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Response of Female Guinea Pigs to Repeated Oral Administration of Quinalphos

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Quinalphos (0,0-diethyl 0-2-quinoxalinyl phosphorothioate) is a broad spectrum organophosphorus insecticide, and in its pure form is available as a white crystalline powder. It is readily soluble in organic solvents but is sparingly soluble in water, about 20 ppm at 20°C. It is unstable both in acid and alkaline media but is more stable under basic donditions. Its half-life is about 32 h at pH 11.0. Technical quinalphos is usually available as a 70% solution in Xylene. Due to its broad spectrum insecticidal action it is used quite extensively in agriculture. Earlier the consumption of this insecticide in India was around 60-300 metric tons (ANONYMOUS 1978). Presently the installed capacity is around 700 metric tons and the projected estimate is about 1000 metric tons (ANONYMOUS 1979).

Information on the toxic effects of quinalphos in species of mammals is very fragmentary. Response of CCl_4 treated male rats to quinalphos and BHC has been studied recently (DIKSHITH et al. 1979). Quinalphos treated goats have shown inhibition of acetylcholinesterase(AchE) activity in red blood cells(RBC). The rate of inhibition of AchE and clinical symptoms of toxicity increased with the additional doses of quinalphos (DIKSHITH et al. unpublished). The significance of measuring cholinesterase activity for diagnostic and treatment of occupational workers in field or work situations is already documented (WHO TECHNICAL REPORT 1967,1973; HOLMSTEDT 1971).

We report here the behaviour of AchE activity in RBC and brain of poisoned female guinea pigs as could be seen in the inhibition and recovery of the enzyme.

MATERIALS AND METHODS

Female guinea pigs of average body weight (250 g) of the Industrial Toxicology Research Centre's colony were housed in the air conditioned room (75±2°F) of the animal house. Animals were maintained on pellet diet (Hind.lever,India) and water ad libitum throughout the period of study. All the animals were separated into five groups, each with 24 animals.

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Preparation of the compound and treatment. Quinalphos (technical grade) obtained from Regional Research Laboratory, Jorhat, India was used. The compound dissolved in peanut oil was administered daily orally to female guinea pigs at amounts of 0,0.5,1.0,2.0 and 4.0 mg/kg for a total period of 30 days.

Animals of group 1 were administered peanut oil (0.2 mL/animal) and were used as control. Guinea pigs of the treated groups and control were killed at regular intervals of 24 and 72 h, 7, 15 and 30 days. Four animals from each group were kept for post-treatment rest. These animals were killed 15 days after the last dose of administration.

Organ body weight ratio. The liver, kidney, adrenal, brain, ovary and uterus were removed and weighed individually. The organ body weight ratio was calculated using statistical method of FISHER (1950).

Histological Studies. Sections of the liver, kidney, adrenal, brain, ovary and uterus were fixed in formalin-saline solution. After routine processing of each tissue, paraffin sections were cut at 6 µm thickness and stained in haematoxylin-eosin.

Acetylcholinesterase estimation. Freshly removed brain was washed free from extraneous material, homogenized in 0.25 M ice cold sucrose solution 10% w/v in a Potter-Elevehjen type homogenizer. RBC was separated by centrifuging the blood at 2500 g for 10 min. Acetylcholinesterase(AchE,Ec3.1.1.7.) activity was assayed by the method of HESTRIN (1949).

RESULTS AND DISCUSSION

Mortality and Morbidity. Guinea pigs exposed to quinalphos (0.5, 1.0 and 2.0 mg/kg/day) for 15 and 30 days did not indicate any symptoms of poisoning or overt toxicity. However, animals dosed with 4.0 mg/kg/day exhibited severe clinical symptoms of toxicity-like tremor, salivation, convulsion and death. Several animals of this group died irregularly within a period of 7 days of administration. Therefore, the tissues of group 5 animals were not used for further study.

Organ body weight ratio. Relative organ body weight of all animals dosed with quinalphos at concentrations of 0.5,1.0 and 2.0 mg/kg/day for 15 and 30 days and also with a post treatment period of rest are shown in Table 1. No significant change was seen in the ratio of liver weight. However a significant fall in the weight of kidney was observed in group 4 after 15 and 30 days of treatment.

