Journal of Innovative Research in Engineering and Science 2(1), March, 2011.
ISSN : 2141-8225
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Organophosphate and Carbamate Pesticide Residues in Beans from Markets in Lagos State, Nigeria *(pp. 50-61.)*

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Abstract: Foods treated with pesticides for protection against destructive pests often contain residues of these chemicals. The levels of pesticide residues in food are often determined as a means of assessing appropriate use as well as the level of human exposure to these chemicals and hence their potential human health hazards. The aim of this study was to determine the concentration of organophosphate and carbamate pesticides in beans samples collected from markets in Lagos State and compare these values with established safety values. Beans (*Phaseolus vulgaris L.*) samples purchased from different markets in Lagos State were analyzed for residues of organophosphate and carbamate pesticides. Analysis was done using gas chromatograph with mass spectrometric detector (GC-MS) after careful extraction and cleanup. It was found that all the beans samples contained residues of one or more organophosphate or carbamate pesticides. Mean concentrations ranged from 19.4 to 455.9 µg/kg. Maximum residue limits (MRL) of the various pesticides (except for parathion) were exceeded in up to 10% of samples. The estimated total diet intake (ETDI) for dichlorvos exceeded its maximum permissible intake (MPI) by 131%. Organophosphate and carbamate pesticide residues are present in beans sold in Lagos markets and maximum residue limits for most of the pesticides are exceeded. There is therefore a need for more stringent monitoring of importation and use of these pesticides in agriculture and food storage in Nigeria.

Keywords: pesticide residues, organophosphate, carbamate, Lagos.

1 INTRODUCTION

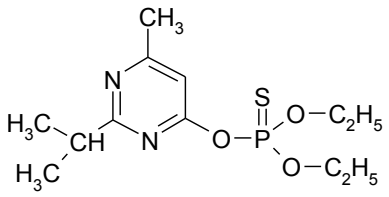
Pesticides are chemicals used in agriculture to protect crops against destructive pests both in the field and during storage. They are also used in public health and other areas for the eradication of disease vectors and other pests. When used, pesticides contaminate the environment and accumulate in the food chain thereby posing hazard to human health (Blasco *et al*, 2003; Leong *et al,* 2007; Pesticide Action Network, 2001).

There are different classes of pesticides but the major ones are organochlorines, organophosphates, carbamates and pyrethroids. Organochlorines being chemically stable and persistent in the environment have been banned in most countries of the world but the

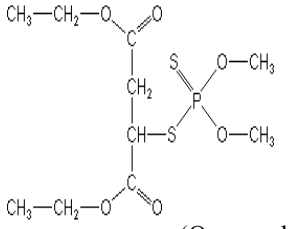
less persistent classes are widely in use. Organophosphates are highly potent compounds used mainly as insecticides especially in the control of storage insects in food crops. They are very toxic and more often involved in acute poisoning than other classes of pesticides (Collins, 2006; Mansour, 2004). Carbamates on the other hand are less toxic but very effective as insecticides, fungicides, herbicides, nematocides and sprout inhibitors. Figure 1 shows structures of some organophosphate and carbamate pesticides.

Organophosphorus and carbamate pesticides exert their toxic action by inhibiting the enzyme acetylcholinesterase (AChE). This enzyme is responsible for the hydrolysis of acetylcholine (ACh), a neurotransmitter that conducts nerve impulses across neuromuscular junctions in the nervous system of vertebrates as well as insects. This enzyme inhibition causes accumulation of ACh leading to generalized cholinergic action and resulting in rapid, uncontrolled twitching of voluntary muscles which eventually leads to paralysis, respiratory failure and death (Podolska and Napierska, 2006; Guilhermino *et al,* 2004).

Chronic exposure of humans to low doses of pesticides through air, water and food may lead to chronic toxicity due to accumulation of residues in the body over a long period of time. Possible health problems associated with chronic pesticide toxicity include cancers, congenital malformations, neurological disorders, infertility, blood dyscrasias, impotence, immunological disorders, liver and kidney damage, skin alterations and worsening of existing health conditions (Sesline and Jackson, 1994; Jobling *et al,* 1995).

