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"No-effect-level" of Melathion (0, 0'-dimethyl Dithiophosphate of Diethyl Mercaptosuccinate) in *Bubalus bubalis*

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With the stepping up in the use of pesticides for enhancing agricultural production, the chances of environmental pollution and their residual concentrations in various foodstuffs, have also increased. Consequently many health hazards in human beings and animals have occurred. FAO/WHO (1965) Joint Expert Committee on Pesticides in Food has emphasized the establishment of the data on maximum safe levels (no-effect-level) of various pesticides in maximum number of species, which could further form the basis of calculating ADI in man. Such information is available only for a few organophosphorus insecticides to a limited extent and is mostly in laboratory animals like rat. However, the information in this regard in livestock, especially in Bubalus bubalis is absolutely not available. It is, therefore, considered to be of great interest to investigate the no-effect-level of malathion in this species.

MATERIALS AND METHODS

In this study a total 17 apperently healthy buffalo calves of non-descriptive breed aged six to nine months were used. Out of this 5 animals were used for conducting a preliminary study, where the calves were orally administered various doses (1.0, 1.5, 2.0 and 2.5 mg/kg/day) of malathion (Technical grade, 95 per cent) for one month and the effect on cholinesterase activity was observed. On the basis of this information a detailed study was conducted in 12 animals divided into four groups with three animals in each. The animals of group II, III and IV were orally administered daily for one year with 0.5, 1.0 and 1.5 mg/kg malathion, respectively and group I was kept as control. The following biochemical and haematological parameters were estimated from jugular blood samples at various time intervals.

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BIOCHEMICAL

Cholinesterase (ChE): The activity of RBC ChE (RChE, E.C. 3.1.1.7) and plasma ChE (PChE, E.C. 3.1.1.8) was assayed according to the electrometric method of MICHEL (1949). The activity was calculated as Delta pH/hr and expressed in terms of per cent activity.

Aminotransferases: The activity of aspartate aminotransferase (E.C. 2.6.1.1.) and alanine aminotransferase (E.C. 2.6.1.2.) was assayed in serum by the colorimetric procedure of REITMAN and FRANKEL (1957) as described by WOOTTON (1964). The activities were expressed as μ mol pyruvate/min/L.

Blood glucose, serum urea and plasma total proteins: Blood glucose was measured according to the method of FRANKEL, et al. (1970). Serum urea was determined according to the urease method of BRENT and BERGMYER (1965). Total protein was determined in plasma according to the method described by WOOTTON (1964).

HAEMATOLOGICAL

Haemoglobin (Hb), packed cell volume (PCV), total red blood cells (tRBC) and total white blood cells (tWBC) were estimated according to the standard methods detailed by SONI, et al. (1967).

EVALUATION OF DATA

The data were analysed by the student's 't' test (LI, 1964).

RESULTS

PRELIMINARY STUDY

There was no inhibition in RChE or PChE with 1.0 mg/kg dose and higher doses (1.5, 2.0 and 2.5 mg/kg) produced inhibition in both RChE and PChE.

DETAILED STUDY

Cholinesterase (ChE): Figure 1 represents the percentage change in RChE and PChE from the control of 100 per cent. The activities of RChE and PChE were not inhibited by the administration of 0.5 mg/kg of malathion daily for one year. With 1.0 and 1.5 mg/kg doses the inhibition in RChE appeared at the end of 8th month (89.7 per cent) and 2nd month (89.7 per cent), respectively and maximal inhibition was recorded at the end of one year (71.0 and 53.3 per cent, respectively). With 1.0 and 1.5 mg/kg

