Journal of Applied Sciences

ISSN 1812-5654

Journal of Applied Sciences 10 (11): 975-980, 2010
ISSN 1812-5654

Pesticides Residues in Smoked Fish Samples from North-Eastern Nigeria

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Abstract: Smoked fish species, *Clarias* sp., *Gymnarchus niloticus* and *Tilapia* sp., sampled from the open markets in North-Eastern Nigeria were investigated for the presence and concentration levels of pesticide residues of DDT, dichlorvos and lindane. The GC-MS and GC-FID techniques were employed in the determination of the pesticide residue. The obtained results showed positive identification of op-DDT (2.844-4.220 μg g^{-1}), pp-DDT (3.821-4.479 μg g^{-1}), dichlorvos (2.844-4.220 μg g^{-1}) and lindane (3.479-9.878 μg g^{-1}). *Gymnarchus niloticus* showed consistently higher pesticide residue levels in the studied smoked fish samples followed by *Clarias* sp. and then *Tilapia* sp.

Key words: Dichlorvos, organochlorine pesticides, smoked fish, health hazard

INTRODUCTION

Smoking fish is an age long tradition of preserving fish from further deterioration after being caught and dried, especially for commercial purposes. In North-Eastern Nigeria immense commercial agriculture exists, particularly around the Lake Chad area, because apart from food crops farming, the production of smoked fish is prominent in this area. These extensively interlaced agricultural activities expose food produce to pesticides.

Generally, fish is a perishable commodity and there has been large scale deterioration and losses in the quality of processed fish due to the combined effects of insect infestation and other biological agents that flourish under the tropics hot and humid conditions (Osuji, 1974; Mohammed and Yusuf, 2001). Control measures against insect infestation of dried and smoked fish include the injudicious use of harmful chemical insecticides such as dichlorvos, DDT and heptachlor to keep away insects and other pests (Eyo and Mdaihli, 1997; Mohammed and Yusuf, 2001; Bhuiyan *et al*., 2008). These pesticides have induced the development of pests resistance (Mohammed and Yusuf, 2001), leading to the applications of higher pesticides doses (UNEP, 2002).

Numerous studies on both human and laboratory animals provide strong evidence of the toxic potential of exposure to pesticides residues (Gladen and Rogan, 1995; Longnecker *et al*., 2001; Torres-Areola *et al*., 2003). Therefore risk characterization of pesticides in environmental samples, foods and dietary products is an important step and a vital tool in the assessment of food safety risk (Renwick, 2002; Duffus and Worth, 2006; Granby *et al*., 2008). The toxic effects of pesticides to man and the environment is a major issue that gives rise to concerns at local, national, regional and global scales and is the basis for the control, monitoring and prohibition of pesticides in food (UNEP, 2002). A number of pesticides residues monitoring researches have been conducted on various samples, such as eggplant (Islam *et al*., 2009), drinking water from household wells (Sabdono *et al*., 2008) and in human breast milk (Ebadi and Shokrzadeh, 2006).

The objective of this study was to determine the presence and concentration levels of DDT, dichlorvos and lindane in some species of smoked fishes from North-Eastern Nigeria. This will provide supportive monitoring information and food safety status of the smoked fishes studied.

MATERIALS AND METHODS

Samples and sampling: Three different species of fish, Gymnarchus (*Gymnarchus niloticus)*, Catfish (*Clarias* sp.) and Tilapia (*Tilapia* sp.) were analysed in this study (Fig. 1a-c). About 1-2 kg sample sizes of each species were randomly weekly purchased from the fish market for 10 weeks (August to December 2008). The fish samples were collected into plain polyethylene freezer bags, frozen within 24 h and stored at -20°C until analyzed.

Preparation and analysis: Sample preparation and analysis were carried out according to standard procedures described by Clifton (1996) and Hopper (1982).

Sample homogenization and extraction: Frozen samples were thawed and individually rinsed with purified water to

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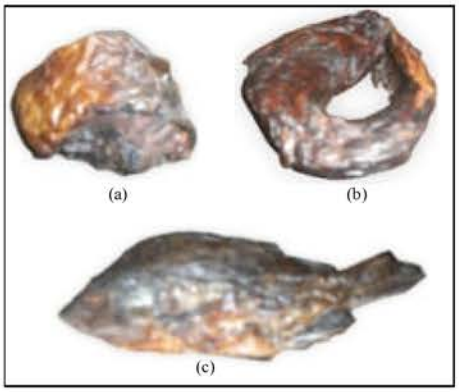

Fig. 1: Smoked fish samples from North-Eastern Nigeria. (a) Gymnarchus, (b) Catfish and (c) Tialpia

remove possible impurities. Edible portions from approximately 20 to 30 specimens of each collected species weekly were pooled and homogenized using Ultra Turrax® T25 (IKA, Canada). About 25 g of the homogenized sample was weighed into a round-bottomed flask and extracted with 150 mL of light petroleum spirit (BDH, England) for 1 min. The extract was dried by passing it through a thin layer of anhydrous Na_2SO_4 in a filter paper. The filter paper was washed with 50 mL of light petroleum spirit and then concentrated in a rotary evaporator to dryness and the weight of the fat recorded.

