaned up and analyzed as per the multi-residue analysis method and the amount of residue was calculated from each of the fortified samples. The retention time was within ± 0.05 % of that of the standard.

Recovery experiments were carried out in order to establish the efficiency of the method and the recoveries[4] were found to be in the acceptable range of 70-110% with RSD $\leq$ 20%. Based on the recovery experiment, the limit of detection of the OCPs was set at 0.001 ppm, with the regression coefficient of 0.9977.

Hazard risk index (HRI) analysis

From a potential health perspective, it is certainly important to compare exposure estimates to established toxicological criteria such as EDI. Actually EDI is a realistic estimation of pesticides residues exposure that was calculated in the agreement with the international guidelines[5-6] EDI of pesticide residues for each combination of pesticide and commodity was calculated by multiplying the residual pesticide concentration (mg/kg) by the food consumption rate (kg/day) and dividing by a body weight of 60 kg for an adult. The average daily vegetable intake for adult (Maharashtra) was considered to be 0.123 kg/person/day[7]. Then HRI of the residues was computed using the results and other statistics followed by equation, modified after EFSA (European Food Safety Authority Scientific Colloquium Summary Report).

$$HRI = EDI/ADI$$

Where, EDI is estimated daily intake, ADI is acceptable daily intake. HRI value more than 1 is considered as not safe for human health[8].

Results and discussion

Occurrence and levels of OCPs in vegetables

Pesticide residues of the present study were compared with MRL established by Ministry of Health & Family Welfare[9] and Codex Alimentarius Commission[10] mentioned in **Table 2**. The arithmetic mean (with standard deviations (±SD)) and range of OCPs in the vegetables are shown in **Table 3**. Among the studied pesticides, quantifiable residues of DDTs were found in virtually all the vegetable samples, whereas frequency of detection was higher in veg IV. The DDT metabolites p,p'-DDE were in general more prevalent than o,p'-DDD suggesting either efficient biotransformation of the parent materials in the plant systems or old sources of DDT contamination. The

Table 2.: Maximum residue limit (MRL) (mg/kg) of identified pesticide residues in the analysed vegetabl (guideline values)

Sr. No.	Pesticide	MRL (Ministry of Health & Family Welfare (India)[9]				MRL CODEX[1(
		Cauliflower	Chili	Brinjal	Carrot	
1	α – HCH	1.00	–	1.00	1.00	–
2	β – HCH	1.00	–	1.00	1.00	
3	γ – HCH	0.10	–	0.10	0.10	–
4	δ – HCH	0.10	–	0.10	0.10	–
5	Aldrin	0.10	0.10	0.10	0.10	0.10
6	Endosulphan	2.00	1.00	1.00	1.00	
7	Dicofol	5.00	1.00	5.00	5.00	–
8	DDT	3.50	3.50	3.50	3.50	3.50

– Not available for commodities analysed

maximum concentration of ΣDDT was detected in veg I (0.281 mg/kg).

Among the HCH isomers, lindane was the most frequently quantified. Maximum concentration of ΣHCH was detected in veg I (0.011 mg/kg). As compared to the other OCPs frequency of detection of HCH isomers was considerably less. Present study of water and soil has also reported DDTs dominance over HCHs. The correspondence between the residue levels of DDT and HCHs in soil, water and their accumulative levels in the edible portions of vegetables could be because DDT is more hydrophobic than HCH. Hydrophobic compounds are strongly bound to root and soil organic colloid surfaces resulting in less absorption and/or translocation[11]. As seen in the other two matrices of study concentration of dicofol was detected higher than the other OCPs. It was recorded maximum in the range of 0.041 – 0.169 mg/kg, in veg IV.

Endosulphan was the most frequently detected compound in all the samples of vegetables. Endosulphan sulphate was recorded maximum in veg III, in the range of 0.003 – 0.067 mg/kg. β isomer of endosulphan was recorded in all the vegetable samples examined. Both the isomers of endosulphan, α-endo. and β-endo. were recorded maximum in veg IV (carrot)

which were in the range of 0.013 – 0.017 mg/kg an 0.022 – 0.031 mg/kg respectively.

