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Studies of Pesticide Residues in Smoked Catfish (Clarias Gariepinus) in Nigeria: Some Health Implications

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Abstract: Heavy post harvest losses are incurred by fish processors and marketers of cured fish products in Nigeria. Consequently, several means of cutting down these losses have been devised. Prominent among the methods is the use of pesticides as "preservatives". Pesticides used in this study were 2,2-dichlorovinyldimethylphosphate (DDVP1000 EC), benzene hexachloride (lindane/gammalin 20^R) and "otapiapia", a locally mixed pesticide. Various concentrations (2%, 5%, 10% and 20%) of each pesticide were applied to the fish samples. The residues contained in the fish samples were analyzed using Gas Chromatography–Mass Spectrometry (GC-MS). The identities of the pesticides and the fish samples were revealed by mass spectra data. It was observed that the active recoverable ingredients of DDVP100 EC were organometallic compounds, which include tungsten, phosphorus, silicon and azafrin. That of gammalin 20^R was lindane, and for "otapiapia", it was pyrazine and dioctyldiphenylamine. The results showed that even after four weeks of exposure to atmospheric air, that these pesticides were still present in the fish. The study concluded that these pesticides should not be used at all in the preservation of smoke- dried fish. The approved pesticides by the WHO, which are actellic dust (pirimiphos-methyl) and synergized pyrethrins are hereby further recommended by the authors.

Key words: Pesticide residues, Smoked fish, Claries gariepinus, Health implication.

INTRODUCTION

Fish and fishery products account for the major animal protein intake in human diets and livestock feeds. Fish protein has a high biological value because it is composed of a very high profile of essential amino acids, notably lysine, methionine and isoleucine, which are relatively deficient in animal protein (Olomu, 1995). Fish is however susceptible to damage as soon it is harvested. Some factors responsible for this include the prevailing high temperatures in Nigeria and the facilities for processing, storing and distributing the fish caught that are frequently inadequate or non existent in most cases. There is therefore enormous waste through spoilage of both fresh fish and dried fish (UNIFEM 1988; FAO 1986; and Rawson 1976). Preservation of fish generally slows down spoilage. Preservation methods are applied with an intention to making the food safer and extend its shelf-life (Ghazala, 1994).

Despite these preservation methods, processed fish products are still susceptible to biodeterioration due to microbial attack and insect infestation during storage or distribution. The most important insect pests of dried fish are beetles of the family Dermestidae (Igene, Abolagba and Ikeme 1998; Abolagba, Okonji and Enobakhare 1996). They will invade fish from the earliest stages of drying but unlike flies will continue to be attracted to, and breed in the dried product (Bostock *et al* 1987).

The insecticides to be used for direct application to dried fish must be sufficiently toxic to the targeted insect pests whilst not leaving residues harmful to the consumer (FAO 1981). Only few insecticides are able to control the major insect pests of dried fish at application rates low enough to ensure low residues. The recommended insecticide treatment is to dip in a solution of 0.125% pyrethrins, 0.25% pi-peronylbutoxide (Morris and Andrews, 1968). The work of Green (1967) showed that treatment by dipping and soaking in 0.0625% malathion solution left high residues of up to 27mg/Kg even after 4 weeks storage for all marine species of fish tested, except shark on which insecticide breakdown was rapid.

Proctor (1972) also reported residues of up to 28ppm for tilapia species diped in a 0.0625% malathion solution and up to 43ppm using a 0.125% solution, both analysis being undertaken after a period of 8 weeks storage.

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In northern Nigeria, smoked dried fish of the species Tilapia, Heterotis, Gymnarchus and Clarias were usually dipped into aqueous solutions containing 0.0125% chloripyrifos methyl, pirimiphos methyl or permethrin and good control of Dermestes maculatus was achieved and adequate protection against this pest was conferred by the three insecticides (Guggenhein 1980). Igene, Abolagba, and Ikeme (1998) confirmed that lindane a (a concentrated gamma BHC formulation) is used to protect fish against insect pests in storage and during transportation and that fish merchants sprinkle handful of BHC and "otapiapia", a locally formulated pesticide onto fish cartons and for protection against insect pests. The claim of the users of these obnoxious methods was that the effect goes off after sometime or after washing with tap water. It is based on this claim that the research was embarked upon.