Extract cleanup: The extract clean-up was achieved by Gel Permeation Chromatography (GPC) procedure developed by Hopper (1982) for organophosphates and chlorinated pesticides. The GPC consists of 6.0 g of SX-3 Bio-Beads (Bio-Rad, UK) in 150 mL of a mixture of ethyl acetate/cyclohexane mixture (1:1) for 5-6 h at a regulated flow rate of between 1.5-1.8 mL min^{-1} and nitrogen carrier gas. The cleaned sample extract was then evaporated to dryness under a gentle stream of nitrogen, made up to 1 mL with hexane and analyzed by gas chromatography.

Instrumentation: Gas Chromatography-Mass Spectrometry (GC/MS) was used for the determination of DDT and lindane using HP 5972 GC/MS (Agilent, USA), while flame ionization detector (GC/FID) was used for dichlorvos determinations. The chromatographic column was fitted with an ultra-low bleed fused silica, 30 m×0.25 mm id ×0.25 μm film thickness and carrier gas was helium.

The external standard method of analysis was used. The sample extract was made up to 1 mL with hexane and analyzed on the GC/MS/FID. The instrument was operated in the selective ion monitoring mode and the computer which controlled the system had an EI-MS library of standards for the target pesticides under the experimental conditions used. In addition, there was retention time match of analyte peak to standard peak for further confirmation of analyte identity. Data acquisition and processing was integrated with Merck D2000 GC workstation.

System performance check was conducted by standard solution containing a mixture of 500 ng mL^{-1} each, of dichlorvos, pp-DDT, op-DDT, lindane and, 500 mg mL^{-1} plant oil was prepared in the elution solution. The recoveries for the individual pesticides were determined from the analysis of fish blank (oil fraction) matrix spiked at 2 levels with analytes; 0.3 and 1.5 mg L^{-1}. the run-to-run (intra-assay) precision was established by replicate injections of fish blank samples spiked at both levels. The day-to-day (inter assay) precision was determined by the analysis of 6 replicates each day on 3 different days. Mixed pesticides standard solutions at 4 concentration levels (0.125, 0250, 0500 and 1.000 μg mL^{-1}) were analyzed to obtain standard curves for all the analytes of interest using external standard method. The instrument detection and quantitation limits were based on a signal-to-noise ratio of 3:1 and 10:1, respectively.

Statistical analysis: The results of analyses are presented as Mean±SD and statistically analysed for significance in multiple variations by Analysis of Variance (ANOVA) with Scheffe's post hoc test, using coupled Microsoft Excel+Analyse-it v. 2.10 software. Variations were considered significant at $p<0.05$.

RESULTS

Figure 2-4 show Box-Whisker plots, indicating concentration spread and mean values of pesticide residues in Gymnarchus, Catfish and Tilapia, respectively. Gymnarchus (Fig. 2) generally, showed consistently higher residue levels of all the pesticides determined in the smoked fish samples. The results on the plots showed that with the exception of Catfish (Fig. 3), which showed no presence of lindane and pp-DDT, the two other smoked fish species revealed the presence of pesticides residues determined. Tilapia (Fig. 4) shows the widest concentration spread for all pesticides residues determined. However, on the basis of pesticides, residues of dichlorvos recorded the highest mean concentration in Gymnarchus (4.220±0.599 μg g^{-1}) followed by catfish (3.323±0.395 μg g^{-1}) and then Tilapia (2.844±0.685 μg g^{-1}). Variations in residue concentration was statistically ($p<0.05$) significant for *Gymnacus* sp. and Tilapia only. For lindane residues recorded between levels found in Gymnarchus (9.878±1.961 μg g^{-1}) and Tilapia

