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Insecticide residues in the blood serum and domestic water source of cacao farmers in Southwestern Nigeria

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ABSTRACT

The blood serum of cacao farmers and their domestic water sources were analyzed for insecticide residues in selected cacao growing communities of Southwestern Nigeria. The farmers were grouped into five exposure periods based on their years of involvement in insecticide application, viz, <5 years, 5–9 years, 10–14 years, 15–19 years and >20 years.

The residue analyses revealed that 42 out of the 76 farmers had residues of diazinon, endosulfan, propoxur and lindane in their blood; and 47.6% out of these farmers belonged in the >20 years exposure duration period. About 34% of the farmers had diazinon with a mean concentration of 0.067 mg kg^{-1}, 29% endosulfan (mean = 0.033 mg kg^{-1}), 23% propoxur (mean = 0.095 mg kg^{-1}), and 17% lindane (mean = 0.080 mg kg^{-1}) in their blood. The residues of lindane, endosulfan and propoxur in all the exposure duration categories were found to be far below the no observable adverse effect level (NOAEL) while diazinon residues detected in the blood serum of the farmers in all the exposure duration categories exceeded the NOAEL of 0.02 mg kg^{-1} for the insecticide. The study also revealed that the sources of drinking water had been contaminated with dazinon and propoxur in some of the farmers' localities; and the concentrations of the insecticides exceeded the acceptable daily intake (ADI).

It is concluded that cacao farmers in Southwestern Nigeria may have been occupationally exposed due to insecticide application for mirid control in their cacao plantations; and the exposure at times is of such magnitude as to be hazardous to the farmers and their respective communities.

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1. Introduction

Insecticides, herbicides and fungicides constitute the major pesticides used in Nigeria. According to FAO (2005), pesticide importation rose steadily from about 13 million dollars in 2001 to 28 million dollars in 2003 with insecticides accounting for about 32% of the imports. The bulk of the pesticides is used in respect of agricultural production for the control of noxious weeds, insect pests and diseases of crops; and as the agricultural production system moves more and more from subsistence to market-oriented large scale farming, a concomitant increase in pesticide usage seems inevitable. Besides, insecticides are used in urban and suburban dwellings rather extensively as aerosols for the control of peridomestic pests (e.g. ants, cockroaches, mosquitoes and other nuisance flies); and as other formulations for the control of structural pests like termites, and other stored product pests.

Pesticide usage generally is fraught with problems of undesirable side effects and food chain involvement. Many pesticides pose substantial short and long-term health risks (WHO, 1990), and cause substantial environmental damage/contamination (Conway and Pretty, 1991). They are known to disturb the biochemical and physiological functions of erythrocytes and lymphocytes (Banerjee et al., 1999). The adverse health effects include a series of chronic end-points including cancer (Settimi et al., 2003; Alavanja et al., 2004), neurotoxic (Kamel and Hoppin, 2004), immunotoxic (Galloway and Handy, 2003) developmental (Colborn, 2006), endocrine (Barlow, 2005) and reproductive (Garcia et al., 1999; Yucra et al., 2006) and neurobehavioural effects (Amr et al., 1993). This has led to the prescription of tolerances [(maximum residue level (MRL) and acceptable daily intake (ADI) as well as no observable adverse effect level (NOAEL)] for various pesticides in food and water, especially by the Codex Alimentarius Commission (CODEX, 2004), and other designated authorities in several developed countries of the world like the US Environmental Protection Agency (EPA) (EPA, 1997).

Biological monitoring of exposure can be carried out by determining intact compounds or their metabolites in the blood serum, plasma or urine (Aprea et al., 2002). Much work has been done on pesticide residues and its cumulative effect on human beings in

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the developed countries. For example, the residues of HCH, DDE and DDT have been shown to produce hazardous effects in humans (Kohan et al., 1994). In less developed countries such as Pakistan, the presence of pesticide residues has been reported in the blood of Karachi people (Azmi et al., 2005). Cruz et al. (2003) also reported the presence of pesticide residue in an urban and two rural populations in Portugal while the effect of pesticide residues on health and different enzyme levels in the blood of farm workers from rural area of Karachi, Pakistan was reported by Azmi et al. (2006).

