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Chronic Toxicity of Phosalone to Rats: Effect on Erythropoiesis

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It is well known that organophosphorus insecticides (OPI) act as nerve poisons by blocking synaptic transmission in cholinergic part of the nervous system (Murphy 1980). Phosalone [O,O-diethyl-S-(6-chloro-1,3-benzoxozol-2(3H)-onylmethyl)phosphorodithioate], an OPI, is being extensively used to kill insect crop pests in this part of India. Studies on the effects of phosalone on physiology of vertebrates are very few. Prolonged exposure to phosalone has been reported to cause marked changes in body weight, liver weight and RBC of rats (FAO/WHO 1973b). Palanivelu (1984) observed a significant diminution in oxygen consumption and electrical activity of different regions of rat brain exposed to phosalone. Sunita et al. (1987) reported a decrease in brain SDH activity of rat dosed with phosalone. Ravi and Selvarajan (1986) reported a decrease in brain acetylcholinesterase (AchE) activity of fish exposed to phosalone. However, the effect of multiple sublethal doses of phosalone on haemopoietic system of rat has not yet been fully evaluated. The present investigation is one such attempt over a 60 day period.

MATERIALS AND METHODS

Pure wistar strain male albino rats, Rattus norvegicus (115 ± 5g) obtained from King Institute, Madras, India, were used as test animals. Before experimentation the rats were housed in clean polythene cages and acclimatized to laboratory conditions (Temperature: 25±2°C; D12hr: L12hr period) for about a week as per the instructions of Behringer (1973). They were fed on formulated rat feed (Hindustan Lever Ltd., Bombay, India) and provided clean drinking water **ad lib**. Feeding was stopped 24hr before commencing experiments to avoid metabolic variations due to diet, if any.

Technical grade phosalone (93% pure), an insecticide and acaricide, was chosen. Since the percent dissolubility of phosalone is more in acetone than in any other solvent (Rhone-Poluenc Co.,) acetone was used as the solvent. The LD_{50} of phosalone to rat calculated by the moving average method of Weil (1952) was found to be 127 mg/kg body weight with 95% confidence

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limits of 91.4 to 176.6 mg/kg (Janardan Reddy 1988). The acclimatized rats, ready for experimentation, were divided into seven groups of six animals each. The first group was the control and received a daily maximum dose of 0.1 ml acetone and the remaining six were experimental groups each of which received a daily dose of 1/10th LD_{50} of phosalone (12.7 mg/kg/day) for 1,3,7,15,30 and 60 days respectively. After the expiry of each dosed period, the rats were anaesthetized with ether and blood was collected into a hypodermic syringe rinsed with heparin through direct heart puncture. Later the blood was transferred into sterilized glass vials (0.4 mg heparin per 2 ml of blood) kept at ice cold temperature and immediately used. Serum was obtained by centrifuging the blood at 1500 rpm for 10 min in separate tubes having no anticoagulant.

The whole animal oxygen consumption and kidney oxygen consumption were determined by Winkler's Iodometric method (Welsh and Smith 1961) and by using constant volume respirometer (Umbriet et al. 1972) respectively. The red blood corpuscles (RBC) count and white blood corpuscles (WBC) count were made by Neubaur crystalline counting chamber (Davidson and Henry 1969); haemoglobin (Hb) concentration was measured by acid haematin method (Sahli 1966) and packed cell volume (PCV) by microhaematocrit method (Schalm et al. 1975). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated after obtaining RBC, Hb and PCV values. Serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and serum acid phosphatase (SAP) were estimated by the methods given by Bergmeyer (1974). Significance of the data was assessed through student's 't' test.

RESULTS AND DISCUSSION

The changes in whole animal oxygen consumption and kidney respiration and haematological parameters of the rats dosed with phosalone are presented in Tables 1 and 2 respectively. Table 3 revealed changes in serum enzyme activities.

It is evident from the results that phosalone caused a gradual decrease ($P < 0.05$) in whole animal oxygen consumption and the rate of oxygen consumption of the kidney. The decrease in whole animal oxygen consumption might be due to damage to the structural integrity of the cells of respiratory organs (Saraswathi and Indira 1986) by phosalone. In general, decrease in whole animal oxygen consumption leads to a decrease in oxygen consumption of body tissues and this, perhaps, explains the decrease in kidney oxygen consumption. Reduced ability of the rat to extract oxygen, after being dosed with phosalone, indicates the existence of hypoxic condition in rat. It is well known that hypoxia constitutes the fundamental stimulus for erythropoiesis and that a diminution in oxygen consumption of the kidney which is the sensing organ for low O_2 tension promotes the production of erythropoietin (Gordon et al. 1967). Erythropoietin thus produced

Table 1. Variations in whole animal oxygen consumption (WAOC) and kidney oxygen consumption (KOC) in male albino rats, **Rattus norvegicus**, dosed for 1,3,7,15,30 and 60 days with multiple sublethal doses of phosalone. Values are mean±SD of six individual observations.

