African Journal of Biochemistry Research Vol.3 (3), pp.048-051 March, 2009
Available online at http://www.academicjournals.org/AJBR
ISSN 1996-0778 © 2009 Academic Journals

Full Length Research Paper

Toxicological effects of chlorpyrifos and methidathion in young chickens

Ojezele, Matthew Obaineh[1]* and Abatan, Oluwole Matthew[2]

[1]Department of pharmacology and therapeutics, Igbinedion University, Okada, Nigeria.
[2]Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, P. O. Box 20711 (U. I.), Ibadan, Oyo State, Nigeria.

Accepted 25 January, 2009

The objective of this work is assessment of the toxicological effects of the organophosphorus compounds (methidathion and chlorpyrifos) and the usefulness of some parameters as bioindicators. Haematology and serum biochemistry were assayed in 15 cockrels at random age of 4 - 6 weeks. The birds were randomly assigned to 3 oral (by mouth) pulse-dose treatments of 0, 3.5 mg/kg chlorpyrifos, and 7.5 mg/kg methidathion, respectively. Haematology and serum biochemistry were used as indices of toxicosis. Significantly ($P < 0.05$) reduced WBC, Neutrophils and Lymphocyte counts were observed in the groups of birds dosed with chlorpyrifos and methidathion. PCV, Hb, and RBC were significantly decreased by methidathion while chlorpyrifos caused a significant increase in these parameters. Results obtained in this study also showed that methidathion and chlorpyrifos caused significant decrease in the levels of ALP, ALT, TP, and ALB. While methidathion caused a significant decrease in the level of AST reverse is the case for chlorpyrifos with respect to AST. It was also observed that both organophosphates had no significant effect on the level of BIL. The study thus showed that the evaluated parameters can serve as useful bioindicators of the sub lethal exposure to organophosphorus compounds.

Key words: Biochemistry, chlorpyrifos, methidathion, bioindicators, cockrels, haematology.

INTRODUCTION

Pests are a menace to the environment (Martin et al., 2003). Mites, lice, fleas and flies are the most common pests of poultry. Activities or infestation by these pests can in many cases severely compromise the health and well being of individual animals and by extension, man. Some of these pests have also been shown to be capable of transmitting leucosis a disease of poultry (Draper, 1967).

The spread of these pests in the environment can make total eradication difficult if not impossible. Hence, there is the need to, on a regular basis, apply pesticides. In some countries, efforts to control or totally eradicate them necessitate the use of pesticides on farms, offices and homes. The consequences of pesticides use in developing countries are fatal (Pesticides action network, 1998).

Studies have shown that not all applied pesticides may actually reach targeted pests and the remaining have potential to get into the soil, water, and the atmosphere (Don et al., 2002; Goodman et al., 2006). Unfortunately, pesticides are designed to alter specific body processes and they are not target specific (Daren, 2007; Lang, 1993). To this end, human and animals are occasionally and unintentionally exposed to lethal and sub-lethal doses of pesticides (Eddlestone, 2000; Martin et al., 2003). Human and animals can be directly exposed to pesticides by inhalation, ingestion, contact with skin and eyes. Apart from the direct exposure, indirect exposure occurs in animals by consuming prey that contains high residues of the pesticides (Caroline, 1991).

In lethal dose exposure, the effects are obvious soon after exposure leading to high economic losses (in animals) and death in extreme cases (Gayathri et al., 1998). Studies have showed that organophosphates (e.g. chlorpyrifos and methidathion) have a very narrow safety margin in birds and accumulate in their system to their detri-

*Corresponding author. E-mail:matlar2002@yahoo.com. Tel.: 234-8053610558/ 8033923332.

ment (Nancy et al., 1994). Birds so poisoned lose muscle control, start to salivate and vomit, develop diarrhea, become unconscious and die.