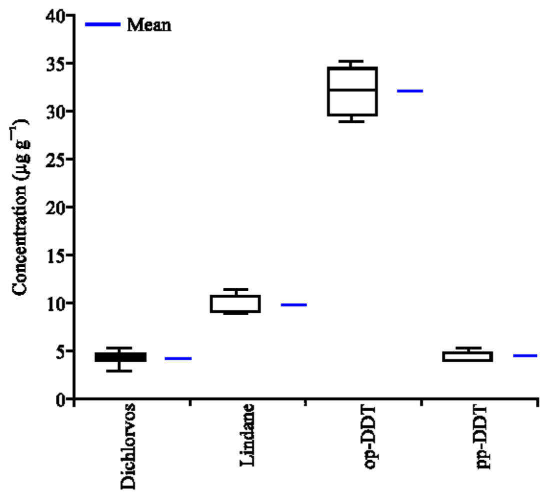

Fig. 2: Box-Wishker plot of pesticides residue in smoked Gymnarchus

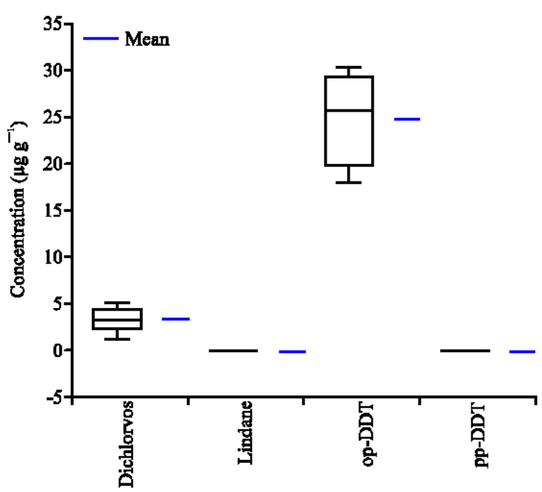

Fig. 3: Box-Wishker plot of pesticides residue in smoked Catfish

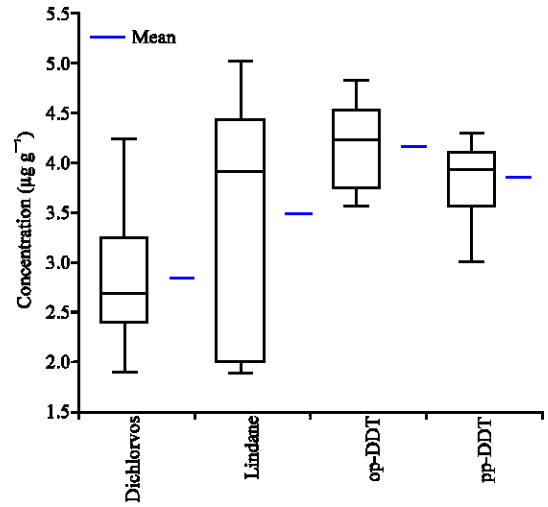

Fig. 4: Box-Wishker plot of pesticides residue in smoked Tilapia

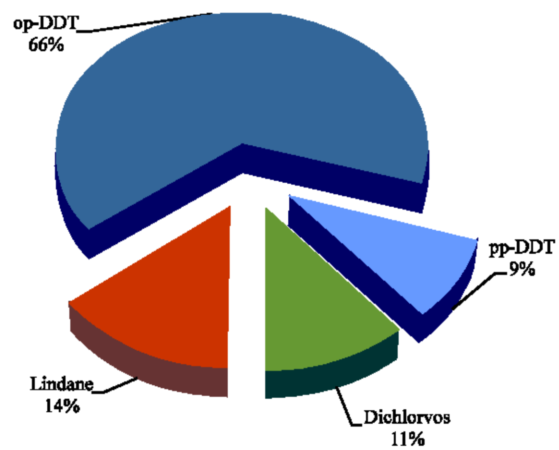

Fig. 5: Average concentration profile of pesticide residue in a smoked fish sample

3.479±0.177 µg g^{-1}) there was a markedly significant difference ($p<0.05$; t-t-test). For op-DDT residue levels, the highest mean concentration was recorded in Gymnarchus (4.220±0.599 µg g^{-1}), followed by in catfish (3.323±0.395 µg g^{-1}) and then in Tilapia (2.844±0.68 µg g^{-1}). A similar trend as observed for lindane residue levels in the fish samples was also observed for pp-DDT residues. However, the variation in pp-DDT residue levels between Gymnarchus (4.479±0.391 µg g^{-1}) and Tilapia (3.821±0.381 µg g^{-1}) was not significant.

Figure 5 shows an average concentration profile for the pesticides residues on a percentage basis, for a smoked fish sample. It simply indicates that in an average smoked fish sample obtained from this region at the time of this study is likely to more DDT than the other pesticides residue.

The QC sample runs for all analytes were within±14% deviation and, percentage recoveries were in the range of 90-105%. The uncertainty of measurement is within the acceptance criteria based on a confidence level of 95%.