1.1 Objectives of the Study:

(i) The specific objective of this study was to determine if residues of lindane, DDVP 1000EC and otapiapia were recoverable after 4 weeks of exposure.
(ii) To determine the effect of some recovered pesticides on some vital organs of rabbits fed graded levels.
(iii) To make recommendations on the health hazards in consuming fish preserved with pesticides based on the outcome of the study.

MATERIALS AND METHODS

2.1 Pesticides and Reagents:

The pesticides and reagents used for the study were obtained from the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Pesticides:

1. DDVP 1000 EC with a chemical formular 2, 2, dichlorovinyl Dimethyl- Phosphate as emulsifiable concentrate (EC);
2. Lindane, which is a gamma isomer of 1, 3, 4, 5, 6 – hexachloro cyclohexane; also known as Benzene hexachloride (BHC). The lindane used was the emulsifiable concentrate (EC) form with "gammalin 20^{R}" as its trade name; and
3. "Otapiapa," a locally mixed pesticide whose composition was not known.

Reagents:

The following reagents were used:

1. Dichloromethane
2. Methanol
3. Anhydrous sodium sulphate

2.2 Fish used:

The fresh fish processed was purchased from Modeh-Noma farms, Sapele Road, Benin City, Nigeria. The fish were adults of *Clarias gariepinus* of between 350-550g in weight belonging to the sub-order Siluridae and the family Clariidae. It is a fatty fish with a fat content ranging from 8-20% (Olomu, 1995).

2.3 Processing and Smoking of the Fresh Fish:

After killing of the fish according to standard procedure, they were gutted whole and then thoroughly washed with clean tap water. Thereafter, a modified Altona kiln was used to smoke the fish for 6 hours. The smoking process was repeated on the second day to ensure proper drying of the fish samples.

2.4 Blending Procedure:

The head of the smoke-dried fish was discarded and the body was chopped into a blender. It was blended for 10minutes at medium speed. After grinding for 1 minute, the sides were scrapped to break up caked material with a spoon. Thereafter blending was continued until very smooth powdered material was obtained.

2.5 Application of Pesticides to the Fish:

2g of the ground portion of fish was weighted into a 100ml beaker each. The pesticides were applied at four (4) different concentrations to the blended fish samples as follows:

0. 2ml or 2%	
0.5ml or 5%	"Otapiapia"
1 ml or 10%	
2 ml or 20%	
0. 2ml or 2%	
0.5ml or 5%	Lindane
1 ml or 10%	
2 ml or 20%	
0.5ml or 2.5%	
1 ml or 5%	DDVP 1000EC
2 ml or 10%	
4ml or 20%	

The reason for the increase in the quantity of DDVP 1000 EC was that the concentration of lindane and "otapiapia" is twice that of DDVP 1000EC

2.6 Extraction Procedure:

The extractions were carried out according to the procedure described by Miller (1975) and Health and Welfare, Canada (1986).

25ml of Methanol and water of ratio 1:1 solution was added to each sample in the beaker. The content in the beaker was gradually stirred for 30minutes. After stirring, the solution was filtered into a conical flask and the filtrate was used to estimate the pesticide residue in each fish sample. 25ml of Dichloromethane (CH_2Cl_2) was added to the filtrate and then stirred. The stirring helped to partition the filtrate into aqueous and non-aqueous residues (Miller 1995). The non-aqueous phase was pipetted into a beaker. Another 10ml of CH_2Cl_2 was added to the filtrate to make sure that no trace element of the pesticides was left in the aqueous residues.

The solution was then filtered and dried using anhydrous sodium sulphate which helped to trap the water present in the filtrate and also to disintegrate the sample (FDA, 1982).

The crystal clear filtrate was left overnight to allow it evaporate to dryness; leaving the pesticides to settle at the bottom of the beaker. This was later reconstituted with CH_2Cl_2 and pipetted into sample vials for GC/MS analysis after labeling appropriately as follows:

OT = "Otapiapia"
GM = Gammalin 20R
DV = DDVP 1000EC

Table 1: Coding of Extracts of smoked fish samples exposed to various concentrations of pesticides for different duration.