The average total concentration of OCPs the vegetable samples analyzed was found in tl order of veg IV > veg I > veg II > veg III (Fig. 2 These were found to be very much within tl prescribed limits. One reason for these lo concentrations can be sprinkled water by the vendo to retain the freshness of vegetables for a long duration. However, the persistent nature of tl pesticides is of great concern due to their bic accumulative behaviour and toxic biological effec on human[12]. Continuous monitoring of residu pesticides level in different food materials from differe
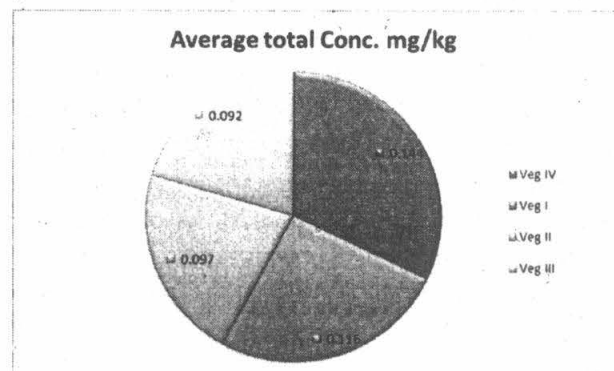

Fig. 2.: Average total concentrations of OCPs vegetable samples

Table 3. : Mean concentrations of OCPs (mg/kg) in the vegetable samples of Nagpur District

Sr. No.	Mean conc. of OCPs	Veg I	Veg II	Veg III	Veg IV
1	α – HCH Mean±SD (Min–Max)	BDL±0.001 BDL–0.002	0.001±0.001 BDL – 0.002	BDL BDL±0.001	BDL – 0.001 BDL – 0.001
2	β – HCH Mean±SD (Min–Max)	BDL	BDL	0.001±0.001 BDL – 0.002	0.001±0.002 BDL – 0.003
3	γ – HCH Mean±SD (Min–Max)	0.001±0.002 BDL – 0.003	BDL	BDL±0.001 BDL – 0.001	BDL±0.001 BDL – 0.001
4	δ – HCH Mean±SD (Min–Max)	0.003±0.003 BDL – 0.006	0.002±0.004B DL – 0.009	BDL	BDL
5	Σ HCH Mean±SD (Min–Max)	0.004±0.005 BDL – 0.011	0.003±0.004 BDL – 0.009	0.001±0.002 BDL – 0.004	0.001±0.003 BDL – 0.006
6	Aldrin Mean±SD (Min–Max)	0.005±0.008 BDL – 0.016	BDL±0.001 BDL – 0.001	BDL±0.001 BDL – 0.001	BDL±0.001 BDL – 0.001
7	α – Endo. Mean±SD (Min–Max)	0.001±0.002 BDL±0.001	BDL – 0.004 BDL – 0.001	0.001±0.001 0.015±0.002	BDL – 0.002 0.013 – 0.017
8	β – Endo. Mean±SD (Min–Max)	0.009±0.001 0.007 – 0.010	0.005±0.002 0.003 – 0.007	0.018±0.012 0.004 – 0.030	0.027±0.004 0.022 – 0.031
9	Endo-SO$_4$ Mean±SD (Min–Max)	0.005±0.003 BDL – 0.008	0.004 ±0.004 BDL – 0.010	0.023±0.03 · 00.003– 0.067	0.001±0.002 BDL – 0.004
10	Σ Cyclodienes Mean±SD (Min–Max)	0.019±0.009 0.010 – 0.031	0.010±0.004 0.005 – 0.015	0.041±0.039 0.009 – 0.097	0.043±0.005 0.038 – 0.050
11	Dicofol Mean±SD (Min–Max)	0.015±0.019 BDL – 0.040	0.063±0.050 0.006 – 0.127	0.049±0.006 0.041 – 0.054	0.081±0.059 0.041 – 0.169
12	p,p'-DDE Mean±SD (Min–Max)	0.071±0.140 BDL – 0.281	0.004±0.006 BDL – 0.013	BDL±0.001 BDL – 0.001	0.018±0.005 0.011 – 0.021
13	OP – DDD Mean±SD (Min–Max)	0.001±0.003 BDL – 0.005	0.001±0.002 BDL – 0.003	0.001±0.001 BDL – 0.002	BDL
14	Σ DDT Mean±SD (Min–Max)	0.072±0.139 BDL – 0.281	0.021±0.038 BDL – 0.077	0.001±0.002 BDL – 0.003	0.018±0.005 0.011 – 0.021
15	Total OCPs Mean±SD (Min–Max)	0.116±0.144 0.015 – 0.328	0.097±0.066 0.018 – 0.157	0.092±0.038 0.060 – 0.147	0.144±0.062 0.101 – 0.237

Table 4: Health risk assessment based on acceptable daily intake (ADI) of pesticides

Sr. No	Pesticide	ADI (mg/kg /day)	Veg I Cauliflower		Veg II Chili Brinjal		Veg III Carrot		Veg IV	
			EDI	HRI	EDI	HRI	EDI	HRI	EDI	HRI
1	γ – HCH	0.001	>	0.006	–	–	0.0001	0.145	>	0.002
2	Aldrin	0.0001*	>	0.328	>	0.020	0.0002	1.540	>	0.030
3	Endo.	0.006	0.0019	0.312	>	0.052	0.0131	2.190	0.0001	0.016
4	Dicofol	0.002	0.0001	0.041	0.0003	0.131	0.0073	3.657	0.0003	0.173
5	DDT	0.01*	0.0006	0.058	0.0002	0.016	0.0005	0.046	>	0.043

– EDI not established for the particular pesticide in particular vegetable
> Very less to be mentioned
* Provisional tolerable daily intake

areas is obvious to understand the trend of contamination.