Effects of sub-lethal exposure to organophosphates may not be grossly visible immediately. In the case of some animals, regular application of some pesticides leads to bioaccumulation (Ling and Huang, 1995). This can result in consumer toxicity (Sun et al., 2006). Nancy et al. (1994) outlined the various sub-lethal effects of pesticides (organochlorines and organophosphates) in animals which include abnormal ovulation and egg-shell formation in chicken; reproductive and developmental toxicity and endocrine disruption. Ramish (2007) and Tejendra (2005) cited depression, reduced feed intake and dullness as clinical symptoms accompanying exposure of birds to sub-lethal dose of a pesticide. In the findings, there was no alteration recorded in haematological parameters but a significant dose dependent increase in serum enzymes was observed.

This study is therefore targeted at identifying the effects of organophosphous pesticides on the haematological and serum biochemical indices of young chickens. Also the study aims at assessing the suitability of the parameters used as indicators in assessing exposure or toxicity status of the animals under investigation.

MATERIALS AND METHODS

Study animals

A pilot toxicity study was carried out by dosing birds with different concentrations of the organophosphates below the lethal dose, generally less than 50 mg/kg (Daniel, 1998) .The working concentration was selected based on the observed concentration that did not cause mortality in the experimental animals.

A set of 15 cockrels were randomly selected from a flock of birds, raised on deep litter system, within the age range of 4 – 6 weeks. The birds were randomly assigned to 3 oral (by mouth) pulse-dose treatments of 0, 3.5 mg/kg chlorpyrifos, and 7.5 mg/kg methidathion, respectively.

The birds were stabilized two weeks prior to the start of the experiment and fed *ad libitum* a basal diet containing 19% crude protein and 2800 kcal/kg metabolizable energy.

Experimental design

Chemical preparation and administration to birds

Chlorpyrifos (480 g/litre) and methidathion (420 g/litre) were purchased locally (Ibadan, Nigeria) and prepared to working dose (sub-lethal) by dilution with distilled water. Each bird was weighed and dosed by weight with the working dose of the chemicals every morning using a cannula for 15 days.

Haematology and serum biochemistry

The birds were bled on the 16th day by jugular venapuncture for collection of blood and serum samples to be used for biochemical and haematological analysis. The blood for heamatology and serum biochemical analyses were collected into sodium ethylene diamine-tetraacetic acid and plain bottles, respectively.

WBC (White Blood Cell Counts) and RBC (Red Blood Cell Counts) were determined with an improved Neubauer haemocytometer (Campbell, 1991). Packed cell volume was determined and Hb (Haemoglobin content) was determined by cyanide free haemoglobin determination (Baker and Silverton, 1985). Mean cell volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentrations (MCHC) were calculated using conventional methods (George and Parker, 2003).

The blood smears were stained with a Diff-Quick stain and a differential cell count was done (Jain, 1986). Total protein (TP) was determined by Biuret method (Pilar et al., 2005). Serum biochemical analyses including liver enzymes were done using commercially available test kits according to Bergmeyer et al. (1986).

Data analysis

Data were recorded as arithmetic mean ± standard error of mean while the degree of freedom = 4. Statistical calculations were performed using SPSS 8.0 (SPSS Inc. Chicago, Illinois, USA) and significance level was set at $P < 0.05$ (Bolton, 1997).

RESULTS

The results of the experiments carried out are shown in Tables 1 and 2. Table 1 shows the results obtained for the effects of the organophosphates on haematological parameters on the animals under study. Significantly ($P < 0.05$) reduced WBC, Neutrophils and Lymphocyte counts were observed in the groups of birds dosed with chlorpyrifos and methidathion. PCV, Hb and RBC were significantly decreased by methidathion while chlorpyrifos caused a significant increase in these parameters.

Results obtained in this study also showed that methidathion and chlorpyrifos caused decreased significant levels of ALP, ALT, TP and ALB. While methidathion caused a significant decrease in the level of AST reverse is the case for chlorpyrifos with respect to AST. It was also observed that both organophosphates had no significant effect on the level of BIL (Table 2).

DISCUSSION

Human and animals are exposed to different levels of pesticides as a result of the use of pesticides to control rodents in homes and offices, and ectoparasitic infestations in animals (Martin et al., 2003). In this study, effects of sub-acute levels of two organophosphorus pesticides have been evaluated in birds 4 - 6 weeks old. These organophosphates, chlorpyrifos and methidathion, are used to control ectoparasites in domestic animals, livestock and poultry (Alberto, 2006)

In this study the dose of the pesticides administered to the birds elicited varying responses. Although no mortality was recorded at the reduced (sub-lethal) dose, the haematology and biochemical changes observed may be indicative of grave danger in cases of inadvertent exposure of birds to such chemicals even at low doses.