Duration:	24hrs	2wks	4wks	Concentrations (%)
	OT 1a	OT1b	OT1c	2
	OT2A	OT2B	OT3C	5
	OT3A	OT3B	OT3C	10
	OT4A	OT4B	OT4C	20
	GM1A	GM1B	GM1C	2
	GM2A	GM2B	GM2C	5
	GM3A	GM3B	GM3C	10
	GM4A	GM4B	GM4C	20
	DV1A	DV1B	DV1C	2.5
	DV2A	DV2B	DV2C	5
	DV3A	DV3B	DV3C	10
	DV4A	DV4B	DV4C	20

2.7 Gas Chromatographic-Mass Spectra Analysis:

The fish extracts were injected at a column temperature of 140^0C. After a period of 10minutes at 140^0C, the oven temperature was increased linearly at 10^0C per minute up to 300^0C, and maintained at this temperature for 20 minutes and which was found optimal for our analyses. The results of analyses reported in this study are only qualitative.

3.0 Results:

Tables 2-4 show the results of the active components in each pesticide recovered from fish samples.

It is evident that pyrazine and dioctydiphenyl amine, which are the active components of 'otapiapia' were recovered even after the fish was exposed to air for 4 weeks as indicated in Table 2. Also, lindane was recovered from the fish samples treated with gammalin 20^R and exposed to air for 4 weeks as seen in table 2.

The result in table 3 indicated that the presence of organometallic compound and azafrin from fish samples treated with DDVP1000EC. It is therefore clear that residues of the pesticides used in treating the fish could be recovered after exposing the fish to air and storing for 4 weeks.

Table 2: Active Components of Otapiapia Recovered from Fish Samples

Concentration	Exposure Period			
	24 hours	2 weeks	4 weeks	Control
2%	Pyrazine + Dioctyldiphenylamine	Pyrazine + Dioctyldiphenylamine	Pyrazine + Dioctyldiphenylamine	Nil
5%	Pyrazine	Nil	Nil	Nil
10%	Dioctyldiphenulamine	Dioctyldiphenylamine	Pyrazine + Dioctyldiphenylamine	Nil
20%	Dioctyltiphenylamine	Dioctyldiphenylamine	Pyrazine + Dioctyldiphenylamine	Nil

Table 3: Active Components of Gammalin 20^R Recovered from Fish Samples

Concentration	Exposure Period			
	24 hours	2 weeks	4 weeks	Control
2%	Lindane	Lindane	Pyrazine + Lindane	Nil
5%	Lindane	Lindane	Lindane	Nil
10%	Lindane	Lindane	Lindane	Nil
20%	Lindane	Lindane	Lindane	Nil

Table 4: Active Components of DDVP1000EC Recovered from Fish Samples

Concentration	Exposure Period			
	24 hours	2 weeks	4 weeks	Control
2.5%	Organometallic compound	Organometallic compound	Organometallic compound + Azafrin	Nil
5%	Organometallic compound	Organometallic compound	Organometallic compound + Azafrin	Nil
10%	Organometallic compound	Organometallic compound	Organometallic compound + Azafrin	Nil
20%	Organometallic compound	Organometallic compound	Organometallic compound + Azafrin	Nil

N.B: Organometallic compounds: tungsten, Phosphorus and Silicon

Figures 1-3 show the structures of the active ingredient recovered from fish exposed to Otapiapia, Gammalin 20^R and DDVP1000EC. The active components of the pesticides found most often were lindane, azafrin, pyrazine, dioctyldiphenylamine and the organometallic compounds, which include tungsten, phosphorus and silicon. The major components of the fish are esters, which are usually 6,7,8 and chain carbon atoms either saturated or unsaturated or as methyl ester namely n-Hexadecanoic acid. 6,9,12, 15 docosatetraenoic acid methyl ester; 5, 8, 11, 14 - eicosotetraenoic acid ethyl ester; and 9 – octadecanoic acid methyl esters. These esters were present in all spectra. A library-based GC – MS data search revealed lindane with molecular ion peak (M^+) at 288 (molecular weight 288) and various fragmentation peaks. "otapiapia" with molecular ion peak ($M^+ + 1$) at 393 (molecular weight 393) and organometallic compound with molecular ion peak (M^+) at 612 (molecular weight 612) and various fragmentation peaks.

It is important to state that traces of pesticides were still recovered by the gas chromatographic and mass spectroscopy techniques applied for all the concentrations. The main component of DDVP, which is organometallic compound, contains tungsten, phosphorous and silicon that are known to be very reactive and very dangerous to health when consumed, as it might possibly cause carcinogenic effects (Hathway *et al* 1991; Gold 1984).

