PAT December, 2013; 9 (2): 1-17 ISSN: 0794-5213

Online copy available at

www.patnsukjournal.net/currentissue

Toxicological Effects of Chlorpyrifos, Dichlorvos and Alpha Cypermethrin on Adult Albino Mice, *Mus musculus*

[1]Kemabonta, K. A and Akinhanmi, F.O

Dept of Zoology, Faculty of Science, University of Lagos. Lagos
[1] kkemabonta@unilag.edu.ng, kennykemabonta@yahoo.com
[1]Corresponding Author

Abstract

The study was carried out to investigate the toxicological effects of three pesticides: chlorpyrifos, dichlorvos and alphacypermethrin on body weight, haematology, systemic, biochemical parameters and semen of adult mice. The mice were used to simulate the incessant exposure of people working with these pesticides, especially the local retailers and farmers who use them on daily basis with no appropriate storage facility and most often stored in poorly ventilated rooms. Mice were exposed 8hrs daily to the fumes of these pesticides and fed pelleted mice feeds for 8weeks. There was a decrease in weight of mice exposed to the insecticides with chlorpyrifos being the most affected. Mice exposed to alphacypermethrin and the control showed no physiological changes while those exposed to chlorpyrifos and dichlorvos showed skin lesions and inflamed bladder respectively. Histology of tissues of the brain, liver, lung and kidney showed edema, inflammations, congestions, nephritis and necrosis. Haematological parameters showed significant decrease in Red Blood Cell (RBC), Haemoglobin (HGB) and Packed Cell Volume (PCV) ($P<0.05$) except the White Blood Cell (WBC), which increased insignificantly ($P>0.05$). Biochemical parameters showed insignificant increase in Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), creatinine and urea levels ($P>0.05$) except the Alanine phosphatase (ASP) which showed a significant increase ($P<0.05$). Semen analysis revealed decreased sperm motility and an increase in abnormal sperm cells. The study showed that exposure to pesticides may lead to adverse health effects as evidenced by the results from the present study and the extrapolation to humans' situations is also discussed.

Introduction

Pesticide usage in developing nations like Africa has increased dramatically in recent times with adverse effects on humans and other non-target organisms (Czarniewska *et al*., 2003). Misuse of highly toxic pesticides, coupled with a weak or a totally absent legislative framework in the use of pesticides are reasons for the high incidence of pesticide poisoning in developing countries (Konradsen *et al*., 2003). Unintended exposure to pesticides can occur during their manufacturing, formulation and application or from environmental residues after application (Jeyaratnam, 1990). Chemical plant workers, transport workers, and pesticide applicators are the major occupational groups that might be exposed to pesticides. In industrial settings, workers

are at increased risk since they handle various toxic chemicals including pesticides, raw materials, toxic solvents and inert carriers (Keifer *et al*., 2010).

Chlorpyrifos is a broad-spectrum, chlorinated organophosphate (OP) insecticide, acaricide and nematicide used on agricultural food crops, and to control public health pests such as mosquitoes and fire ants (USEPA, 2006). Dichlorvos or 2,3-dichlorovinyl dimethyl phosphate (Trade Names: DDVP) on the other hand is a highly volatile organophosphate, widely used to control household pests, in public health, and protecting stored products from insect infestation. Alpha-cypermethrin is a synthetic, broad spectrum pyrethroid used against a wide range of chewing and sucking insects present in fruits, vegetables, oil seeds, beans, cotton & other crops. It also has domestic applications in public health for the control of cockroaches, mosquitoes and flies (PAN, 2005). These three pesticides are often used by farmers, pest control officers and stored food processors. Most of these workers do not store the pesticides appropriately. They sometimes store the containers in their homes thus, breathing air containing the pesticides which may be detrimental to their health in the long run.

The objectives of this research were to evaluate the effect of exposure to the three pesticides mentioned through the physiological processes, internal organs and systems, biochemical and hematological indices assessments and on the reproductive capability of the adult mice.

Materials and Methods

Experimental animals

The experiment was carried out in the Zoological Gardens laboratory of the Department of Zoology, University of Lagos, Lagos Nigeria. Forty adult mice were housed in four customized rectangular cages with ten mice in each cage. The average weight of the mice was 27g and their average age was 13 weeks old and fed with pelleted mice feed 32.26mg/kg per body weight per day.

Acclimatization

The mice were purchased from Lagos University Teaching Hospital (LUTH) Idiaraba, Lagos.

They were kept in customized wooden cages (45x30x30cm^3) for 14 days to acclimatize to laboratory conditions (29±5ºC Temperature and 80±5% Relative Humidity). Wood shaving (bedding) was put inside the cages for easy movement and to keep the animals warm. The mice were fed and given tap water every two days and weighed. The uneaten food, and left over water were changed every two days. To ensure proper hygiene, their bedding was changed every week.

Selection of Animals For Bioassay

Adult mice (12-14 weeks old with body weight 27-31g) were selected randomly from their cages for the experiment. Sizes and age are known to affect physiological responses of animals to chemicals.