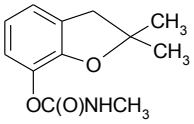


Diazinon

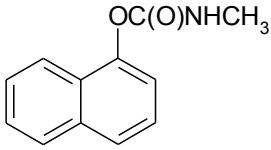


(Organophosphate)

Malathion
(Organophosphate)



Carbofuran
(Carbamate)



Carbaryl
(Carbamate)

Figure 1: Structures of Some Organophosphate and Carbamate Pesticides.

Acute and sub-acute toxicity may also arise from exposure to high doses among people who are directly involved in the manufacture, formulation, mixing and application of pesticides or in suicide and homicide cases. Human exposure may be through dermal contact, inhalation or accidental ingestion. Symptoms of acute toxicity vary with the individual chemical involved but may generally include dizziness, headaches, sweating, fatigue, numbness, vomiting, cramps, chemical burns of the eye and skin, neurological effects, respiratory tract irritation, liver and kidney damage, coma or death (Koprucu *et al*, 2006; Turgut, 2007).

Inspite of their benefits to man, pesticides are poisons and must be properly used to minimize human exposure and reduce health risks. There is therefore governmental regulation of pesticide use all over the world and analysis of pesticide residues in food is one way of monitoring effectiveness of regulatory control measures.

2 MATERIALS AND METHODS

2.1 Collection of Samples

Samples of brown beans were purchased from wholesale markets across Lagos State. The samples were code named and stored in glass bottles with tight covers to protect them from moisture and contamination. They were then stored in the refrigerator until ready for use.

2.2 Preparation of Samples

The samples were cleaned by picking out stones and other extraneous materials. Each sample was thoroughly mixed and a 200.0g portion was taken and milled to 20 mesh particle size to produce a good homogenate. The milled samples were then stored in glass bottles with appropriate labels in a refrigerator at 4^0C. Duplicate portions (200.0g) of the samples were stored as whole grains in labeled glass bottles in the refrigerator as backup samples.

2.3 Extraction and Clean-up of Samples

Extraction of samples for the analysis was according to the methods of Wei-Guo *et al.*, (2006) and Zawiyah *et al.*, (2006) with slight modifications. The milled sample was properly mixed and 2.0g was weighed into a 20.0ml sample vial. Anhydrous sodium sulphate (1.0g) was added and mixed with the sample to absorb any moisture present. The sodium sulphate was previously heated at 650^0C for one hour and stored in a desiccator. Ethyl acetate (10.0ml) was added to the vial. The mixture was vortex mixed for 5min and then allowed to stand for 45min. It was mixed again and centrifuged for 5min. at 2500rpm. The supernatant was carefully transferred into a flask. The residue was further extracted twice as described above, using 10.0ml ethyl acetate each time. The supernatants were combined and reduced to about 5ml using a rotary evaporator at 35^oC. The solution was then transferred into a sample tube and reduced to about 1ml under a gentle stream of nitrogen gas using a nitrogen evaporator at 36^0C. This was then taken for florisil cleanup.

For the clean-up, solid phase extraction cartridges (florisil, 500mg/6ml) were used. Each cartridge was conditioned with 5.0ml of the eluting solvent mixture (hexane/ethyl acetate 50:50) and the sample extract (1ml) was loaded on the florisil. The sample tube was rinsed

three times with 1.0ml eluting solvent, and the rinses added to the florisil column. The sample was then eluted with 5.0ml of the same solvent mixture into a receiving glass tube. The florisil column was rinsed with another 3.0ml of the eluting solvent mixture into the same receiving glass tube. The eluant was then evaporated to dryness under a gentle stream of nitrogen gas and the residue reconstituted in 1.0ml ethyl acetate for GC-MS analysis.

