Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 98, No. 2, March 1989, pp. 133–137.

Insecticide induced hematological changes in pigeons

ADITYA MANDAL and PULAK LAHIRI
Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Calcutta 700 019, India

MS received 10 November 1986; revised 24 May 1988

Abstract. Hematological responses of bluerock pigeon (*Columba livia* Gmelin) were studied after oral administration of chlordane (a cyclodiene), fenitrothion (a phosphothioate) and carbaryl (a carbamate) for one week. Comparable hematological disorders were induced by these insecticides which include reduction in total count of peripheral erythrocytes, hemoglobin content, hematocrit and total cellularity of spleen. Total count of peripheral leucocytes, on the otherhand, increased with marked heterophilia together with lymphopenia and monocytopenia. Both bleeding and clotting time became conspicuously prolonged in the experimental birds. The results indicate potential to use hematological responses for rapid on the spot assay of insecticide toxicity in non-target animals.

Keywords. Hematology; anemia; insecticide; spot-assay; pesticide monitoring.

1. Introduction

Abundant use of insecticides in the field has indeed posed serious risks to wild life, particularly for the more sensitive animals like birds (Stickel 1973). India is one of the largest users of agricultural insecticides in recent times (Allen *et al* 1984). Of the organic insecticides used in India, organochlorines constitute the largest group, followed by phosphatic and carbamate compounds (Anon 1984).

We have been studying the feasibility of using hematological responses to predict early warning of insecticide-toxicity in non-target animals. Pigeon is found to be quite sensitive to insecticides (Mandal 1986; Mandal and Lahiri 1985). The present paper reports hematological responses of pigeons following short duration exposure to selected insecticides representing 3 principal classes.

2. Materials and methods

Sexually mature bluerock pigeon (*Columba livia* Gmelin) of either sex trapped from the same geographical areas were procured locally during the months of March and April. They were acclimatized to laboratory conditions atleast for 7 days and were maintained on adequate balanced diet (Chakrabarti 1986). In pilot experiments dietary influence was investigated and no variation in hematological parameters was noticed with the balanced diet supplied to the birds. Five birds were kept in each cage. The cages were sufficiently large to allow the pigeons a reasonable freedom of movement. Ten birds were evenly divided into control and experimental groups for each set of experiment. Olive oil suspension of fenitrothion [0 0-dimethyl-0(3-methyl-4-nitrophenyl) phosphothioate], chlordane (β-cis-octa chlorometheno-tetrahydroindane) and carbaryl (1-naphthyl-N-methyl-carbamate) were administered intragastrically twice per week for one week only, as per the following schedule (Mandal 1986):

Control — Vehicle only
Group I — Chlordane 5 mg/kg body wt
Group II — Fenitrothion 0·1 mg/kg body wt
Group III — Carbaryl 0·1 mg/kg body wt

Following termination of experiment after one week, blood samples were collected from the pectoral vein in tubes previously rinsed in heparin for analyses of hematological indices. The birds were then killed by decapitation and the spleens were quickly dissected out for total count of cellularity. Peripheral blood counts of red blood cells (RBC) and white blood cells (WBC) were done with a hemocytometer by the method of Natt and Herrick (1952). Bleeding time (BT) was recorded according to Duke's method (Kolmer *et al* 1969). Clotting time (CT) was measured by the capillary glass tube method (Kolmer *et al* 1969) and haemoglobin (Hb) was determined by the Hellige method (Halaz 1967). Avian blood cells settle very slowly and therefore to determine erythrocyte sedimentation rate (ESR), sedimentation tubes (Westergren) were positioned at 45° (Washburn and Meyers 1957), thereby substantially increasing the sedimentation rate. Hematocrit (hct) was determined by microhematocrit method (Wintrobe *et al* 1976). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from the RBC count, hemoglobin content and hematocrit (Dacie and Lewis 1975). WBC differential counts were made from Wright stained blood smears (Lucas and Jamroz 1974). The number of nucleated cells per spleen was determined with a hemocytometer after mincing the organ in cold phosphate-buffer saline and sequential passage through 20–, 22– and 23 gauge needle. Since avian spleen produces only lymphocytes (Payne 1971; Hodges 1974), differential counts of spleen were not done. Statistical analyses were done by Student's '*t*' test.

3. Results

The birds remained active and healthy throughout the experiment. Apetite and food intake was normal and body weight remained unaltered in the experimental birds. There was no pathological symptoms to suggest any overt toxicity due to insecticide administration.

Results of hematological studies have been presented in tables 1 and 2. Mild to moderate changes of hematological indices were observed in the pigeons following intake of all the 3 insecticides. It is evident from table 1 that in all the groups of birds significant reduction of total peripheral RBC, Hb content and hct was noticed. Also, ESR and MCV remained unchanged in these birds. MCH and MCHC were reduced in chlordane and carbaryl-fed pigeons. However, there was significant increase in the total counts of peripheral WBC in the insecticide fed pigeons. Simultaneously, prolongation of both bleeding time and clotting time was observed in such birds. Differential counts of formed elements in the experimental pigeons is characterised by both marked heterophilia and significant lymphopenia and monocytopenia (table 2). Eosinophilia was observed only in chlordane and carbaryl-fed pigeons. Total splenic cellularity was reduced significantly in all the insecticide fed birds, though there was no significant change of weight of this organ (table 3).