Peripheral blood film is an important indicator of haematology and other diseases. The increase in RBC for birds dosed with chlorpyrifos may be an indication of a

Table 1. Changes in haematological parameters following the oral administration of methidathion and chlorpyrifos in cockrels

Haematological parameters	PCV%	Hb g/dl	WBC X 1O^6 mm^3	RBC X IO^6 mm^3	NEUT	LYMP	MCVμ/3	MCHC %	MCH pg
Control	23.3±0.	7.33±0.1	4.66±8.7	1.88±4.2	2970.7±694.1	1696±181.9	124.3±5.1	31.5±0.2	39.1±1.4
Methidathion	20±1.5*	6.53±0.3*	2.86±5.6*	1.35±2.4*	1496±417.4	1370.7±154.9*	147.5±8.5*	32.7±0.5*	48.2±2.0*
Chlorpyrifos	24±0.5*	7.60±0.2*	2.53±3.5*	1.98±30.0*	1060±133.1*	1466.7±231.3*	126.5±2.0*	31.7±0.2*	40.0±5.0*

*= Significant values ($P < 0.05$)

PCV- Packed cell volume
Hb- Haemoglobin concentration
RBC- Red blood cell counts
WBC- White blood cell counts
MCV- Mean corpuscular volume
MCH- Mean corpuscular haemoglobin
NEUT- Neutrophils counts
LYMP- Lymphocyte counts
MCHC- Mean corpuscular haemoglobin concentration

Table 2. Changes in serum biochemical parameters following the oral administration of methidathion and chlorpyrifos in cockrels.

	BIL (mg/dl)	ALP(U/l)	AST(U/l)	ALT(U/l)	TP(g/dl)	ALB(g/dl)
Control	0.2±0.03	1135.33±17.3	287.2±59.8	11.4±2.4	4.06±1.1	1.16±0.2
Methidathion	0.2±0.03	1063.2±455.2*	228.4±38.6*	6.8±1.9*	2.24±0.3*	0.8±0.1*
Chlorpyrifos	0.2±0.06	824.6±229.5*	292.4±23.6*	10.2±0.8*	2.24±0.3*	0.68±0.2*

* = Significant values ($P < 0.05$).

BIL- Bilirubin
ALB- Albumin
TP- Total protein
ALP- Alkaline phosphatase
ALT- Alanine aminotransferase
AST- Aspartate aminotransferase

hypoxic condition, which developed after organophosphate exposure. This might have led to a stress-linked release of new erythrocytes and synthesis of more haemoglobin in older erythrocytes to improve the ability of exposed birds in carrying oxygen. This correlates with the findings of Fernando and Moliner (1991) who reported that a hypoxic white blood cell (WBC) counts condition developed in fish after pesticide exposure. The reduction in RBC in methidathion treated group, on the other hand, may be attributed to intravascular haemolysis, anaemia or depression of the haemopoiesis. However, the apparent non significant effect of both organophosphate on bilirubin (a by- product of RBC lyses) ruled out the possibility of haemolysis. Anaemia and suppression of the haemopoietic system could be implicated. This was further buttressed by the reduction of WBC of birds exposed to methidathion.

The reduction in WBC of birds exposed to chlorpyrifos and methidathion may be due to depression of production of WBC by the haematopoietic system. This can also be linked to reduction of PCV, Hb, and RBC observed in methidathion treated group. In different study, Alkahem (1994) observed that exposure of fish to nickel led to low leukocyte counts which was attributed to reduction in lymphocytes and neutrophils. Reduction in the number of lymphocytes may be due to decreased production or rapid removal from circulation and subsequent destructtion. The reduction of lymphocytes is indicative of immunosuppressive effects of organophosphates which may require other studies to assess the levels of immunoglobulin in exposed birds. Such birds can also be challenged by exposing them (experimentally) to infections and then to assess their ability to withstand or fight invading microorganisms (Janeway, 2005).