2.4 Preparation of Calibration Curves

Pure standards of organophosphate (chlorpyrifos, diazinon, dichlorvos, fenitrothion, malathion, parathion, pirimiphos-methyl) and carbamate (carbaryl and carbofuran) pesticides were sourced from Sigma-Aldrich, Germany and all were above 99% purity. Stock solutions of the pesticides standards were prepared and then serially diluted to produce different concentrations of pesticides. Stock standard solutions were stored in amber coloured bottles at 4^0C in a refrigerator while working standard solutions were prepared fresh before use.

Standard solutions of the pesticides were run on GC/MS under the set chromatographic conditions and mean peak areas were plotted against concentrations to obtain calibration curves of individual pesticides. The chromatograms of standard pesticide mixtures are shown in Figures 2 to 4.

2.5 Limits of Detection (LOD)

To determine the limit of detection of the equipment for each pesticide, an air blank sample was run under the experimental conditions to obtain the detector baseline noise. A detectable ion should produce a signal that is at least three times the baseline noise [that is, signal-to-noise (S/N) ratio = 3][14]. The LOD of each pesticide was determined by running serially diluted solutions of the pesticide at the set chromatographic conditions and finding the concentration at which S/N = 3. Retention times, limits of detection and coefficient of determination of the various pesticides are presented in Table 1.

2.6 Analysis of Pesticide Residue Content

2.6.1 GC-MS Conditions

All compounds were determined and quantified with the aid of a gas chromatograph equipped with a mass-selective detector (GC-MS), an autosampler and a split-splitless injector. The DB-5 fused silica capillary column of 30m x 0.25µm i.d. x 0.25µm film thickness was coated with cross-linked 5% phenyl dimethyl polysiloxane. The carrier gas was helium (99.999% purity) at a flow rate of 1.0ml/min. Oven temperature was maintained initially at 70^0C for 1min, increased at 15^0C/min to 175^0C, then at 2^0C/min to 215^0C, at 10^0C/min to 265^0C and finally at 20^0C/min to 290^0C and held for 8min. Injection volume was 1µL, injected in splitless mode at injection temperature of 250^0C.

2.6.2 Identification and Quantification

Pesticide residues were identified if the retention times matched those of the standards and the relative abundances were within 10% of those of the standards. Identified pesticides were quantified using the external standard method of comparing sample peak areas with

those of the pesticide standards under the same conditions. Each sample was analyzed three times and the mean values obtained. The pesticide content of each sample was calculated as:

$$Pesticide\ Content = \frac{As\ x\ Vf}{\text{Wts x CF}}$$

Where As = peak area of sample
Vf = final volume of clean extract
Wt_s = weight of sample extracted
CF = calibration factor.

$$The\ CF\ of\ each\ pesticide\ was\ calculated\ as = \frac{Peak\ Area\ of\ Standard}{\text{Total Amount of Standard Injected}}$$

2.8 Estimation of Daily Intakes

The pesticide residue levels determined in the study and an assumed consumption rate were used to estimate daily intakes of the various pesticides (Handa *et al.*, 1999). Estimated daily intakes (EDIs) were then used to calculate estimated total diet intake (ETDI) of residues as:

$$ETDI = EDI \times 25$$

(If a total diet of 1.5kg per person per day is assumed, then beans is contributing only 4% of a person's diet). The MPIs were calculated from acceptable daily intakes (ADIs) fixed by FAO/WHO using an average body weight (Wt) of 60kg as follows:

$$MPI = ADI \times W_t$$

Values of ETDI obtained for the various pesticides were compared with their MPI values. The results are shown in Table 3.