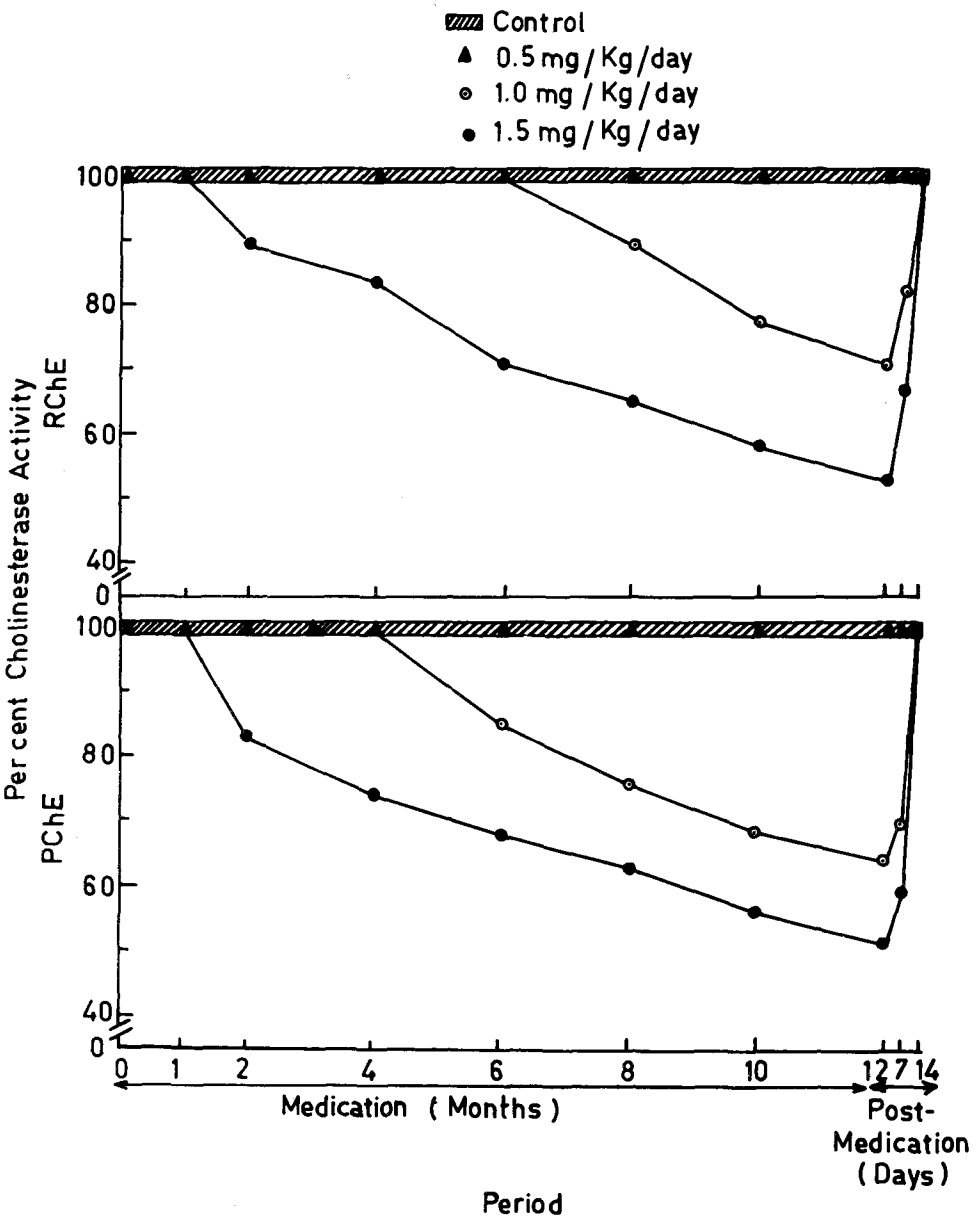

FIGURE 1 - Effect of continuous oral administration of malathion in different doses for one year and during post-medication period on RBC cholinesterase (RChE) and plasma cholinesterase (PChE) in buffalo calves.

doses the inhibition of PChE appeared at the end of 6th month (85.7 per cent) and 2nd month (83.3 per cent) respectively and the maximal inhibition was recorded at the end of one year (64.1 and 51.4 per cent, respectively)

Aminotransferases, blood glucose, serum urea, and plasma total proteins (Table 1)

With 0.5 mg/kg dose, there was no significant ($P > 0.05$) change in aminotransferases, blood glucose, serum urea and plasma total proteins. With 1.0 and 1.5 mg/kg doses the significant ($P < 0.05$) elevation in alanine aminotransferase at the end of 12th month (49.5 $\pm$ 0.3 μ mol pyruvate/min/L) and 8th month (54.3 $\pm$ 0.7 μ mol pyruvate/min/L) respectively. Significant ($P < 0.05$) increase in aspartate aminotransferase was observed with 1.5 mg/kg dose at the end of one year (70.6 $\pm$ 1.0 μ mol pyruvate/min/L).

With 1.5 mg/kg dose significant elevation of blood glucose (85.6 $\pm$ 2.4 mg/100 ml) and serum urea (38.9 $\pm$ 2.2 mg/100 ml) occurred at the end of 12th month and 4th month, respectively. Plasma total proteins showed significant ($P < 0.05$) reduction with 1.0 and 1.5 mg/kg doses (7.0 $\pm$ 0.0 and 6.6 $\pm$ 0.1 g/100 ml, respectively) at the end of 8th month.

Haematological parameters: There was no significant ($P > 0.05$) change in any of the haematological parameters studied (Hb, PCV, tRBC and tWBC) with 0.5 mg/kg dose. With 1.0 and 1.5 mg/kg doses significant ($P < 0.05$) increase in total white blood cells appeared at the end of 12th month (12.4 $\pm$ 0.5 and 15.5 $\pm$ 0.8 thousands/cu mm, respectively) in comparison to control (8.8 $\pm$ 0.9 and 8.9 $\pm$ 0.6 thousands/cu mm, respectively).

DISCUSSION

The data for ADI (daily intake of compound, which during an entire life-time appears to be without appreciable risk) have been calculated from the basis of no-effect-levels in various sensitive species, viz. rat and dog (FAO/WHO, 1965). From these data permissible and tolerance limits for residues of pesticides in various food stuffs have been calculated. In an attempt to find the no-effect-level of malathion in Bubalus bubalis, the index selected was not only conventional

TABLE 1

Effect of continuous oral administration of malathion in different doses for one year and during post medication period on aminotransferases, blood glucose, serum urea and plasma total proteins, in buffalo calves.