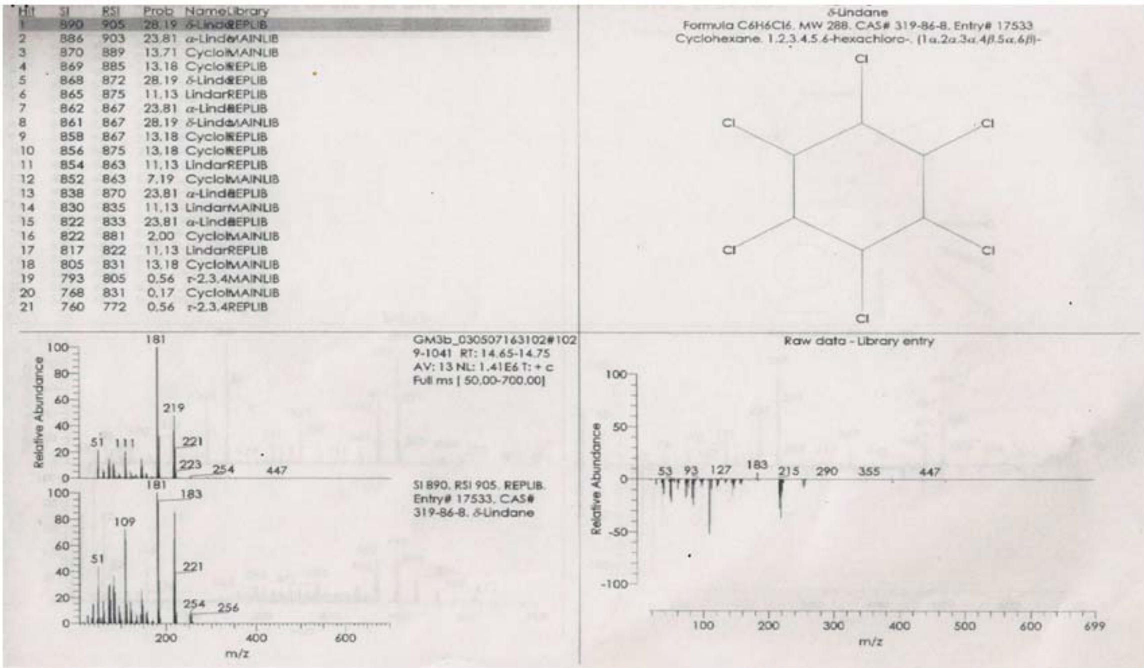

Fig. 1: GC-MS of Fish Sample Exposed to Gammalin 20^R for 2 weeks.