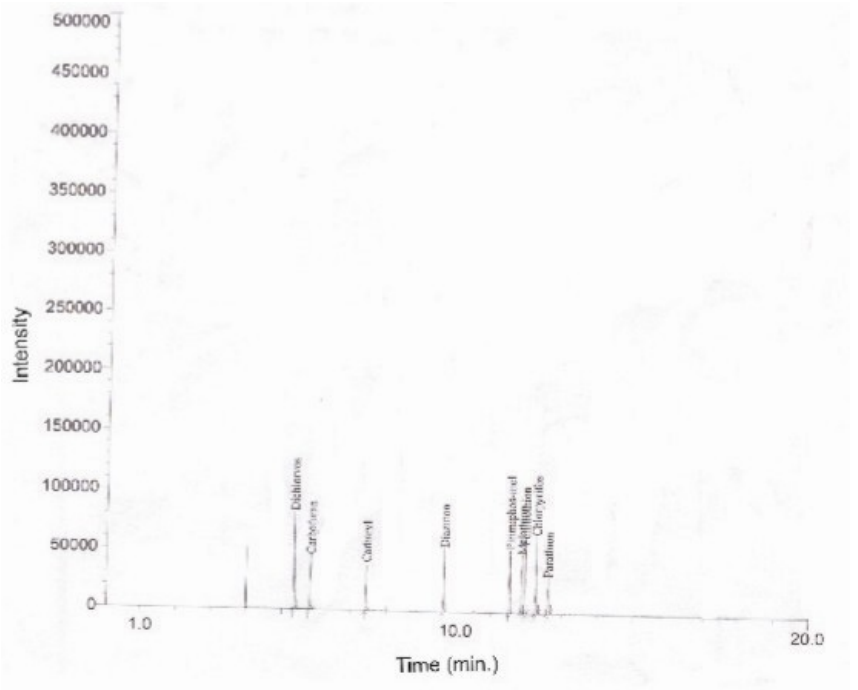
3 RESULTS

The results of the analysis are presented in the following tables and figures.

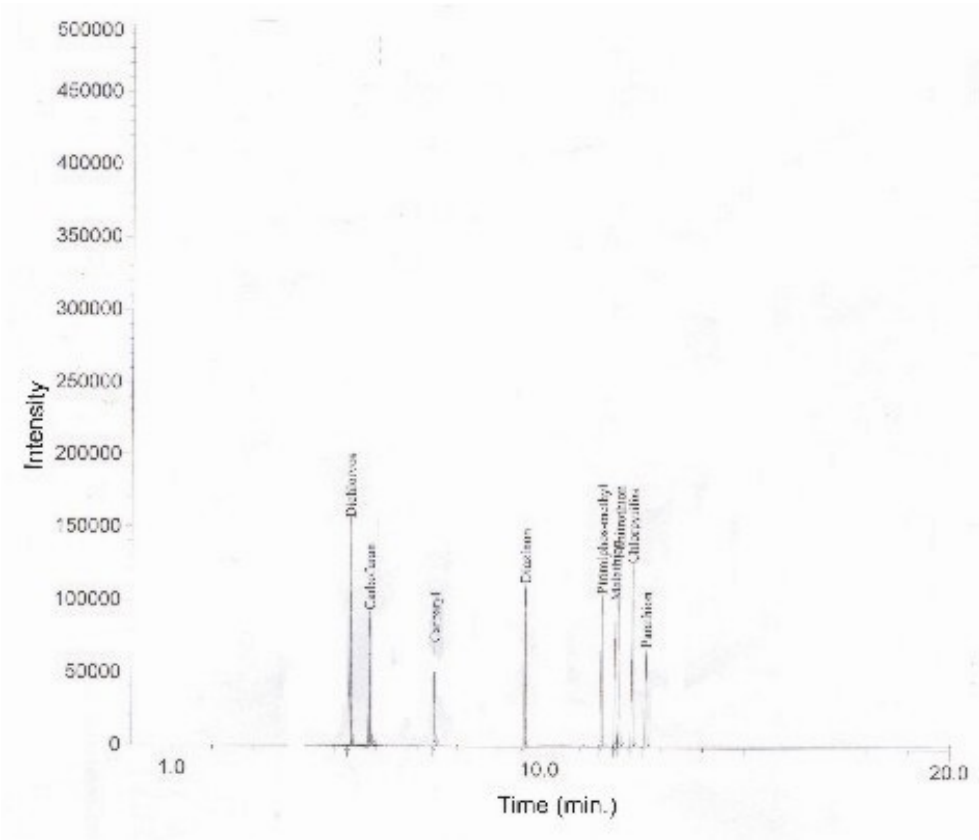
Table 1: Names, Retention Times (RT), Limits of Detection (LOD) and Coefficients of Determination of the Pesticides.