Seasonal variation

The residue levels and detection rates of the OCPs are given in Fig. 3. It is giving a statistic where total concentration of OCPs in vegetables was higher in monsoon season, whereas all the compounds of OCPs were detected in almost all the vegetable samples in summer season. This may be because more pests were active during summer when the vegetables were growing, which might have necessitated the illegitimate use of some of the OCPs. It is also possible that the high temperatures in summer volatilized the OCPs from their reservoirs such as soil or vegetation and that the edible parts of the crops may have trapped some of the evaporating pesticides.

It was also noticed that residues of α-HCH, β-HCH, γ-HCH (lindane) and hence ΣHCHs were more prevalent in summer samples in comparison to the other seasons. In particular, presence of the insecticide lindane was computable in all the summer vegetables, which suggests that the vegetables might have been contaminated at the beginning of spring or earlier when they were planted.

Daily intake and health risk assessment

ADI, EDI and HRI of pesticide residues are given in **Table 4**. Health indices of aldrin and endosulphan in cauliflower were calculated to be 0.328 and 0.312, respectively. The highest health indices were reported for aldrin (1.540), endosulphan (2.190) and dicofol (3.657) in brinjal only. Therefore the main health risk may be posed by these recorded compounds, while the remaining pesticide residues present no risk in the other vegetables analyzed.

It is noteworthy that dietary pesticide intakes estimated in this study considered only exposures from vegetables and did not include other food
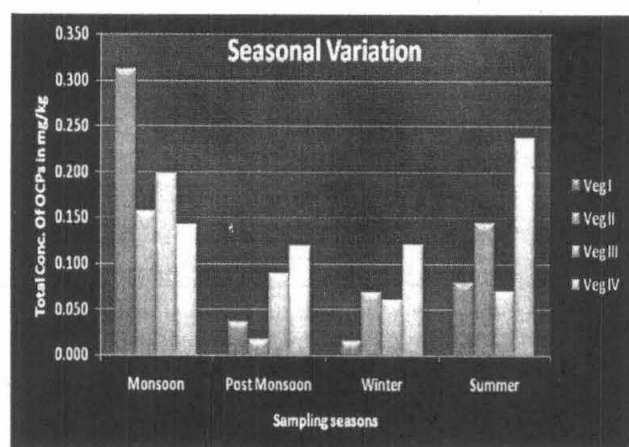

Fig.3.: Seasonal variation of OCPs in vegetable samples

products including fruits, grains, dairy, fish, meats, etc. Therefore, estimates are not considered as total dietary exposure to the pesticides, nor do we consider drinking water, residential or occupational exposures. So, it is an underestimation of the total exposure of pesticides studied. Moreover, not all registered pesticides and all vegetables usually consumed were measured in this study. At the same time, processing factors were ignored, whereas fruits and vegetables are often peeled, cooked or boiled before consumption, resulting in an overestimation of the actual exposure to pesticide residues. Furthermore, the effect of pesticides on more vulnerable groups such as children and pregnant women could all affect these calculations[13-14]. At the same time, as per the health risk assessment done, no detectable amount of pesticide residues was found but this does not necessarily mean that the content is truly zero (Table 4). The content may just be too low for detection with the currently available methods and technology.

Conclusion

This research revealed high occurrence rates but low residue levels of OCPs in vegetables produced in farmlands of Umred region, Nagpur District. Since most of the determined residue levels are far below the prescribed national and international residue limits, it can be concluded that there was a good observance of limitations in agricultural application of the chlorinated pesticides. However, the high prevalence of contamination in the other studied matrices of environment was worrisome considering the cumulative nature, level of persistence of these pesticides and, especially, the high amounts of vegetables in the diets, at Maharashtra region. Consumption of pesticide free vegetables and elimination of the vegetable OCP contamination are recommended. Moreover, controlling the pesticide levels especially by educating the farmers about the judicial and restricted use of pesticides may curtail the severity of problem up to certain extent.

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