Table 1. Hematological indices of the whole blood of control and insecticide-fed pigeons.

	Control	Chlordane	Control	Fenitrothion	Control	Carbaryl
Total count of RBC ($10^6/\mu l$)	2·60 ± 0·11	2·20 ± 0·10^b	2·66 ± 0·05	2·25 ± 0·16^b	2·60 ± 0·11	2·20 ± 0·10^b
Hemoglobin (g/dl)	12·80 ± 0·50	9·02 ± 0·88^a	14·13 ± 0·34	12·01 ± 0·76^b	12·80 ± 0·50	9·02 ± 0·88^a
Hematocrit (%)	38·33 ± 1·20	32·25 ± 1·85^b	35·16 ± 0·75	30·00 ± 1·40^b	38·33 ± 1·20	32·25 ± 1·85^b
ESR (mm/h)	40·50 ± 1·85	37·16 ± 1·50^{NS}	42·10 ± 1·30	39·00 ± 1·03^{NS}	40·50 ± 1·85	37·16 ± 1·50^{NS}
MCV (fl)	147·42 ± 1·11	146·60 ± 0·98^{NS}	132·18 ± 4·10	133·33 ± 2·20^{NS}	147·42 ± 1·11	146·60 ± 0·98^{NS}
MCH (pg)	49·23 ± 2·21	41·00 ± 2·32^b	53·12 ± 1·40	53·37 ± 1·22^{NS}	49·23 ± 2·21	41·00 ± 2·32^b
MCHC (g/dl)	33·39 ± 1·35	27·96 ± 1·40^b	40·19 ± 1·60	40·03 ± 0·87^{NS}	33·39 ± 1·35	27·96 ± 1·40^b
BT (s)	50·25 ± 4·28	70·83 ± 3·53^a	48·83 ± 2·53	58·00 ± 2·65^b	50·25 ± 4·28	70·83 ± 3·53^a
CT (s)	26·75 ± 1·18	31·00 ± 1·24^b	30·66 ± 1·86	36·00 ± 1·26^b	26·75 ± 1·18	31·00 ± 1·24^b

Values are mean ± SE. P values: $^a < 0{\cdot}01$, $^b < 0{\cdot}05$. NS, Not significant.

Table 2. Total and differential count of WBC in the whole blood of control and insecticide-fed pigeons.

	Control	Chlordane	Control	Fenitrothion	Control	Carbaryl
Total count of WBC ($10^3/\mu l$)	15·75 ± 0·58	18·30 ± 0·88^b	15·90 ± 0·73	17·68 ± 0·24^b	15·75 ± 0·58	18·30 ± 0·88^b
Differential count (%)						
Lymphocyte	63·60 ± 2·44	54·55 ± 2·10^b	62·20 ± 1·62	52·90 ± 2·50^b	63·60 ± 2·44	54·55 ± 2·10^b
Monocyte	2·53 ± 0·13	2·10 ± 0·10^b	3·10 ± 0·20	2·30 ± 0·10^a	2·53 ± 0·13	2·10 ± 0·10^b
Heterophil	26·89 ± 2·15	35·54 ± 1·40^a	27·52 ± 2·10	37·24 ± 1·60^a	26·89 ± 2·15	35·54 ± 1·40^a
Eosinophil	5·80 ± 0·25	6·79 ± 0·32^b	6·18 ± 0·35	6·70 ± 0·20^{NS}	5·80 ± 0·25	6·79 ± 0·32^b
Basophil	1·18 ± 0·09	1·02 ± 0·01^{NS}	1·00 ± 0·06	0·86 ± 0·05^{NS}	1·18 ± 0·09	1·02 ± 0·01^{NS}

Same notations as in table 1.

Table 3. Total cell count and weight of spleen of control and insecticide-fed pigeons.

	Control	Chlordane	Control	Fenitrothion	Control	Carbaryl
Total count of splenic cells (10^9/spleen)	3·00 ± 0·10	2·05 ± 0·24^a	3·09 ± 0·15	2·59 ± 0·11^b	3·00 ± 0·10	2·05 ± 0·24^a
Weight of spleen (mg)	417·50 ± 7·61	391·20 ± 8·70^{NS}	418·20 ± 20·14	400·33 ± 19·30^{NS}	417·50 ± 7·61	391·20 ± 8·70^{NS}

Same notations as in table 1.

4. Discussion

Organic insecticides belonging to different classes induced more or less similar hematologic disorders in pigeon even when administered for short duration. The most common response being the development of mild to moderate anemia as evidenced by significant reduction of total count of RBC, Hb content and hct in the insecticide-fed birds. Reduction in Hb content can be attributed to the decreased RBC number. While Hb content and hct decreased in the insecticide-fed pigeons, there was no marked change in MCV. MCH and MCHC decreased only in chlordane and carbaryl-fed pigeons (table 1). It thus appears that anemia is secondary to possible accelerated hemolysis, hemorrhage and/or reduced erythropoiesis inflicted by these insecticides in the pigeons. Similar effects have also been found in birds chronically exposed to insecticides (Mandal and Lahiri 1985; Mandal 1986). However, the mechanism of anemia in fenitrothion-fed pigeons might differ from other two groups since MCH and MCHC did not change as in chlordane or carbaryl-fed birds.