Pesticide Name	RT (min.)	LOD (µg/ml)	Coefficient of Determination (r^2)
Carbaryl	7.42	0.0075	0.9995
Carbofuran	5.83	0.0066	0.9999
Chlorpyrifos	12.28	0.0025	0.9999
Diazinon	9.65	0.0009	1
Dichlorvos	5.35	0.0168	0.9999
Fenitrothion	11.96	0.0007	0.9985
Malathion	11.87	0.0082	0.9999

Parathion	12.62	0.0062	0.9984
Pirimiphos-methyl	11.53	0.0014	1



(A)



(B)

Figure 2: Chromatogram of Mixed Organophosphate and Carbamate Pesticide Standards (Dichlorvos, Carbofuran, Carbaryl, Diazinon, Pirimiphos-methyl, Malathion, Fenitrothion, Chlorpyrifos, and Parathion) at 2.5 µg/ml (A) and 5µg/ml (B).

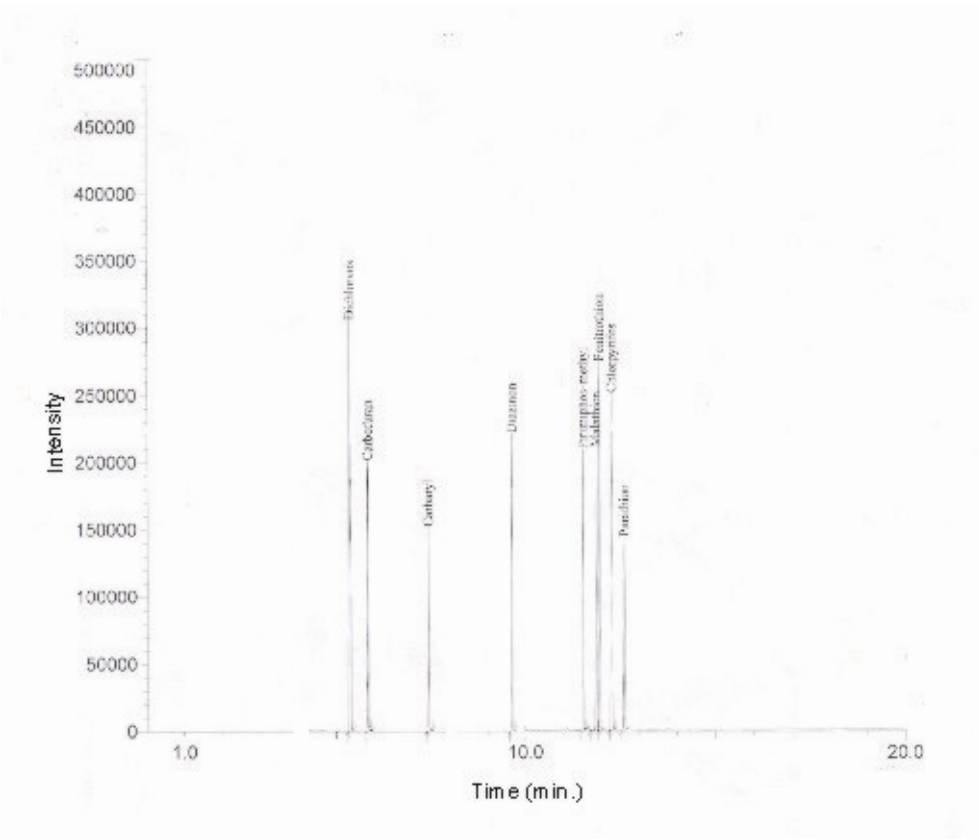


Figure 3: Chromatogram of Mixed Organophosphate and Carbamate Pesticide Standards (Dichlorvos, Carbofuran, Carbaryl, Diazinon, Pirimiphos-methyl, Malathion, Fenitrothion, Chlorpyrifos, and Parathion) at 10 µg/ml