Parameter	Dose (mg/kg/day)	Medication period (months) 0	4	8	12	Post-medication (days) 14
SGOT (u mol pyruvate/min/L)	0.0	67.0±0.0	67.0±0.0	65.0±1.0	64.2±0.5	64.4±0.7
	0.5	64.4±0.6	64.9±1.4	63.2±1.2	64.0±0.4	64.6±0.6
	1.0	65.5±2.3	62.7±1.6	65.0±2.1	65.9±0.6	63.7±1.2
	1.5	61.3±1.3	66.8±0.7	67.4±0.2	70.6±1.0*	62.6±0.8
SGPT (u mol pyruvate/min/L)	0.0	45.3±4.5	44.7±3.7	45.1±2.6	45.0±1.2	45.1±1.3
	0.5	38.4±1.7	39.7±2.8	42.7±0.0	43.6±0.4	43.8±0.1
	1.0	40.2±2.7	43.0±0.5	47.0±3.3	49.5±0.3*	40.7±0.3
	1.5	42.8±3.7	45.7±2.8	54.3±0.7*	58.7±1.1*	41.4±1.8
Blood glucose (mg/100ml)	0.0	70.3±1.3	70.1±0.4	70.4±0.9	70.5±0.1	70.2±0.4
	0.5	70.0±3.6	69.6±0.4	69.0±0.5	69.7±1.1	69.3±1.2
	1.0	68.3±2.3	68.0±1.2	71.3±0.9	74.8±0.9	68.8±0.3
	1.5	70.0±4.0	72.0±0.8	73.1±0.8	85.6±2.4*	70.5±0.6
Serum urea (mg/100ml)	0.0	52.9±3.6	56.1±1.8	56.0±0.0	55.9±0.4	55.5±0.2
	0.5	56.3±3.6	59.8±1.5	59.1±0.6	58.4±0.3	58.5±0.4
	1.0	59.8±3.2	58.2±0.2	40.1±0.3	42.2±1.3	58.5±0.5
	1.5	53.2±2.2	58.9±2.2*	41.9±0.2*	47.1±1.1*	53.2±2.8
Plasma total protein (g/100ml)	0.0	7.7±0.1	7.5±0.0	7.3±0.1	7.5±0.1	7.6±0.1
	0.5	7.7±0.1	7.9±0.1	7.3±0.0	7.5±0.1	7.5±0.0
	1.0	7.9±0.2	7.8±0.1	7.0±0.0*	6.9±0.0*	7.6±0.1
	1.5	7.2±0.0	8.0±0.0	6.6±0.1*	6.5±0.0*	7.5±0.1

* Statistically significant ($P<0.05$) difference when compared with the pre-treated values (0 month).

ChE enzyme inhibition but also other biochemical and haematological parameters were taken into consideration, so that a more authentic information is derived.

The perusal of the data from this study reveal that a dose of 0.5 mg/kg administered continuously for one year, did not cause inhibition of RChE and PChE and also did not affect the other measured biochemical or haematological parameters. However, doses higher than (1.0 and 1.5 mg/kg) caused inhibition of RChE and PChE and also produced other biochemical and haematological changes but no clinical manifestation of toxicity was observed with these doses. In comparison to the no-effect-level of malathion in rat (5 mg/kg/day) and man (2 mg/kg/day) compiled by FAO/WHO (1965), the level obtained in this study in Bubalus bubalis is much lower (0.5 mg/kg/day) which shows that this species is much more sensitive to malathion toxicity than rat and man.

The other biochemical changes seen with higher doses of malathion were elevation of aminotransferases, blood glucose and serum urea and decrease in plasma total proteins. These changes have also been observed during subacute oral toxicity studies in buffalo calves after feeding malathion sprayed fodder at different levels (VADLAMUDI, 1974 and GUPTA and PAUL, 1977). The possible mechanism involved in the elevation of aminotransferases may be due to tissue damage (ROUILLER, 1964), or due to increased synthesis or decreased catabolism of aminotransferases (DENMANN, et al., 1963). The exact mechanism involved in hyperglycaemia is still obscure, however, the possibility of gluconeogenesis (KACEW, et al., 1972) cannot be ruled out. In this study increase in serum urea and decrease in plasma total proteins are suggestive of gluconeogenesis for the observed hyperglycaemia.

The haematological defect in the form of leucocytosis seen in this study may be due to increased formation of leucocytes as proposed by FLOREY (1970) for other drugs (sulphonamides, arsenic and chloramphenicol).

SUMMARY

An oral dose of 0.5 mg/kg/day malathion continuously administered for one year has been investigated to produce no change in biochemical (RChE, PChE, aspartate- and alanine aminotransferases, blood glucose, serum urea and plasma total proteins) or haematological (Hb, PCV, tRBC and tWBC) parameters and, therefore, considered to be "no-effect-level" in Bubalus bubalis. However, the administration of higher doses (1.0 and 1.5 mg/kg/day)

produced significant ($P/0.05$) changes in all biochemical parameters studied. With these doses leucocytosis was evident among haematological parameters measured.

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