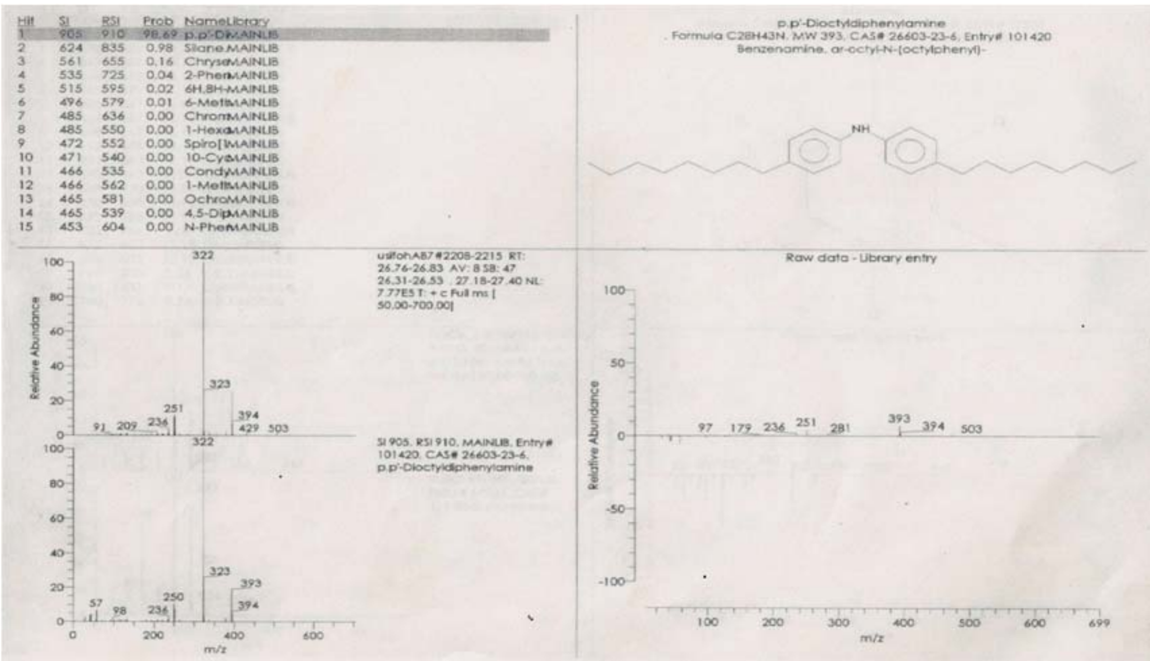

Fig. 2: GC-MS of Fish Sample Exposed to "Otapiapia for 24 Hrs.

Ecobichon and Joy (1982) observed that when organophosphorous compounds inactivate acetylcholinesterase at muscarinic receptors for acetylcholine, the chemical symptoms observed include cold sweating, salivation, nausea, bronchoconstriction and tightness of chest with a decrease in blood pressure. Levin and Rodnitzky (1976) quoted examples of cases where manifestation of anxiety and irritability have persisted for months after acute exposure to organophosphorous compounds. Injury to the liver is the most serious toxicity of pyrazine. Elevations of the plasma alanine and aspartate amino-tranferases (enzymes in the liver) are the earliest abnormalities produced by this compound (Dillon *et al* 1994).

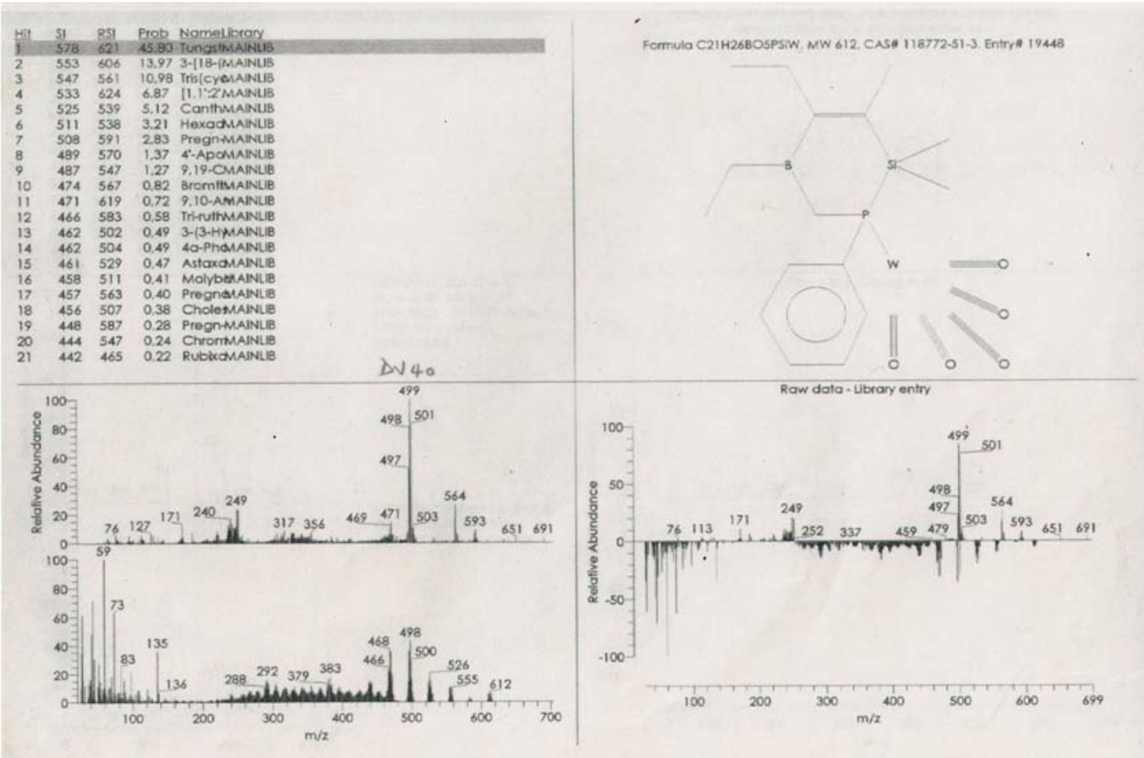

Fig. 3: GC-MS of Fish Sample Exposed to DDVP (20%).