Table 2: Pesticide Residue Concentrations in Beans Samples Versus Maximum Residue Limits of the Various Pesticides

Pesticide	MRL (µg/kg)	Maximum Concentration (µg/kg)	Mean Concentration (µg/kg)	% Occurrence (of pesticide in sample)	% Samples above MRL
Carbaryl	50	73.5	42.9	29	6
Carbofuran	100	135.3	98.5	35	7
Chlorpyrifos	50	98.2	50.1	44	10
Diazinon	20	27.8	19.4	29	6
Dichlorvos	100	740.5	455.9	42	5

Fenitrothion	10	Nd	Nd	0	0
Malathion	100	Nd	Nd	0	0
Parathion	50	44.9	31.8	16	Nil
Pirimiphosmethyl	50	92.5	43.5	54	10

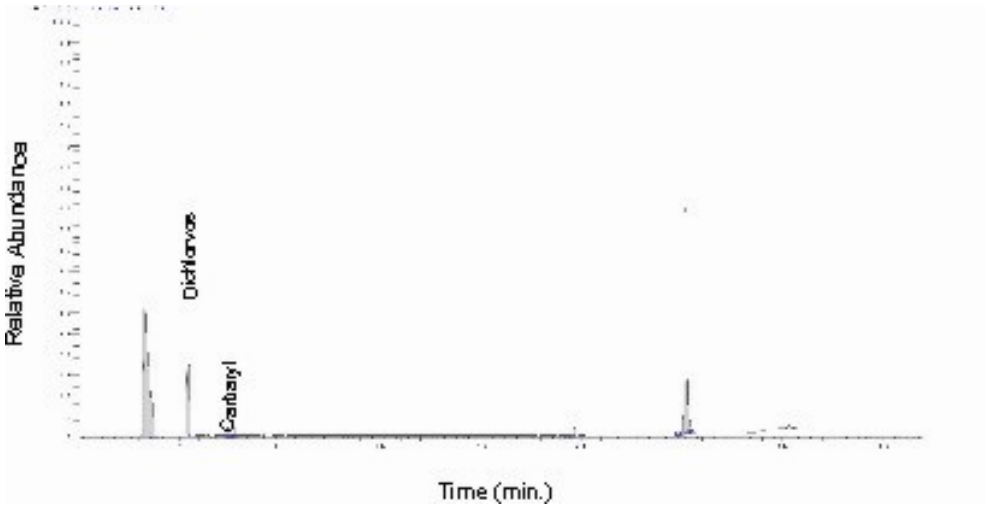


Figure 4: Chromatogram of a Sample Showing Dichlorvos and Carbaryl.

Table 3: Acceptable Daily Intakes (ADIs), Maximum Permissible Intakes (MPIs), Estimated Daily Intakes (EDIs) and Estimated Total Diet Intakes (ETDIs) of the Various Pesticides

Pesticide	ADI (µg/kg)	MPI (µg/person/day)	EDI (µg/person/day)	ETDI (µg/person/day)
Carbaryl	10	600	2.205	55
Carbofuran	3	180	4.059	102
Chlorpyrifos	10	600	2.946	74
Diazinon	2	120	0.834	21
Dichlorvos	4	240	22.215	555*
Fenitrothion	5	300	-----	-----
Malathion	20	1200	-----	-----
Parathion	1	60	1.347	34
Pirimiphos-Methyl	10	600	2.775	69

* ETDI that are above the MPI.