TEST COMPOUNDS

Termicid® (active ingredient: 0,0-diethyl 0-(3,5,6-trichloro-2-pyridinyl-phosphorothioate (Chlorpyrifos) 200g/L; Sniper® (active ingredient: 2,3-dichlorovinyl dimethyl phosphate (Dichlorvos) 100g/L and Alpha Cypermethrin (active ingredient pyrethroid) 100g/L ,were used for the bioassay. All these pesticides were gotten from the Department of Zoology, University of Lagos, Yaba, Lagos.

BIOASSAYS

In order to evaluate the effects of the insecticides fumes, 4 similar cages, each having 10 mice, the male and female were used. The exposure treatments were as follows: The first, second, third and fourth set of mice labeled TCD, DDVP, ACP AND CN were exposed to 2ml each of Chlorpyrifos, Dichlorvos, Alphacypermethrin and Control respectively for 8 hours a day (8am to 4pm) for 8 weeks. The animals were analyzed twice during the course of the experiment. The first analysis was carried out after four (4) weeks of exposure and the second analysis, after 8 weeks.

COLLECTION OF BLOOD AND ORGANS

Three animals from each group were sacrificed after four and eight week exposure. Blood samples were collected by ocular puncture via heparinized capillary tubes, into properly bottles. Blood samples to be used for biochemical analysis were collected into

lithium heparinized bottles while blood samples to be used for haematological analysis were collected into EDTA bottles. The lungs, kidney, liver and brain were excised and transferred into plain bottles containing 10% formalin to preserve and to fix the organs, getting them ready for histopathological examinations.

MORPHOLOGICAL CHANGES

Weight change- the body weight was measured using a table scale at the end of each week for eight weeks.

HISTOPATHOLOGICAL ANALYSIS

The lungs, liver, kidney and brain were prepared for histological studies. The tissues (organs) were cut into thin slices with a scalpel blade and placed in embedding cassettes. These cassettes were labeled accordingly to avoid mix up. The tissues were cut into slices of about 0.3-0.5cm thickness in order to ensure penetration of processing agents. These were fixed in 10% formalin for 6 hours. It was then dehydrated with grades of alcohol from 70% to 90% (Absolute) alcohol. Wax impregnation removed the clearing agent from the tissue and allowed for the tissues to be permeated by the molten paraffin wax (impregnation reagent) which was subsequently allowed to harden to produce a block from which section was cut. Thereafter, a solid block containing the tissue was obtained. This was done by filling a mould of suitable size with molten paraffin wax, orientating the tissue in the centre of the mould to ensure it was cut in the right plane and the mass was finally cooled to enhance solidification. A microtome was used in the cutting of sections. The specimen was stained with haematoxylin for 20 minutes and excess stain was removed with alcohol and rinsed with tap water for 20 minutes. The slide was put in eosin for 2-3minutes and rinsed with distilled water. It was brought into alcohol- 70%, 90%, and 100% respectively at each stage for 2 minutes. The slide was cleared in xylene and mounted with Canada balsam; mounting was by dropping the balsam on the section on the slide. The slides were later covered gently with cover slip, air bubbles were avoided. Slides were allowed to dry on hot plate. Observation was done under compound microscope and photomicrographs.

HAEMATOLOGICAL ANALYSIS

Blood sample of mice (1ml) were collected into (Ethylene diamine tetra-acetate) EDTA anticoagulant containers and full blood count was done. This was done with the aid of

an automated Mind Ray BC 3200 analyzer. The blood sample was centrifuged at 5000 r.p.m and the serum was placed in the analyzer and the counts for each blood parameter were taken.

BIOCHEMICAL ANALYSIS

Blood samples of mice (1ml) were collected into lithium heparinized bottles and centrifuged at 5000 r.p.m. The serum was then placed in a Roche Itache auto analyser. The values for each biochemical parameter were taken.

SEMEN ANALYSIS

This was conducted using the extracted contents of the epidydimis of sacrificed males from each group of mice. The epidydimis was cut open with a surgical blade and emulsified in 0.5mls of physiological saline. A drop of diluted semen was then placed on a glass slide and cover with a cover slip. This was viewed under the microscope and the percentage motility of the sperm cells was recorded after three fields were viewed. The sperm morphology, which is the percentage of abnormal sperm cells was also observed.

STATISTICAL ANALYSIS

The experiment was undertaken using a complete randomized block design. Statistical analysis of the effects of different pesticide treatments was done by comparing all the means, including control by analysis of variance (ANOVA). Additional analysis comparing each treatment with the control, and comparing the test compounds were done by T-test using the graph pad software at 0.05level of significance.

RESULTS

WEIGHT CHANGES IN ADULT MICE

The weight of mice in the control progressively increases from 27g at the onset of the experiment to 35g after 8 weeks. On the other hand, there was a decrease in weight of mice exposed to the insecticides with chlorpyrifos being the most affected. However, the weight changes within the exposed groups were relatively the same. (Figure 1)

PHYSIOLOGICAL CHANGES

The mice exposed to alphacypermethrin and the control showed no physiological changes while those exposed chlorpyrifos and dichlorvos showed skin lesions and inflammed bladder respectively after one week exposure (Plates 1 and 2).