TABLE 1

RELATIVE ORGAN WEIGHTS OF QUINALPHOS (0, 0.5, 1.0 and 2 mg/kg) IN FEMALE GUINEA PIGS AFTER DAILY ORAL FEEDING FOR 15 AND 30 DAYS.

Group/ Tissue	15 days				30 days				Post Treatment Rest 15 days		
	I	II	III	IV	I	II	III	IV	II	III	IV
Liver	3.43 ± 0.05	3.44 ± 0.23	3.75 ± 0.16	3.04 ± 0.17	3.16 ± 0.07	3.90[b] ± 0.14	3.24 ± 0.22	3.45[d] ± 0.08	3.34[c] ± 0.08	3.79 ± 0.15	2.20[c] ± 0.31
Kidney	1.19 ± 0.04	0.98[c] ± 0.05	1.02[c] ± 0.04	0.67[a] ± 0.05	1.16 ± 0.06	0.91[c] ± 0.05	1.24 ± 0.04	0.85[b] ± 0.03	0.76[d] ± 0.02	0.84[b] ± 0.06	0.64[c] ± 0.05
Adrenal	0.032 ± 0.001	0.04[b] ± 0.002	0.05[b] ± 0.004	0.032 ± 0.004	0.047 ± 0.004	0.042 ± 0.004	0.04 ± 0.002	0.047 ± 0.002	0.04 ± 0.002	0.05 ± 0.005	0.04 ± 0.004
Brain	1.48 ± 0.02	1.45 ± 0.17	1.48 ± 0.10	0.89[a] ± 0.03	1.19 ± 0.04	1.25 ± 0.06	0.85[a] ± 0.02	0.98[b] ± 0.01	0.92[b] ± 0.04	1.02[c] ± 0.05	0.95 ± 0.04
Ovary	0.042 ± 0.004	0.03 ± 0.002	0.03[b] ± 0.002	0.01[a] ± 0.002	0.02 ± 0.001	0.015 ± 0.003	0.012[d] ± 0.001	0.013[d] ± 0.001	0.016 ± 0.002	0.02[b] ± 0.001	0.01 ± 0.002
Uterus	0.10 ± 0.02	0.06 ± 0.004	0.06 ± 0.01	0.13 ± 0.02	0.18 ± 0.05	0.13 ± 0.02	0.14 ± 0.04	0.16 ± 0.01	0.17 ± 0.02	0.15 ± 0.02	0.08 ± 0.04

a - $P < 0.001$; b - $P < 0.01$; c - $P < 0.02$; d - $P < 0.05$
All values represent the mean ± SE of 4 animals.

Weight changes in the adrenal after 0.5 and 1.0 mg/kg dose and exposed for 15 days was found to be a marginal increase. 1.0 and 2.0 mg/kg of quinalphos caused significant fall in the weight of brain after 30 and 15 days of treatment, respectively. The weight ratio of the ovary was highly significant in 2 mg/kg dose and uniformly lowered in all treatments except in 0.5 mg/kg/day for 15 days. There was no marked change in the uterine weight ratio.

Histological studies. Gross as well as microscopic observations of the different vital organs suggested no significant pathological change in any of the reatments.

Acetylcholinesterase estimation. The activity of AchE in the brain and RBC of quinalphos treated and control guinea pigs at 0, 24 and 72 h, 7, 15 and 30 days and the pattern of recovery are summarised in Table 2. The percent inhibition of AchE in RBC was more than 70% in all the treatments. The increased period of exposure with 2.0 mg/kg has shown a gradual fall in AchE inhibition. Brain has shown relatively less significant inhibition in the activity of AchE as compared to RBC.

The recovery study suggested that the activity of this enzyme could be normalised after 15 days of rest. Thus, the enzyme activity was quickly restored to about 100% within 15 days in lower dose (0.5 mg/kg), while in higher dose regime (1.0 and 2.0 mg/kg) its recovery was comparatively slower.

Female guinea pigs dosed with low levels of quinalphos for 24 h to 30 days have shown no clinical symptoms of insecticide poisoning. There was also no death in any of the animals. In contrast animals exposed to high concentration of quinalphos (4.0 mg/kg/day) died within the first week with severe symptoms of organophosphorus poisoning. The organ body weight ratio of the trated animals, however, did suggest significant changes in comparison to control. It is of significance, however, that none of the organs suggested any morphological change in the poisoned animals.