Normally in pigeons, lymphocytes constitute approximately 62% of the WBC population. Concomitant with increase in the total number of circulating leucocytes in the insecticide-fed pigeons, differential counts of these formed elements have undergone a shift from lymphocytosis to heterophilia. Such virtual reversal of lymphoid-myeloid ratio in the insecticide-fed pigeons possibly indicates systemic reaction to these chemicals. In birds both lymphocytes and monocytes are formed in the spleen (Lucas and Jamroz 1974); thus observed lymphopenia and monocytopenia in the experimental birds possibly result from decreased cellularity of the spleen (table 3).

There was consistent prolongation of both bleeding and clotting time in such birds. Increase in clotting time was reported in rats (Srinivasan and Radhakrishnamurthy 1983), humans (Barsel'yants 1969), mice (Gupta *et al* 1983), pigeons and other birds (Mandal and Lahiri 1985; Mandal 1986), particularly after long exposure to various insecticides. It is evident from the present study that all the 3 classes of insecticides are quite hematotoxic and evoke common disorders in pigeon even when exposed for relatively short duration. Comparable hematologic response was reported for rather longer exposure to such chemicals (Mandal and Lahiri 1985; Mandal 1986). Hematological responses of pigeons to insecticides thus seem to be quite effective for rapid detection of prepoisoning cases and may be used for on the spot-assay of toxicity of insecticides particularly in the field situation.

Acknowledgements

The authors are grateful to the University Grants Commission, New Delhi for the

financial help. Thanks are due to Dr J Chakraborty of the Central Food Laboratory, Calcutta for fenitrothion and carbaryl. Mr M R Ray of the Chittaranjan National Cancer Research Centre, Calcutta deserves special thanks for his assistance in this study.

References

Allen J R, Hargraves W A, Hsia S M T and Lin F S D 1984 *Differential toxicities of insecticides and halogenated aromatics* (ed.) F Matsumura (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press) Chapter 12, pp 469–503

Anonymus 1984 Working group on pesticides; *Pestic. Inf.* **10** 34

Barsel'yants G B 1969 Effect of toxic chemicals on human blood serum proteins; *Gig. Tr. Prof. Zabol.* **13** 50

Chakrabarti S 1986 *Organochlorine pesticide effects on gonadal function in domestic duck*, Ph.D. thesis, Calcutta University, Calcutta

Dacie J V and Lewis S M 1975 *Practical haematology* Fifth edition (Edinburgh, London, New York: Churchil Livingstone)

Gupta M, Sasmal D, Bandopadhyay S, Bagchi G, Chatterjee T and Dey S 1983 Haematological changes produced in mice by Ochratoxin A and Citrinin; *Toxicology* **26** 55

Halaz M 1967 A contribution to the study of the blood picture in some kind of breeds of ducks; *Acta Zootech. Univ. Agric. Nitra (Czechoslavakia)* **16** 91

Hodges R D 1974 *The histology of fowls* (London, New York, San Francisco: Academic Press)

Kolmer J A, Spaulding E H and Robinson H W 1969 *Approved laboratory technique* Fifth edition (first Indian edition), (New Delhi: Appleton-Century-Crofts) pp 39–126

Lucas A M and Jamroz C 1974 *Atlas of avian hematology* United States Department of Agriculture, Monograph 25, (Indian edition) (New Delhi: Oxford and IBH Publishing Co.)

Mandal A 1986 *Studies on physio-pathological effects of pesticides on some common Indian birds*, Ph.D. thesis, Calcutta University, Calcutta

Mandal A and Lahiri P 1985 Hematological responses to sumithion (fenitrothion) in the blue-rock pigeon *Columba livia* Gmelin; *Indian J. Exp. Biol.* **23** 702

Natt M P and Herrick C A 1952 A new blood diluent for counting the erythrocytes and leucocytes of the chicken; *Poult. Sci.* **31** 735

Payne L N 1971 The lymphoid system; in *Physiology and biochemistry of the domestic fowl* (eds) D J Bell and B M Freeman (London: Academic Press) Vol 3, pp 985–1037

Srinivasan K and Radhakrishnamurthy R 1983 Effect of dietary intake of HCH isomers on some hematological parameters; *J. Fd. Sci. Technol.* **20** 322

Stickel L F 1973 Pesticide residues in birds and mammals; in *Environmental pollution by pesticides* (ed.) C A Edwards (London, New York: Plenum Press) pp 254–312

Washburn A H and Meyers A J 1957 The sedimentation of erythrocytes at an angle of 45 degrees; *J. Clin. Lab. Med.* **49** 318

Wintrobe M M, Lee G R, Boggs D R, Bithell T C, Athens J W and Foerster J 1976 *Clinical hematology* Seventh edition (Philadelphia: Lea and Febiger)