HISTOLOGICAL CHANGES

Histological examination of the lungs, liver, kidney and brain of exposed animals showed varying effects which include oedema, congestions, inflammations, interstitial nephritis and necrosis (Plates 3 to 25). Mice exposed to Chlorpyrifos showed chronic inflammation in the liver and mild hepatitis at 4 and 8wks respectively (Plates 3 and 15). The lungs mild and moderate congested blood vessels at 4 and 8wks respectively (Plates 9 and 21) while the brain and kidney showed no pathology at 4th and 8wks respectively (Plates 6, 12, 18 and 24). Mice exposed to dichlorvos by the 4^{th} and 8^{th} weeks showed chronic inflammation and mild congestion in the liver respectively (Plates 4 and 16) while the lungs revealed mild congestion at both times (Plates 10 and 22). On the other hand, the kidney and brain at 4^{th} and 8^{th} weeks showed no significant pathology (Plates 7, 13, 19 and 25). In mice exposed to alphacypermethrin, the liver section revealed periportal hepatitis (Plate 5) after 4 weeks, dilated blood vessels and congestion at 8wks (Plate 17), while the kidney showed interstitial nephritis and chronic inflammation during the same period (Plates 14 and 26) The brain section showed no pathology at 4^{th} and 8^{th} weeks (Plates 8 and 20).

HAEMATOLOGICAL CHANGES

Effects of pesticides on haematological parameters.

The blood parameters of mice exposed to chlorpyrifos, dichlorvos and alphacypermethrin at 4 weeks and 8 weeks showed some differences as shown in Table 1. Except at 4wks for treatment with Chlorpyrifos, white blood cell count, Red blood cell count, heamoglobin, and PVC levels decreased significantly at 8wks after treatment (Table 1)

BIOCHEMICAL CHANGES

Effects of pesticides on biochemical parameters

The exposure of *M. musculus* to pesticides showed evidences of stress (Table 2), at ($P<0.05$) significance level. The liver enzymes in all the exposed groups showed

varying changes. The level of Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), creatinine and urea levels were either insignificantly lower or higher than in the control mice except for treatment with chlorpyrifos that was significantly higher than in the control and other treated mice

SEMEN ANALYSIS

The sperm cell count showed a significant count of sluggish and dead cells among treated mice. There was also a higher number of abnormal cells among treated mice as compared to the control. In both cases (motile and abnormal sperm cells count), the worst hit were mice exposed to chlorpyrifos. (Figure 2 and 3)

DISCUSSION

Three pesticides, chlorpyrifos, dichlorvos and alphacypermethrin, were studied to evaluate their toxicological effects on adult mice. These pesticides were chosen because they are widely used in Nigeria by farmers (Kemabonta *et al* in Press) as well as in many parts of the world. In order to simulate the incessant exposure of people working with these pesticides, especially the local retailers and farmers who use them often in Lagos and have no appropriate storage facility for them on a daily basis and most often stored in poorly ventilated rooms, mice were exposed 8hrs daily to the fumes of these pesticides for 8weeks. This experiment also typifies real life situation in which an ever-increasing circle of Nigerians, cutting across different socio-economic classes now use these pesticides domestically. The animal model used in this study was used to assess the adverse effects of pesticides on laboratory animals (Costa *et al.,* 1989).

The result presented in this study clearly indicates that the inhalation of chlorpyrifos, dichlorvos and alphacypermethrin for eight weeks caused a slight decrease in the average body weight gain in the test mice. This finding agreed with what had been reported in earlier studies by Yoshida *et al*.,(1985); Barna *et al*., (1990) , who observed a drastic loss in weight of rats exposed to . chlorpyrifos. This reduction in body weight gain is a clear indication of general toxicity (Ambali, S.F., 2009).

Histology sections of the tissues (liver, brain, lungs and kidney) of the exposed mice showed various pathological changes. The transverse section of tissue of the brain and kidney of mice exposed to chlorpyrifos showed no pathological changes. Section

through the liver and lungs showed chronic inflammation, congestion which could lead to jaundice and death of hepatocyte (necrosis). This agreed with results of experiment carried out by Rady (2009) who found that the lungs of guinea pigs exposed to chlorpyrifos showed congestion of blood vessel and infiltration of lymphocytes in the tissues.

Histology section of the kidney of mice exposed to dichlorvos showed interstitial nephritis, a kidney disorder in which the spaces between the kidney tubules becomes inflamed, while sections through the liver revealed chronic inflammation. This result is consistent with necrosis of liver hepatocytes, inflammation, highly vacuolated hepatic cells, inflammatory cell infiltrate in the kidney and thickening of blood vessels in the kidney by Matthew (2010)

Histology section of kidney of mice exposed to alphacypermetnrin also showed interstitial nephritis. Blood vessels in the lungs of mice exposed to alphacypermethrin were congested. Periportal hepatitis, inflammation of the portal vein in the liver, was observed in the animals exposed to alphacypermethrin. Histology section through the tissue of mice exposed to alphacypermethrin showed a widened perivascular space which could lead to cerebral oedema. This agrees with the report documented by Muthuviveganandavel *et al.*, (2011), that albino rats' exposure to pyrethroids resulted in cerebral edema, loss of lobar architecture and congestion of blood vessels.