In Nigeria, apart from the work of Ivbijaro (1977) which evaluated insecticide residues in kolanuts, there seems to be no monitoring of pesticide residues in the country. Cacao farmers in Nigeria have a long history of pesticide usage on their farms. Cacao being a plantation crop had been subjected to large volumes of insecticides annually since 1957 especially for the control of the brown cacao mirid, *Sahlbergella singularis* Haglund. These insecticides included at various times till present, lindane (γ-BHC), endosulfan or thiodan (6,7,8,9,10,10-hexachloro-1,5,5a, 6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), diazinon {*O*,*O*-diethyl-*O*-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl]phosphorothioate}, propoxur(2-isopropoxyphenyl-*N*-methylcarbamate) and dioxacarb {2-(1,3-dioxolan-2-yl)-phenylmethylcarbamate}. Several of the cacao farmers have been involved in the pesticide spraying operations on their cacao farms for variable periods of 5 to over 20 years; but none has ever had their blood or domestic water source analyzed for pesticide residues. In this study, we report investigations carried out on cacao farmers in selected villages of Osun and Ondo States of Nigeria.

2. Materials and methods

2.1. Selection of study sites and subjects

The present study was carried out in 11 selected villages of Osun and Ondo States which are the major cacao producing areas in southwestern Nigeria. The villages were Ologiri, Onigbodogi, Egbejoda, Oniperegun, Omi-Nla (from Osun State) and Uloen, Bamkemo, Lipanu, Agopanu Igbatoro, Kajola and Olobi (from Ondo State). A field survey was initially carried out in which questionnaires and oral interviews were administered on 150 farmers. The survey was designed to elicit information on occupational history of the farmers including years of involvement in insecticide application, duration and frequency of application, types of insecticides used, factors associated with exposure and risk of pesticide poisoning. Based on the number of years of involvement in insecticide spraying, the farmers were subsequently categorized into exposure duration periods as <5 years, 5–9 years, 10–14 years, 15–19 years and over 20 years so as to facilitate the data analysis.

2.2. Collection of blood samples

Only 76 out of the 150 farmers referred to above gave oral consent and hence, participated in the trials. Their blood samples were collected with technical assistance from the Department of Haematology and Immunology, Obafemi Awolowo University, Ile-Ife, Nigeria. The skin of each subject was properly cleaned with a swab of methylated spirit so as to minimize sample contamination from possible insecticide residue adsorbed to the skin. Thereafter, using butterfly needles, blood samples were drawn from the veins in the inner forearms of each subject. Eight milliliters of blood was collected and dispensed into 10-ml labeled plain decontaminated bottles, preserved in a Jablo box with ice pack and transported back to the laboratory. In the laboratory, the serum was separated by centrifugation and frozen at −20 °C in decontaminated and labeled plastic vials.

2.3. Collection of water samples

Domestic water samples (from well and stream) were also collected from the various villages depending on the source(s) of drinking water available in each community. Five hundred milliliters of water from any of the sources was drawn into labeled plastic bottles and transported to the laboratory for the residue analysis.

2.4. Insecticide residues determination

The analyses for insecticide residues in the serum and water samples were performed using the method of Banerjee et al. (1999) with high performance liquid chromatography (HPLC) (Model Cecil 1100), UV detector with variable wavelength and stainless steel column (C_{18} Reverse Phase) packed with octadecylsilylsilica. The eluent was prepared by mixing HPLC grade methanol and water (1:1) and degassed before being poured into the column. The flow rate was set at 1 ml/min. The insecticide standards used were diazinon (98.5% purity), endosulfan (99% purity), lindane (99.5% purity), and propoxur (99% purity) and were all obtained from Chem Service, West Chester, UK. All other reagents were of analytical grade and they were obtained from Sigma Aldrich, UK.

The serum sample was extracted by the precipitation of the serum protein using 1% sodium tungstate and 1 N H_2SO_4. The precipitate was washed thrice with 0.1 N H_2SO_4 and the filtrate containing insecticide residue was extracted thrice with *n*-hexane, using a separating funnel, and passed through sodium sulphate column. The organic solvent (*n*-hexane) was evaporated using a water bath and the residue was dissolved in 1 ml methanol before being loaded and injected into the valve of the chromatography system. The insecticides in the water samples were extracted by shaking the sample with *n*-hexane thrice, using a separating funnel. The solvent layer was separated and concentrated. The organic solvent was then evaporated using a water bath and the residue was dissolved in 1 ml methanol before being loaded and injected into the valve of the chromatography system. The detection limit of each of the insecticide was calculated using derived blank concentrations and the corresponding area count of each of the internal standards following the procedures of Miller and Miller (2000). Thus, the detection limit for lindane = 0.0047 μg/ml, endosulfan = 0.009 μg/ml, diazinon = 0.0032 μg/ml and propoxur = 0.0028 μg/ml.

The data on the serum residue analysis were analyzed based on the categorisation of the farmers on their years of exposure duration. The data on residues in water were analyzed based on locations and source of the samples. Descriptive statistics (means, and percentages), was used to analyze the two data sets.