Parameter	Control	Dosed (days)					
		1	3	7	15	30	60
WAOC (ml O_2/hr)	34.77 ±1.36	33.15 ±1.46	32.41 ±1.75	30.63 ±1.68	27.84 ±1.27	26.07 ±1.32	24.28 ±1.24
% Change	-	-4.66	-6.78	-11.91	-19.93	-25.02	-30.17
KOC ($\mu L O_2$/g wet tissue)	3428 ±186	3216 ±175	3156 ±213	2987 ±163	2741 ±196	2535 ±157	2316 ±165
% Change	-	-6.18	-7.93	-12.86	-20.04	-26.05	-32.44

might have caused an increase in RBC count of the rats since it is known that erythropoietin promotes erythropoiesis by inducing primitive cells in bone marrow to differentiate into erythroblasts that subsequently mature and form non-nucleated red cells (Hodgson 1970). The erythropoietin, produced under hypoxic condition, could also have activated pyridoxal phosphate enhancing the rate of synthesis of haemoglobin in the developing red cells resulting in an increase in Hb concentration. Increase in PCV in the present study could be attributed to an increase in RBC since PCV was found to increase when red cell population increases and decrease when red cell population declines (Davidson and Henry 1969). Decrease in MCV reflecting variation in red cell volume implies the occurrence of exosmosis as indicated by increased electrolyte concentration inside the red cell after insecticidal treatment (Janardan Reddy 1988). Damage to red cell membrane by the insecticide might have resulted in leakage of some amount of Hb into the plasma leading to a decrease in MCH. Increase in MCHC might well be correlated with an increase in Hb and PCV, since MCHC represents the relationship between Hb and PCV.

The serum enzymes are a sensitive index to changes in ecological conditions and can constitute an important diagnostic tool in toxicological studies (Dabrowska and Wlasow 1986). A progressive increase in the activities of SGOT, SGPT and SAP till day 60 (Table 3) is indicative of liver damage and thus alterations in liver function since these are the enzymes that reflect liver damage when their serum levels are enhanced (Balazs 1981). Apparently liver damage in the present study seems to have a potentiating effect on the quantities of erythropoietin produced in the kidney since it was found that erythropoietin will appear

Table 2. Variations in red blood corpuscles (RBC), haemoglobin (Hb) concentration, packed cell colume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and white blood corpuscles (WBC) of male albino rats, **Rattus norvegicus**, dosed for 1,3,7,15,30 and 60 days with multiple sublethal doses of phosalone. Values are mean±SD of six individual observations.

Parameter	Control	Dosed (days)					
	1	3	7	15	30	60	
RBC (m/mm_3)	8.35 ±0.26	8.69 ±0.24	8.95 ±0.32	9.36 ±0.37	9.55 ±0.42	10.79 ±0.53	11.93 ±0.47
% Change	-	4.07	7.19	12.09	14.37	29.22	42.87
Hb (g/100 ml)	15.37 ±0.05	16.72 ±1.12	18.35 ±1.42	19.84 ±1.35	21.33 ±2.51	23.84 ±1.93	25.17 ±1.82
% Change	-	10.08	19.39	29.08	38.78	55.10	63.76
PCV (vol %)	69.52 ±3.34	72.85 ±2.42	75.33 ±3.25	77.54 ±3.53	79.31 ±2.50	81.42 ±2.31	85.34 ±4.49
% Change	-	4.79	8.36	11.54	14.08	17.12	22.75
MCV (cμ)	84.58 ±3.25	80.72 ±2.86	73.48 ±5.04	68.33 ±7.05	65.99 ±3.46	64.16 ±5.57	61.07 ±4.61
% Change	-	-4.56	-13.12	-19.21	-21.98	-24.14	-27.79
MCH (μμg)	18.59 ±1.15	17.12 ±1.20	16.25 ±1.21	15.92 ±1.53	15.13 ±1.42	14.46 ±1.34	14.05 ±1.26
% Change	-	-7.91	-12.58	-14.36	-18.61	-22.21	-24.42
MCHC (%)	20.84 ±1.32	22.91 ±1.46	23.63 ±2.13	23.98 ±2.17	24.49 ±2.12	24.91 ±2.34	25.35 ±2.14
% Change	-	9.93	13.39	15.07	17.51	19.53	21.64
WBC (Thousands/ mm_3)	7.95 ±0.42	9.27 ±0.37	9.78 ±0.44	10.56 ±0.42	11.58 ±0.51	11.89 ±0.62	12.64 ±0.58
% Change	-	16.60	23.02	32.83	45.66	49.56	59.49

more consistently in the blood of those hypoxic rats which display considerable liver damage (Wickramasinghe 1975). These observations suggest that multiple sublethal doses of phosalone

Table 3. Variations in serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and serum acid phosphatase (SAP) activities in male albino rats, **Rattus norvegicus**, dosed for 1, 3, 7, 15, 30 and 60 days with multiple sublethal doses of phosalone. Values are mean±SD of six individual observations.

Parameter	Control	Dosed (days)					
		1	3	7	15	30	60
SGOT (µmoles/ hr/ml serum)	16.55 ±0.75	18.86 ±0.42	19.21 ±0.38	19.75 ±0.44	19.98 ±0.53	20.34 ±0.33	28.86 ±0.46
% Change	-	13.95	16.07	19.34	20.73	22.90	26.04
SGPT (µmoles/ hr/ml serum)	14.95 ±0.44	15.75 ±0.25	16.32 ±0.34	16.73 ±0.52	16.91 ±0.22	17.42 ±0.37	17.84 ±0.47
% Change	-	5.35	9.16	11.91	13.11	16.52	19.33
SAP (µmoles/ min/ml enzyme)	0.284 ±0.040	0.332 ±0.031	0.370 ±0.007	0.411 ±0.005	0.463 ±0.041	0.498 ±0.062	0.522 ±0.052
% Change	-	16.90	30.28	44.72	63.03	75.35	83.80

besides producing hypoxia could have promoted erythropoiesis in rat by increasing the production of erythropoietin.

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