Mice exposed to chlorpyrifos and dichlorvos exhibited a significant decrease in the RBC after 4 weeks and even a further decrease after 8 weeks. Decrease in RBC results in anemia. This agreed with the observed result in a previous study (Ambali *et al*., 2011). They studied the effects of organophosphates on hematological changes in adult mice and their result showed an insignificant increase in RBC, PCV and HGB levels. Decrease in RBC level in mice exposed to dichlorvos can be a useful biomarker of exposure to organophosphate poisoning in human. This is in support with Tayser (2005) who reported biological marker such as monitoring of serum cholinesterase and cholinesterase enzymes in red blood cells (RBC) can assess actual exposure to pesticides particularly organophosphates. On exposure to alphacypermethrin, mice exhibited a significant decrease in the RBC after 4 weeks and even a greater decrease after 8 weeks. Haemoglobin measures the amount of oxygen-carrying protein in the

blood. The HGB level in all the exposed mice decreased insignificantly after 4 weeks. However there was a significant decrease in HGB level of mice exposed to dichlorvos and alphacypermethrin after 8 weeks while the HGB decrease in mice exposed to chlorpyrifos remained insignificant. A low hemoglobin count may also result in anemia.

The increased levels of serum creatinine and urea are consistent with findings from previous studies (Kossmann *et al.,* 2001; Al Qarawri and Adam, 2003).The semen analysis of mice exposed to pesticides revealed varying adverse effects. Since the testes contain rapidly proliferating cells, they are particularly susceptible to damage. The semen analysis presented a well demonstrated picture of these sublethal effects of exposure to pesticides, producing such effects as high levels of sluggishness, morphological abnormalities and death of sperm cells as was seen among the exposed mice. This result is consistent with the report of Kata (2008), which inferred that pesticide exposure to males caused a significant decrease in movement and increase of number of abnormal sperm cells. This poses a potential threat of sterility, infertility and gradual extinction of people exposed continuously to pesticides.

In conclusion, the data presented in this work showed that chronic exposure to chlorpyrifos, dichlorvos and alphacypermethrin fumes can lead to reduction in body weight, various degrees of histopathological lesions in lungs, liver, kidney and brain of the experimental animals. Decreased red blood cells, hemoglobin and packed cell volume are all anormalies that could lead to anaemia. Moreover, the pesticides caused depletion in sperm motility and an increase in the number of abnormal sperm cells, which is an indication of infertility. These health implications of these abnormalities can be threatening and can affect the well-being of humans exposed to inhalation of the pesticides in for a long time. Occupational health should be paramount by providing effective protective equipments. Domestic use of these pesticides should also be reduced to the barest minimum.

References

Al-Qarwai, A. A. and Adam, S.E. (2003). Effects of Malathion plus superphosphate or urea on Najdi Sheep. *Veterinary and Human Toxicology*. 45:3-6.

Ambali, S.F., Akanbi, D.O., Oladipo, O.O., Yaqub, L.S. and Kawu, M.U. (2011). Sub-chronic chlorpyrifos inducd clinical, hematological and biochemical changes in Swisa albino mice: Protective effect of vitamin E. *International journal of Biological and Medical Research*. 2(2):497-503.

Ambali, S.F. (2009). Ameliorative effects of vitamins C and E on neurotoxicological, haematological and biochemical changes induced by chronic chlorpyrifos in Wistar rats. PhD Dissertation, Ahmadu Bello University, Zaria, Nigeria. 201pp.

Barna-Lloyd, T., Szabo, J.R. and Davis. N.L. (1990). Chlorpyrifos-methyl (Reldan R) rat subchronic dietary toxicity and recovery study.Unpublished Report TXT: K-046193-026 from Dow Chemical, Texas, USA. Submitted to WHO by Dow Elanco, Indianapolis, USA. 23pp.

Costa, L.G., Olibet, G., Wu, D. and Murphy, S.D. (1989). Acute and chronic effects of the pesticide amitraz on a 2- adrenoceptors in the mouse brain. *Toxicological Letters*. 47(2):135-143.

Czarniewska, E., Kasprzyk, A. and Ziemnicki, K. (2003). Effect of paraquat and metoxychlor on antioxidant enzymes in frog Rana esculenta. L. liver. *Biological Letters.* 2:125-133.

Jeyaratnam J (1990). Acute pesticide poisoning: a major global health problem. *World Health Stat Q* 43(3): 139–44.

Kata, F.S. (2008). Effect of dichlorvos pesticide on fertility of laboratory male mice Mus musculus. *Basrah Journal of Veterinary Research.* 7(1): 1-10.

Kemabonta *et al* (in Press)

Kossmann, S.; Tustanowski, J. & Kołodziej, B. (2001). Renal dysfunction in chemical plant workers producing dust pesticides. *Med PR.* 52(4):253-6.

Matthew, M. (2010). Histopathological changes in the gonad, liver and kidney of adults exposed to sublethal concentrations of some pesticides. 79-109.

Muthuviveganandavel, I, Muthuraman, P., Muthu, S. and Srikumar, K. (2011). Individual and combined biochemical and histological effect of Cypermethrin and Carbendazim in male albino rats. *Journal of Applied Pharmaceutical Science.* 1(9):121-129.