3. Results

The grouping of cacao farmers whose blood samples were taken for insecticide residue analysis is presented in Table 1. Slightly over half (52.6%) of the farmers had been involved in insecticide application for more than 20 years, while 13–18% had been similarly involved for variable periods ranging from 5 to 19 years. The table also shows the distribution of the farmers that had insecticide residues in their blood, on the basis of their years of involvement in insecticide application (i.e. duration of exposure). A relatively high percentage (47.6%) of the farmers was in the over 20 years exposure duration period; 23.8% and 21.4% were in the 10–14 years, and the 5–9 years exposure periods, respectively. Only 7.1% belonged in the 15–19 years exposure period.

Table 2 shows the distribution of the farmers and the mean concentration of insecticide residues in their blood serum based on

Table 1
Number and presumed exposure periods of cacao farmers in some selected villages of Southwestern Nigeria, that have insecticide residues detected in their blood serum

Duration of exposure (years)	No. farmers assessed	Proportion (%)	No. with residues	Proportion (%)
5–9	12	15.8	09	21.4
10–14	14	18.4	10	23.4
15–19	10	13.2	03	7.1
Over 20	40	52.6	20	47.6

Table 2
Number of selected cacao farmers in Southwestern Nigeria and mean concentration of insecticide residues in their blood based on their years of exposure

Duration of exposure (years)	No. with residue	Number with mean insecticide concentration (mg kg^{-1})			
		Lindane	Endosulfan	Diazinon	Propoxur
5–9	09	00 (0.0)	05 (0.040)	03 (0.089)	01 (0.073)
10–14	10	02 (0.066)	04 (0.011)	02 (0.051)	02 (0.131)
15–19	04	02 (0.080)	00 (0.0)	02 (0.029)	00 (0.0)
Over 20	21	03 (0.089)	03 (0.050)	08 (0.073)	07 (0.088)

() = Figures in parenthesis indicate mean concentration of each insecticide in (mg kg^{-1}).

Table 3
Types of residues detected and their concentrations in water sources taken from cacao farmers' localities in selected villages of Southwestern Nigeria

Location	Sources of water	Residues detected	Concentration (mg kg^{-1})
Egbejoda	Well A	Unknown	–
Egbejoda	Well B	Unknown	–
Egbejoda	Well C	None	–
Ologiri	Well D	Unknown	–
Ologiri	Well E	Unknown	–
Oniperegun	Stream F	Diazinon	0.023
Oniperegun	Well G	Unknown	–
Omi-Nla	Stream H	Unknown	–
Omi-Nla	Stream I	Unknown	–
Uloen	Stream J	Diazinon	0.091
Uloen	Well K	Unknown	–
Bamkemo	Well L	Unknown	–
Bamkemo	Well M	Unknown	–
Lipanu	Well N	Unknown	–
Lipanu	Well O	Diazinon	0.125
Lipanu	Well P	Unknown	–
Olobi	Stream Q	Unknown	–
Olobi	Stream R	Propoxur	0.029
Kajola	Stream S	Diazinon	0.094
Kajola	Well T	Unknown	–

their years of exposure. About 34% of the farmers had diazinon residue in their blood while about 29%, 23% and 17%, respectively, had residues of endosulfan, propoxur and lindane. All farmers in the 5–9 years exposure group had no residue of lindane in their blood. Also in the 15–19 exposure duration group, none of the farmers had endosulfan and propoxur residues detected in their blood. However, two farmers showed multiple contamination. Residues of 0.085 mg kg^{-1} lindane and 0.04 mg kg^{-1} diazinon were detected in one farmer in the 15–19 years exposure period group. A second farmer in the over 20 years exposure period group also had 0.139 mg kg^{-1} and 0.092 mg kg^{-1} of lindane and propoxur, respectively.

Table 3 shows the villages, the sources of water sampled, and the concentrations of the insecticide residues detected. Diazinon was detected in stream water at Oniperegun (Osun State), Uloen and Kajola (Ondo State). It was also detected in well water at Lipanu (Ondo State). Propoxur was detected in stream water at Olobi (Ondo State). The concentration of diazinon in the affected samples ranged from 0.023 mg kg^{-1} to 0.125 mg kg^{-1}.

4. Discussion

The residue analysis revealed that 42 out of the 76 farmers involved in this study had residues of diazinon, endosulfan, propoxur and lindane in their blood. The aforementioned insecticides are the chemicals used by the farmers for mirid control on their cacao farms. This is indicative of occupational exposure.