The most striking and important property of an organophosphorus insecticide is the capacity to combine with cholinesterase even at very low levels of exposure. It has been reported that all the potent organophosphates inhibit one or the other cholinesterase to a level of 50% or more at a concentration of 10^{-6}. Majority of them are effective at 10^{-6}-10^{-9} M (O'BREIN 1969). The inhibition of AchE activity in the RBC and brain of all

TABLE 2

INHIBITION** PATTERN OF ACETYLCHOLINESTERASE IN RBC AND BRAIN OF QUINALPHOS TREATED FEMALE GUINEA PIGS

Group/ Period	I		II				III				IV			
	Brain	RBC	Brain	Inhibition (%)	RBC	Inhibition (%)	Brain	Inhibition (%)	RBC	Inhibition (%)	Brain	Inhibition (%)	RBC	Inhibition (%)
24 h	0.92 ± 0.04	12.29 ± 2.69	0.81 ± 0.01	12.0	3.35 ± 0.05	73.0	0.68 ± 0.08	26.0	2.85 ± 0.08	77.0	0.63 ± 0.02	32.0	3.02 ± 0.15	75.0
72 h	0.92 ± 0.04	12.29 ± 0.04	0.62 ± 0.07	33.0	2.54 ± 0.05	79.0	0.75 ± 0.02	19.0	2.77 ± 0.08	77.0	0.69 ± 0.01	25.0	3.51 ± 0.02	71.0
7 Day	0.86 ± 0.01	14.40 ± 0.89	0.75 ± 0.06	13.0	3.12 ± 0.23	78.0	0.83 ± 0.01	3.0*	4.15 ± 0.06	71.0	0.67 ± 0.04	22.0	4.13 ± 0.16	71.0
15 Day	0.85 ± 0.03	13.12 ± 1.35	0.75 ± 0.05	12.0	2.68 ± 0.40	80.0	0.84 ± 0.02	2.0*	3.43 ± 0.02	74.0	0.54 ± 0.02	37.0	4.11 ± 0.31	69.0
30 Day	0.87 ± 0.03	13.27 ± 1.64	0.72 ± 0.02	17.0	2.34 ± 0.14	82.0	0.71 ± 0.02	19.0	3.02 ± 0.20	77.0	0.77 ± 0.03	11.0	4.56 ± 0.88	66.0
Post Treatment Rest 15 days	0.85 ± 0.026	13.27 ± 1.64	0.82 ± 0.019	3.0	12.01 ± 0.80	2.0	0.69 ± 0.06	19.0	9.38 ± 1.57	29.0	0.64 ± 0.023	25.0	9.66 ± 1.18	27.0

* The percent inhibition was much irregular in comparison to other treatments.
** All values represent the mean ± SE of 4 animals.

the animals dosed with different levels of quinalphos was highly significant. It is, however, interesting to observe here that lower concentrations of quinalphos produced maximum level of inhibition in comparison to higher concentrations of the insecticide. It is possible that lower concentrations faciliated maximum absorption of the compound and therefore the inhibition was also higher.

Studies with laboratory rats and domestic goat also suggested that quinalphos inhibits the activity of AchE in these animals quite significantly (DIKSHITH et al., unpublished). Phosphamidon, another organophosphorus insecticide, has caused significant inhibition of AchE activity in the rat and rabbit (DIKSHITH et al.,unpublished). Quinalphos although produced no discernible morphological change in any of the tissues of female guinea pigs, yet caused maximum inhibition of AchE activity in RBC and brain. Similar observations have been made in the carbaryl treated rats where the inhibition of AchE activity was relatively low (DIKSHITH et al. 1976). In contrast methyl demeton produced marked morphological changes in liver and also caused highly significant inhibition of AchE activity in RBC brain (DIKSHITH et al. 1979). It has been observed in the present study that cessation from further treatment of quinalphos for 15 days would lead to marked recovery of the enzyme. In the lower concentration the recovery was around 100 percent and also more significant. It has been noticed in the methyl demeton treated rats that a period of rest following organophosphorus poisoning, a major restoration of AchE activity was possible(DIKSHITH et al. 1979). The present study thus confirms our earlier observations. The study, therefore, is of great importance and suggests that occupational workers who get exposed to organophosphorus sprays and formulations require rest at regular intervals. Given a brief period of rest, it is possible for the workers to cope up with any kind of abnormality with the severe inhibition of AchE activity and to minimize the danger from organophosphorus intoxication.

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