Lindane has been found to adversely affect reproductive function in animals (MSDS 2001). It may be harmful if absorbed through the skin. It may cause irritation of nose, throat or skin if ingested in addition to the change of liver and kidney (Sang, Petrovic and Cuddeford 1999). Acute exposure may cause dizziness, headaches, vomiting, diarrhea, tremors, weakness, convulsions, dyspnea, and circulatory collapse. Furthermore, pulmonary oaedema, dilation to the heart, extensive necrosis to the blood vessels in the lungs. Liver and kidney have been reported for mammals (Sang et al 1999). We confirmed some of these observations with animal studies using rabbits.

From the foregoing, it is evident that the use of any of the pesticides in the preservation of fish could be harmful to public health.

Conclusion:

This study confirmed that residues of gammalin 20 (lindane), Otapiapia, (pyrazine, dioctyldiphenylamine), and DDVP1000EC (Organometallic compounds) were still recovered from fish samples after exposure to air for 4 weeks.

The claim by the fish processors that washing the fish with water and exposing it to sunlight for few days makes them safe from the Pesticides could therefore not be correct.

The consumption figures of 3.25kg/week on the average from all the zones in Nigeria for smoked fish indicates that there may be high levels of ingestion of pesticides with the cumulative effects on the long run. The study therefore concluded that the health implications for those that consume pesticide-laden fish is grave as the animal studies showed negative impacts on the vital organs such as the liver, kidney and stomach. The authors further advised that the pesticides in question should not be used at all in the preservation of smoke-dried fish.

The approved pesticides by the WHO, which are actellic dust (pirimiphos methyl) and synergized pyrethrins are further supported for use by the authors.

REFERENCES

Abolagba, O.J., V.A. Okonji and D.A. Enobakhare, 1996. Salting of Fish as a Means of Reducing Damage by *Dermestes maculatus* (coleopteran). Nig. J. of Entomology, 13: 81-86.

Bostock, T.M., D.J. Walker and C.O. Wood, 1987. Reduction of Losses in Cured Fish in the tropics - Guide for Extension Workers. Report 0 theTropical Development and research Institute G., 204: 47.

Dillon, R.O., T.R. Patel and A.M. Martin, 1994. Microbiological Control for Fish Smoking. In: Fisheries Processing Biotechnological Applications. ED A. M. Martin Chapman and Hall London, pp: 51-80.

Ecobichon, D.J. and R.M. Joy, 1982. Pesticides and Neurological Disease. CRC Press Boca Raton, Florida, pp: 250.

Eyabi-Eyabi, G.O., 1998. Technologies for Fish Preservation and Processing in Cameroon. FAO Fisheries report, 574: 88.

FAO, 1986. Pesticide residues in Food -1985: Evaluations Part 1. Residues. FAO Plant Production Paper 72/1.

FAO, 1981. The Prevention of Losses in Cured Fish. FAO Fish. Tech. Paper, (219): 87, Rome 1981.

FDA, 1982. Food and Drug administration: Pesticides Analytical Manual, 1.

Ghazala, S., 1994. New Packaging Technology for Seafood Preservation Shelf-life extension and Pathogen Control. In: Fisheries processing – Biotechnological Applications. Ed. A.M. Martin Chapman and Hall, London, 83-110.

Gold, C.A., 1984. An Introduction to Insect Pests and their Control. Macmillan Press ltd. London, pp: 250.

Green, A.A., 1967. The Protection of Dried Sea Fish in South Arabia from Infestation by Dermestes fuschii Kug (Coleoptera, Dermestidae). Journal of Stored Products Research, 2: 331-350.

Guggenhein, H., 1980. Introduction to Better Processing and Packaging Methods for Dried Fish. Project G.C>P/RAF/157/DEW Field.

Hathaway, G.J., N.H. Proctor, J.P. Hughes and M.L. Fischman, 1991. Proctor and Hughes Chemical Hazards of the work place 3rd Edition N.Y Van Nostrand Reinhold.

Igene, J.O., O.J. Abolagba and A.I. Ikeme, 1998. Use of Pesticides on Cured Fish in Nigeria. Report and Proceedings of the Sixth FAO Expert Consultation on Fish Technology in Africa, Kisumu,Kenya, 126-130.

MSDS, 2003. Safety Data for Aluminium Phosphide. Material Safety Data Sheets. August 19, 2003.

Sang, P. and V. Cudderford, 1999. Lindane: A review of Toxicity and Environmental Fate. World Wide Fund, Canada, pp: 65.