PAN (Pesticide Action Network). 2005. PAN Pesticides Database (Version 6) – Alpha-Cypermethrin. Available at http://pestticideinfo.org/ Detail_Chemical.jsp? Rec_Id = PC37663.

U.S. Environmental Protection Agency. (USEPA). (2006). Interim Reregistration Eligibility Decision (IRED) Document for Dichlorvos (DDVP). Office of Pesticide Programs. Washington, DC. 167pp.

Yoshida, A., Kosaka, T., Miyaoka, T., Maita, K., Goto. S. and Shirasu, Y. (1985) Chlorpyrifosmethyl: 28-day oral toxicity study in mice. Submitted to Dow Elanco, Indianapolis, USA by the Institute of Environmental Toxicology, Tokyo, Japan 21pp.

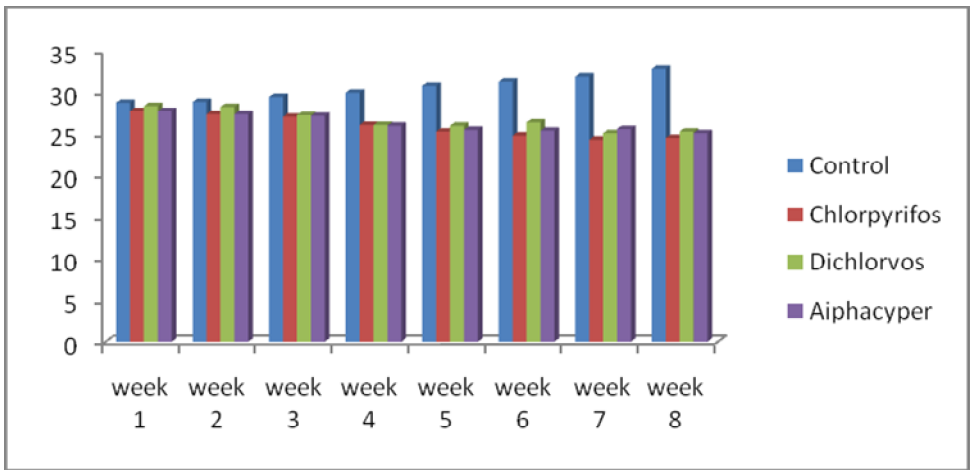


Figure 1: Mean weekly weight (g) of exposed mice.

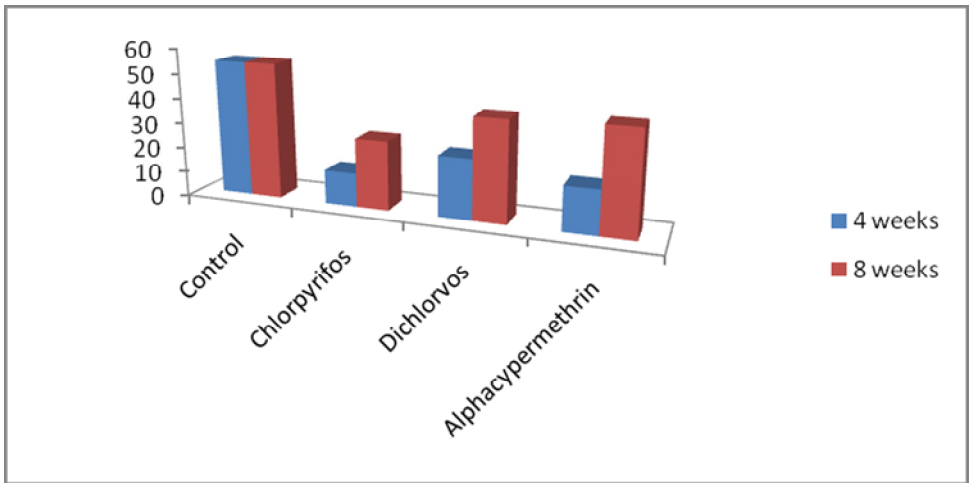


Figure 2: The percentage motility of sperm cells.