The reduction of serum levels of protein for all the birds dosed with the organophosphates correlates with the findings of Das and Mukherjee (2000), which indicated that toxicants may cause stress-mediated mobilization of protein to cope with the detrimental condition so imposed. The protein so mobilized is one of the strategies employed to meet the energy required to sustain increased physical activity, biotransformation and excretion of the toxicants.

Aminotransferases (ALT and AST) are produced in the liver and are good markers of damage to liver cells but not necessarily the severity of the damage (Olav et al., 2007; Rej, 1989). They are normally present at low levels in the blood so if the liver cells are damaged, it would be expected that some of the enzymes leak into the blood and increase the levels. To this end therefore, the reduced levels of AST, ALB and ALT in methidathion treated group could be as a result of suppression of production by the liver apparently an earlier damaging effect before progression to cell death and leakage of enzymes. Increase in serum level of AST as observed in birds dosed with chlorpyrifos may reflect damage of liver cells as a result of a slightly more advanced effect compared with the reduction in levels observed in other enzymes in the same treatment group and methidathion treated group.

In contrast to serum enzymes, albumin apart from being a useful indicator of the integrity of glomerular membrane is also an important indicator in determining the severity of disease (Lawrence and Amadeo, 1989; Mukut et al., 2001). Decrease albumin may be due primarily to reduction in synthesis by the liver and seconddarily to reduced protein intake which further confirms hepatic damage (Luskova et al., 2002; Jyotsna et al., 2003). To this end, decrease albumin observed in organophosphate administration may be attributed to hepatic dysfunction.

In conclusion, the varied changes in the levels of the haematology and biochemical parameters relative to the control group can affect the overall performance of the birds in terms of health and production. From the outcome of this study, the parameters can be used as bioindicators of exposure and effects in instances where organophosphates are used as pesticides.

REFERENCES

Alberto BB (2006). Veterinary clinics of North America: ectoparasite control. Food Ani. Pract. 22(2):463-474.

Alkahem HF (1994). Toxicity of nickel and the effect of sub-lethal level on haematological parameter and behavior of fish, *Oreochromis niloticus*. J. University of Kuwait. Science 21:243-252.

Baker FJ and Silverton RF (1985). Introduction to medical laboratory technology.Butterworth and co publishers.pp 316-334.

Bergmeyer Hu, Horder M, Rej R (1986). IFFC Recommendation. J. Clin. Chem. Clin. Biochem .24: 481-497.

Bolton S (1997). Nonparametric methods. In Pharmaceutical statistics, 3rd Edition. J. Swarbrick (Ed). Marcel Dekker, Inc., NY. pp 537-588.

Campbell, T. W. (1995). Avian haematology.In avian haematology and cytology, 2nd Edition. T. W. Campbell (Ed). Iowa State University Press, Ames, Iowa. pp 3-25

Caroline C. (1991). Pesticides and birds: from DDT to today's poision. J. Pesticide Reform.11 (4): 3-5.

Daniel AP (1998). Destructive turf grass insects: safeguarding the environment. In biology, diagnosis and control, 1st edition. Daniel A. Potter (Ed).John Wiley and sons Inc. New Jersey. pp. 108-109.

Darren MR, Cynthia KA (2007). Management of acute organophosphorus pesticide poisoning .BMJ. 334: 629-634

DAS BK, Mukherjee SC. (2000). Asian fisheries science Asian fisheries society, Manilla, Philippines.13; 225-233.

Don RN, Simon Y, Jan B, Regina K, Keiji T, Baruch R, Arata K, Werner K, Michael L, John BU (2002). Pesticide soil sorption parameters: theory, measurement, uses, limitations and reliability. Pest Manage Sci.58 (5): 419-455.

Draper CC (1967). A yellow fever vaccine free from avian leucosis viruses. J. Hyg. Camb.65:505-513.

Eddleston M (2000). Patterns and problems of deliberate self-poisoning in the developing world. Q .J. Med. 93:715-731.

Fernando MD, Moliner EA (1991). The effect of time on physiological changes in eel Anguilla induced by lindane. Compr. Biochem. Physiol.100C: 95-98.