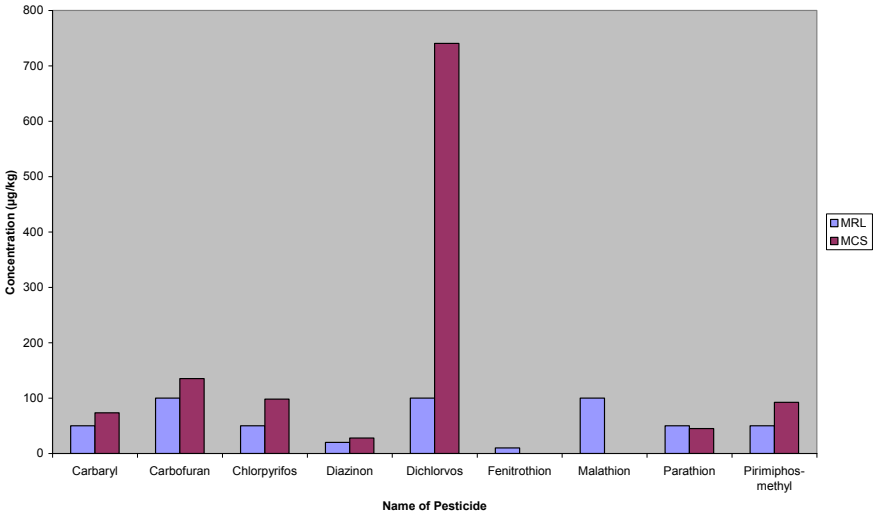


Figure 5: Chart Showing Maximum Residue Limits (MRL) versus Maximum Concentrations in Samples (MCS) for the Various Pesticides.

4 DISCUSSION

Results of the analysis show that all the samples contained one or more organophosphate or carbamate pesticides. Maximum concentrations found ranged from 27.8 to 740.5µg/kg while mean concentrations were 19.4 to 455.9 µg/kg. Residues of pirimiphos-methyl were the most frequently encountered, occurring in 54% of samples while residues of fenitrithion and malathion were not detected in any of the samples. These results are reflected in Table 2.

All the residues detected were above the maximum residue limits (MRL) for the various pesticides except parathion (Figure 7). Dichlorvos had the highest violating concentration which was 640% above its MRL. MRL of a pesticide is the maximum concentration of its residue that is legally permitted to remain in food after it has been treated with the pesticide. It is not expected to be exceeded in any food if the pesticide was applied in accordance with directions for its safe use. If a pesticide residue is found to exceed the MRL in a given foodstuff, the food commodity is said to be adulterated because it contains an illegal amount of the residue.

The occurrence of pesticides above MRLs in the beans samples is therefore an indication of some form of misuse/abuse of these chemicals. Non-compliance with MRLs can impact negatively on international trade in agricultural produce as each commodity must meet international standards or standards of the receiving country. The goal of monitoring of

pesticide use in agriculture should therefore be directed at ensuring appropriate use of recommended products.

Results of this study also reveal that estimated total diet intakes (ETDI) for the various pesticides (except dichlorvos) were lower than their maximum permissible intakes (MPI). Such residues are not likely to cause significant health hazards even on a long term basis. On the other hand, the ETDI for dichlorvos exceeded its MPI by 131%. This indicates excessive or inappropriate use of this chemical. Farmers and other pesticide users therefore need to be educated on dangers of abuse/misuse of pesticide products.

Most pesticides are known to be neurotoxic, especially organophosphates and carbamates which inhibit the enzyme acetylcholinesterase. Others have been found to be carcinogenic, teratogenic and to depress immune responses while some have been identified as endocrine disruptors, meaning that they can affect human growth and reproduction (Mansour, 2004; Jobling *et al.,* 1995; Koprucu *et al.*, 2006)The toxicological importance of pesticide residue data depends, not only on the residue content of food but also on the quantity of contaminated food consumed and the length of time over which the consumption occurs (Petersen, 2000). Therefore, large studies on food consumption patterns of Nigerians and residue contents of other food varieties are required to determine the exact total intake of pesticides and the actual toxicological importance of these residue levels.

Beans as a foodstuff is widely consumed in Nigeria. Consumers of this commodity should be advised to wash it thoroughly before other forms of processing to reduce the pesticide residue content at the point of consumption residues.

5 CONCLUSION

The results of this study show that there is a high incidence (100%) of pesticide residues in beans sold in Lagos markets. Most of the residues were found to be above the maximum residue limits with legal implications while one had concentrations above safety levels. There is a need for the relevant agency to strictly control the importation, sale, use and disposal of these toxic chemicals.

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