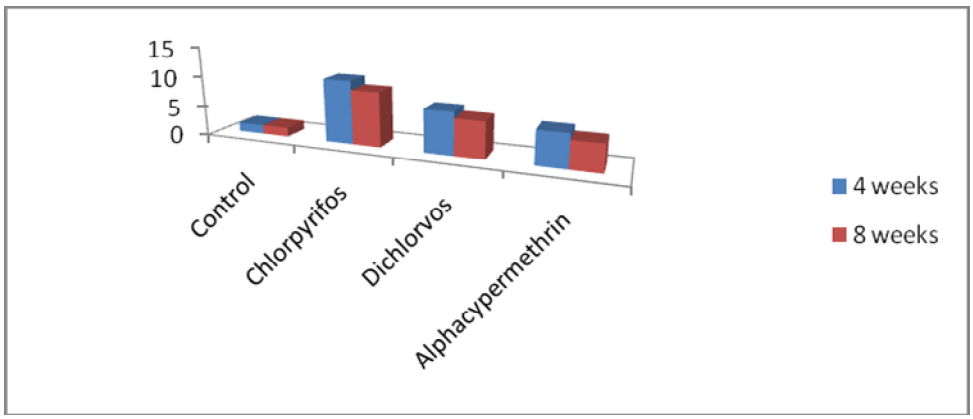


Figure 3: Percentage of abnormal sperm cells

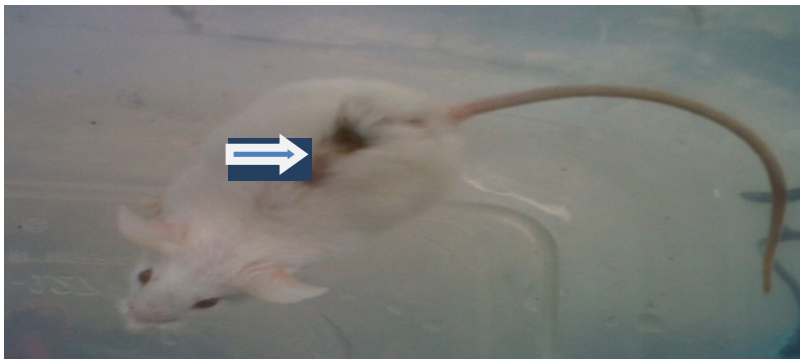


Plate 1: Life Mouse showing skin lesion after one week exposure to chlorpyrifos.

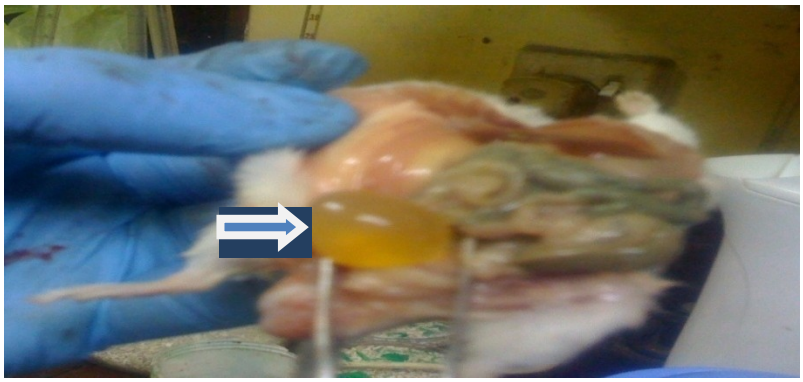
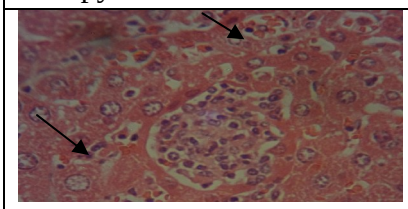
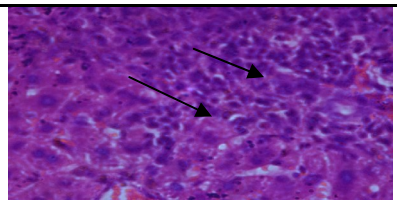
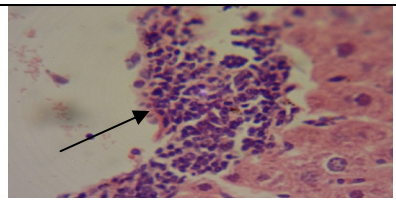
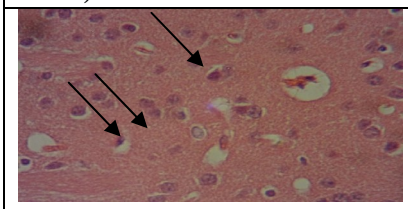
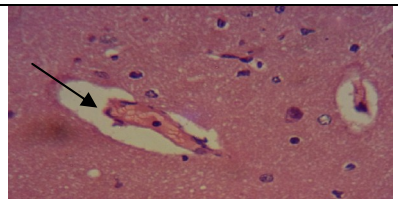
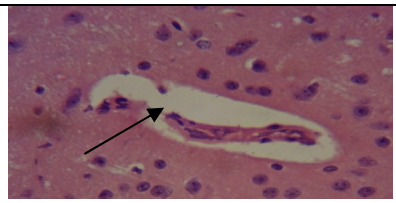
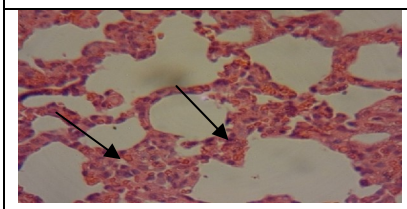
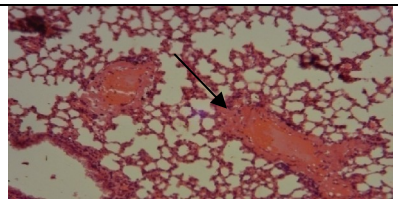
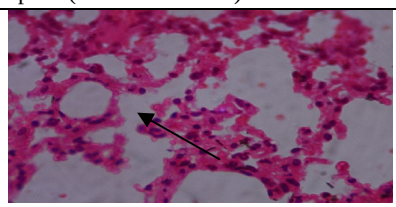
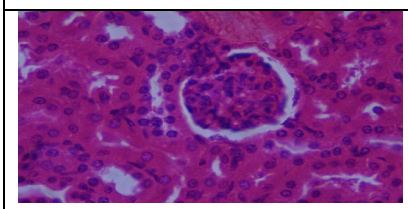
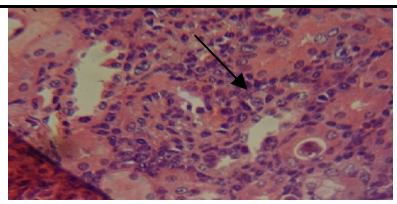
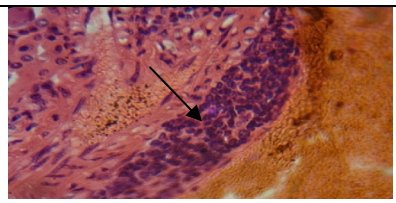