Gayathri MR, Pilla KS, Balakri-Shramuthy P, Ramamoorthy S.(1998).Organophosphate toxicity-a review. J. Experiment Zool.1:2, 73-79.

George GB and Parker K. (2003). Understanding the complete blood count with differential. J. Perianesth Nurs. 19:96-114.

Goodman BA, Allison MJ, Oparka KJ, Hillman JR (2006). Xenobiotics: Their activity and motility in plants and soils.J. Sci. Food Agric. 59:1-20.

Jain N C. (1986). Schalmis Veterinary Haematology, 4th Edn.Lea and Febiger, 600 Washington square, Philadelphia, PA 19106-4198, USA.pp 30-34.

Janeway CA (2005). Immunubiology.In the immune system in health and disease, 6th edition. Eleanor Lawrence(Ed).Garland science NY, USA.pp 461-463.

Jayasree U, Gopala Reddy A, Reddy KS, Kalakuma B (2003).Study on the mechanism of toxicity of deltamethrin in poultry. Indian .J. Toxicol.10 (2): 111-114

Jyotsna AP, Arun JP, Sanjay P. (2003). Biochemical effects of various pesticides on sprayers of Grape garden. Indian. J. Biochem.18/21, 16-22.

Lang L (1993). Are pesticides a problem? Envtal Health Persp. 101:578-583(Pub Med).

Ling YC, Huang IP (1995). Multiresidue-matrix solid-phase dispersion method for determining 16 organochlorine pesticides and polychlorinated biphenyls in fish.Chromatographia 40(5-6): 259-266.

Lawrence AK, Amadeo JP (Ed). (1989). Clinical chemistry: Theory, analysis and correlation, 367.The C.V. Mosby Company.

Luskova V, Svoboda M, Kolarova J. (2002). The effect of Diazinon on blood plasma biochemistry in carp (cyprinus carpio L).ACTA VET.BRNO 71:117-123.

Martin B, Stephaine K, William W, Kim MB, Kathy P, Lorrie B, Carol R (2003). Childhood pesticide exposures in the Texas-Mexico border: clinical manifestations and poison centre use.Am. J. Public Health 93(8): 1310-1315.

Mukut S, Ran S, Xiu LG, Brian LF, Ellen TM, Virginia JS , Eric PC, John EM (2001).Early detection of radiation-induced glomerular injury by albumin permeability assay. Bio .one.155(3):474-480.

Nancy BM, Kathleen RA and Anneta PW (1994). Thinking of the organophosphate chemical warfare agents GA, GB and VX: implication for public protection. Envtal Health Persp.102:1

Olav AG, Ralf W, Axel MG (2007). Biomarkers of liver fibrosis: clinical translation of molecular pathogenesis based on liver-dependent malfunction test. Clinica Chimica Acta.381 (2):107-113.

Pesticides Action Network (PAN). Europe Conference. (2000). PAN
Europe position on pesticides with endocrine disrupting effects.

Pierre Mineau. (2002). Pesticides and wild birds. Canadian wildlife services hinterland who's who.cw 69-4/98

Pilar Lanzarot M, Victoria Barahona M, Manuel I S, Manuel F and Cassidy R. (2005). Haematologic, protein electrophoresis, biochemistry and cholinesterase values of free-living black stork nestlings (*Ciconia nigra*) .J. Wildlife diseases 41(2), 379-386.

Rej R (1989). Aminotransferase in Disease, Clin. Lab. Med, 9(4):667-87.

Sun F, Wong SS, Li GC, Chen SN (2006). A preliminary assessment of consumer's exposure to pesticide residues in fisheries products.62 (2):674-680.

Ramish CG (2007). Veterinary toxicology. In insecticides and molluscicides, 1st edition. Ramish C. Gupta(Ed).Academic press publishers, NY, USA.39-40.

Sievers G, Palacios P, Inostroza R, Doeiz H.(1995).Evaluation of the toxicity of 8 insecticides in *Salmo salar* and the in-vitro effects against the isopode parasite *Ceratothoa gaudichaudii*;Aquacult 134:9-16