DISCUSSION

In general, fish accounts for only a small proportion of human diet but it presents a major route of human exposure to organic contaminants (Yang *et al*., 2006). Accumulation of organochlorines in fish body is indirectly the result of rainfall flushing the organochlorines from cultivated lands, industrial wastes or used directly into water bodies for disease vector control (Anyakora *et al*., 2008). Rivers, lakes and lagoons generally reflect the extent of pollution in the areas they drain and the fish is a representative indicator of pollution of the aquatic ecosystem. In this study, the residual concentrations of the organochlorines (DDT and lindane) and the chlorinated organophosphate, dichlorvos in the three fish species studied shows that DDT was the predominant contaminant in the fish samples analyzed. These suggest that bioaccumulation of DDT and lindane in fish was highly specific probably due to different ecological characteristics for different aquatic species such as feeding habits and habitats (Yang *et al*., 2006). In matrices with high fat content the levels of organochlorine pesticides are dependent on the age of the animals (Glynn, 2000) and the fat content of the product (Frenich, 2006). In this study, the nature of the sample did not allow for age verification of the fish species sampled but the residues of the organochlorines determined correlated positively with the fat content of the species (Frenich, 2006). The Tilapia samples were scaly and had little fat and Gymnarchus were fleshy and very oily.

In Nigeria, the usages of lindane were much more than those of DDT in the study area (Osibanjo, 1994; Anyakora *et al*., 2008). The discrepancy between the usages of lindane and DDT and their accumulative levels in the fish species found in this study might be due to the differences in physicochemical and biochemical properties between lindane and DDT, wherein lindane has higher biodegradability and lower lipophilicity compared to DDT (Guo *et al*., 2007). Dichlorvos was detected in all the three species studied, but at relatively much lower residual levels than those of DDT and lindane. This was in a pattern that reflected an order of magnitude that closely resembled the fat contents of the species studied that is the highest residual level of dichlorvos was found in the species having much more fat content. This observation is consistent with the ability of lipophilic pesticides to accumulate in matrices with high fat content (Naccari, 2004). Thus, the topical application of Ota-piapia, whose predominant content is dichlorvos (Musa *et al*., 2010) for the preservation of smoked fish might be responsible for the small variability observed in the mean concentrations of dichlorvos in the different species since the organophosphate pesticides were not known to be of significance as environmental pollutants (Manahan, 2005).

Consequently, the pesticide residues levels obtained in this study were to a large extent greater than all the three assessment standards for food safety risk due to the dietary exposure of populations to pesticides; the most stringent Chinese standard (10 ng g^{-1}) (Chen *et al*., 2002) on marine biological quality, the food consumption standard (14.4 ng g^{-1}) recommended by USEPA (2000) and the maximum admissible concentration (50 ng g^{-1}) recommended by the European Union on the basis of lipid percentage of food (Binelli and Provini, 2003). Thus, the perceptible health risks associated with the consumption of analyzed smoked fish species cannot be neglected.

In Nigeria several studies have reported the presence of pesticide residues such as DDT, DDE and lindane in fish (Osibanjo and Bamgbose, 1990; Unyimandu and Udochu, 2002), but these results gave no indication of health risks associated with the consumption of these fishes from the Nigerian markets. Nonetheless numerous epidemiological evidence have established that exposure to organochlorines is associated with a wide array of adverse effects on human health including lifetime cancer risk especially to residents of coastal regions who often consume more seafood products than those living inland (Toft *et al*., 2004; Rogan and Chen, 2005). Pesticide residue levels could be decreased by washing but these insecticides were never completely eliminated by washing (Bhuiyan *et al*., 2008). Traditionally, fish is vigorously washed with hot water before cooking but such practice does not completely remove the pesticides from the tissues and dietary intake could still be significant; organochlorine pesticides are lipid soluble and can only be drained out with lost fats and oils. In addition to this, the most pertinent concern however, is that these smoked fishes are sometimes eaten without having to cook them, because they have been smoked and are edible. There are no daily allowable tolerances for dichlorvos in fish. Accordingly, risk assessment should be carried out to ensure intakes are below the Acute Reference Dose (ARfD). Therefore, the presence of dichlorvos, high residual DDT and lindane concentrations in fish from the Lake Chad region deserve continuous monitoring efforts to ensure long-term safety of consumers. Consequently, the values recorded in this study would be useful for comparative purposes since the effects of a regular intake of pesticide residues in food are hard to detect and quantify.

CONCLUSION

This study revealed that pesticide residue levels in smoked fish were above permitted tolerances and dietary intake could be an important process of transferring residues to humans. It also indicated the extensive presence of these pesticides ensuing from widespread usages, which include recent use of pesticides for pest control, fishing and in fish preservation. Thus, illegal use of these pesticides to preserve fish by traders with little or no knowledge of public health policy must be checked through adequate control of the trade and use of pesticides and the enforcement of appropriate sanctions.

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