Plate 2: Anatomy of Mouse showing inflammed bladder after one week exposure to dichlorvos

Histology sections of Mice exposed to the insecticides at 4 weeks

Chlopyrifos	**Dichlorvos**	Alfacypermethrin
 Plate 3 TS of liver with Chronic inflammation (blue arrow) and mild necrosis (black arrows)	 Plate 4 **T.S. of** the liver, with chronic inflammation	 Plate 5 **T.S** of the liver, with periportal inflammation (periportal hepatitis)
 Plate 6 TS of brain – Normal Histology	 Plate 7 **T.S** the brain, with mild cerebral oedema	 Plate 8 T.S of the brain, with widened perivascular space (cerebral oedema)
 Plate 9 Lungs with mild congestion	 Plate 10 T. S of the Lungs, with congested blood vessels.	 Plate 11 T.S of the lungs, with no pathology.
 Plate 12 T.S. of the Kidney, with normal histology	 Plate 13 T.S of the Kidney with mild interstitial inflammatory infiltrate (interstitial nephritis).	 Plate 14 T.S of the kidney, with mild interstitial nephritis.

Histology sections of Mice exposed to the insecticides at 8 weeks

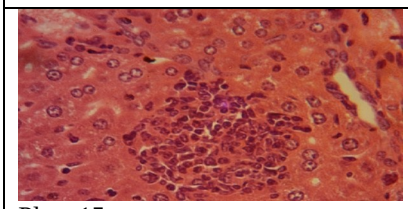
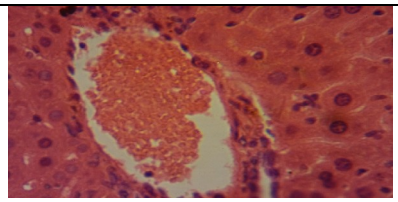
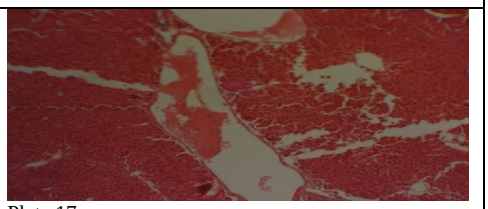
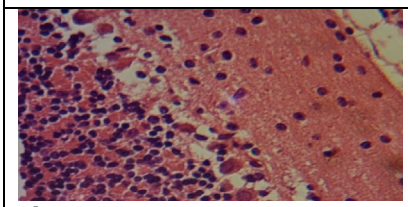
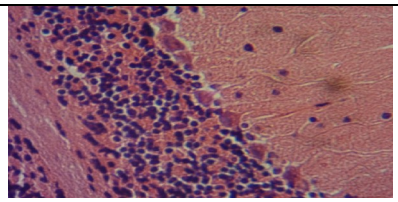
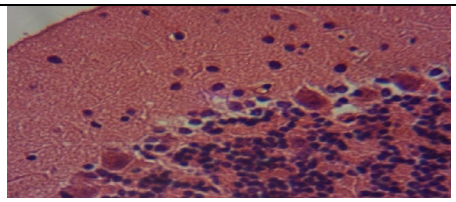
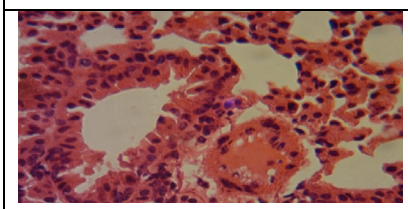
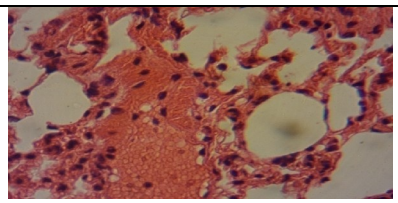
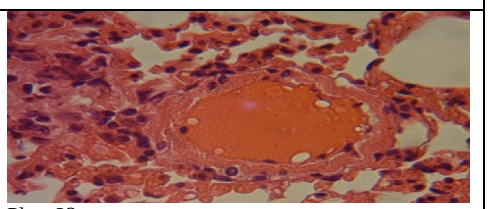
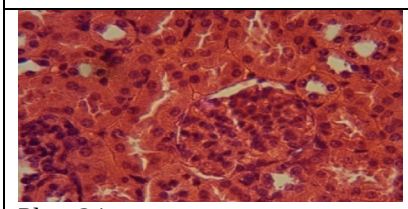
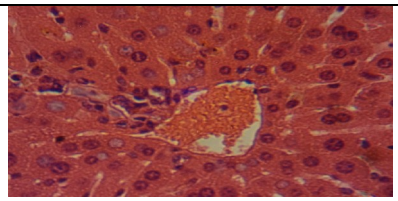
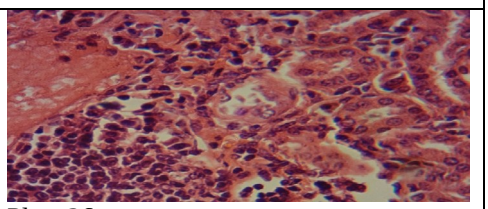
Chlopyrifos	**Dichlorvos**	Alfacypermethrin
 Plate 15 T.S of the liver, with mild hepatitis	 Plate 16 T.S of the liver, with mild congestion	 Plate 17 T.S of the liver, with dilated blood vessel (white arrow) and mild congestion (black arrow).
 Plate18 T.S of the brain, with no pathology	 Plate 19 T.S of the brain, with no pathology	 Plate20 T.S of the brain, with no pathology
 Plate 21 T.S of the lungs, with moderately congested blood vessel	 Plate 22 T.S. of the lungs, with mild congestion	 Plate 23 T.S of the lungs with, mild to moderate congestion of blood vessels.
 Plate 24 T.S of the kidney, with no pathology	 Plate 25 T.S of the kidney, with no pathology	 Plate 26 T.S of the kidney, with chronic inflammation.