Endosulfan, lindane, diazinon and propoxur are all WHO Category II insecticides (i.e. moderately hazardous with acute oral LD $\geqslant 50 \leqslant 500$). They are all capable of causing acute as well as chronic intoxication; and endosulfan is particularly known to be easily absorbed by the stomach, the lungs, and the skin. Proper protective clothing (safety goggles, gloves, masks, overalls with long sleeves, rubber boots etc.) need to be worn when applying these insecticides in order to prevent contamination and consequent poisoning. However, many of the cacao farmers, unfortunately, did not see the use of protective clothing as a necessity. They also manifested habits such as smoking or drinking water during insecticide application without recourse to proper hygiene, which readily predispose them to exposure contamination through oral route. None of the farmers, whose blood samples were analyzed for residues, had been involved in insecticide application for less than 5 years; but over half had been involved for upwards of 15 years. Also, 47.6% of those that had residues in their blood, had been involved in insecticide application for over 20 years while 23.8% and 21.4% had been involved in insecticide application for 10–14 years and 5–9 years, respectively. Thus, there is a trend in insecticide accumulation in the blood of the farmers that seems dependent on the years of exposure.

Relating the symptoms experienced by the farmers as detected during the earlier field survey, with the residues found in their blood, it was discovered that most farmers that complained of severe headache, general body weakness or tiredness, restlessness, nausea and diarrhoea, had insecticide residues in their blood. All these symptoms are characteristic of lindane and endosulfan poisoning (WHO, 1992; Pesticide News, 2000a).

Comparing the residue levels with the no observable adverse effect level (NOAEL) for the insecticides, it was found that residues of lindane, endosulfan and propoxur in all the exposure duration categories were far below the NOAEL. However, diazinon residues detected in the blood of all the categories of farmers exceeded the NOAEL of 0.02 mg kg^{-1}. Acute intoxication of insecticides is generally what is of immediate concern but there are also equally worrisome long-term effects that could result from their chronic accumulation. The breakdown product of diazinon in animals known as diazoxon is a strong cholinesterase enzyme inhibitor (Pesticide News, 2000a). Hence the presence of diazinon residue at levels equal or beyond NOAEL calls for concern and steps need to be taken to prevent further residue accumulation in the body of the farmers. The residue levels of endosulfan and lindane detected in the blood of the farmers were below the NOAEL. Notwithstanding, necessary steps would need to be taken to prevent further accumulation of the insecticides in their bodies. Long-term exposure to low doses of endosulfan has been shown from animal studies to affect the kidneys, liver, and developing foetus. It has been implicated in a decrease of semen quality, as well as increase in testicular and prostate cancer (Pesticide News, 2000b). It also has endocrine disrupting potential and does induce oxidative stress leading to inhibition of cellular respiration (Ho-Yong et al., 2004). Similarly, lindane is a known endocrine disruptor (Pesticide Trust, 1995).

The distribution of the farmers on the basis of the percentage with particular insecticide residues in their blood, reveals that diazinon, endosulfan and propoxur were the major insecticide residues found in the blood of most of the farmers with only a few having lindane residue. This is a welcome development, which is an indication of a gradual phasing out of lindane from the cacao pesticide market in Nigeria. It is recalled that mirid resistance to lindane was reported in the study area as far back as 1965 (Booker and Gerard, 1967) and hence, propoxur, dioxacarb and diazinon were recommended as substitutes. Besides, being an endocrine disruptor and a persistent organic pollutant, lindane had been banned in several countries of the Western world (Maroni et al., 2000).

The diazinon and propoxur residues detected in the water samples taken from farmers' sources of drinking water, indicate that these insecticides must have found their way after application through underground seepage into the water, thereby contaminating it. The cacao farmers' household and their entire community also utilize water from these same sources for drinking. Hence, apart from the cacao farmers, the risk of poisoning due to contamination extends to the entire community. The residue levels are above the acceptable daily intake (ADI) of 0.002 mg kg^{-1}, being markedly so at Uloen, Lipanu and Kajola (Ondo State). This portends a high risk of poisoning for the communities and hence, a necessity for appropriate actions to prevent further contamination of their drinking water sources.

In conclusion, our study has shown that the cacao farmers have been occupationally exposed due to their use of insecticides for mirid control in their plantations. In view of the types of insecticides commonly used and the residues detected in their blood serum and domestic water sources, there is a need to revitalize the pesticide regulation in Nigeria. The Regulatory Agency needs to become more alive to its responsibilities in enforcement and the prescription of standard safety measurements such as acceptable daily intake (ADI) and no observable adverse effect level (NOAEL) for various pesticides being used in the country.

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