Table 1: Means and their standard deviations (SD) for peripheral blood cell indicators in *M. musculus*

Chlorpyrifos			Dichlorvos			Alpha Cypermethrin		
WBC			WBC			WBC		
Control	4 weeks	8 weeks	Control	4 weeks	8 weeks	Control	4 weeks	8 weeks
7.47±1.21	13.23±5.75*	5.57±1.14	7.47±1.21	5.03±1.60	4.97±0.75	7.47±1.21	5.13±2.65	6.43±1.12
RBC			RBC			RBC		
Control	4 weeks	8 weeks	Control	4 weeks	8 weeks	Control	4 weeks	8 weeks
9.73±0.55	8.47±0.88	7.64±0.79*	9.73±0.55	7.26±0.98*	7.23±0.98*	9.73±0.55	7.15±1.04*	7.36±1.08*
HGB			HGB			HGB		
Control	4 weeks	8 weeks	Control	4 weeks	8 weeks	Control	4 weeks	8 weeks
15.53±0.81	13.87±0.35	13.60±0.27	15.53±0.81	13.03±1.10	11.70±1.11*	15.53±0.81	13.23±0.21	12.00±1.63*
PCV			PCV			PCV		
Control	4 weeks	8 weeks	Control	4 weeks	8 weeks	Control	4 weeks	8 weeks
46.27±0.85	44.23±0.65	42.73±2.23*	46.27±0.85	43.40±0.85*	42.43±1.29*	46.27±0.85	43.37±1.30*	42.37±0.85*

* indicate significant (P<0.05) difference in the blood cell indicators when compared to the control

Table 2: Means and standard deviations (SD) for biochemical parameters in *M. musculus*

Chlorpyrifos			**Dichlorvos**			**Alphacypermethrin**		
AST			AST			AST		
Control	4 weeks	8 weeks	Control	4 weeks	8 weeks	Control	4 weeks	8 weeks
137.5±21.57	193.4±52.88	244.3±58.31	137.5±21.57	216.8±55.05	163.9±114.7	137.5±21.57	256.4±104.1	206.6±141.4
ALP			ALP			ALP		
Control	4 weeks	8 weeks	Control	4 weeks	8 weeks	Control	4 weeks	8 weeks
138.7±33.48	126.5±26.35	301.0±21.50**	138.7±33.48	178.3±29.06	112.6±49.50	138.7±33.48	167.7±22.32	197.1±23.33
ALT			ALT			ALT		
Control	4 weeks	8 weeks	Control	4 weeks	8 weeks	Control	4 weeks	8 weeks
72.00±30.90	66.00±25.78	55.43±40.24	72.00±30.90	35.07±17.72	61.27±39.24	72.00±30.90	92.10±67.34	47.10±39.56
CREA			CREA			CREA		
Control	4 weeks	8 weeks	Control	4 weeks	8 weeks	Control	4 weeks	8 weeks
18.93±4.99	27.26±7.60	37.89±2.58	18.83±4.99	26.64±5.19	27.41±8.08	18.83±4.99	29.45±9.41	27.25±10.88
UREA			UREA			UREA		
Control	4 weeks	8 weeks	Control	4 weeks	8 weeks	Control	4 weeks	8 weeks
5.93±1.01	8.7±1.87	9.23±2.37	5.93±1.01	8.5±1.99	10.00±3.37	5.93±1.01	8.03±3.64	10.00±3.01

* indicate significant level ($P<0.05$) difference in biochemical parameters compared to the control