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Kinetics of Malathion Penetration into *Periplaneta americana* L.

S. C. Saxena and Prabhu N. Saxena

Toxicology Laboratory, Department of Zoology, University of Rajasthan, Jaipur-302004, India

The penetration of an insecticide applied topically to the insect body is usually measured on the assumption that entrance is made through the cuticle into the haemolymph. In these experiments groups of cockroaches (Periplaneta americana L.) treated topically with a known amount of malathion were subsequently rinsed with solvent to determine the amount of chemical remaining outside the body at various times after treatment. This technique (O'Brien, 1967) actually measures the amount of chemical that does not enter the body. It involves application of insecticide to the integument, (to which the insecticide was applied) which is later excised and analysed for residual insecticide.

Materials and Methods:

Technical grade malathion (98.7%) was diluted with benzene to the desired concentrations. 0.02 ml of 5% solution of malathion was applied to the profemur and the 4th abdominal segment of each of 20 newly emerged adults (2 hours after the emergence) from inbred colony. A nectrotomy was performed at 5, 7, 10, 15 and 30 minutes to obtain cuticle and haemolymph in which the presence of malathion was determined by thin layer chromatography. The thin layer plates 20 x 20 cm of silica gel G (thickness 250 nm) were prepared by the technique of Stahl (1958) and activated at 120°C for 30 minutes. The activated plates were spotted with malathion and developed by the ascending technique at 25°C. Following solvent systems were tried:

Cyclohexane	-	acetone 40:5
Cyclohexane	-	acetone 40:15
Cyclohexane	-	ethyl methyl ketone 40:1
Benzene		

Of these solvents benzene was found to be the most suitable. The plates were dried at room temperature and examined under ultraviolet light (2540 nm). They were then sprayed with 0.5% solution of Rhodamine B, dried and exposed to bromine vapour. Malathion develops as pinkish violet spots. These spots were then scraped off and malathion was estimated quantitatively using a colorimeter (Norris et al., 1954). The rate of entry of malathion for each time interval following treatment was calculated by dividing the percent of insecticide that entered the body by time. The results for each of the several time intervals (Fig. 1) were plotted. Similar studies were done with 1 & 2% solution of malathion (Saxena Prabhu Narain, 1982).

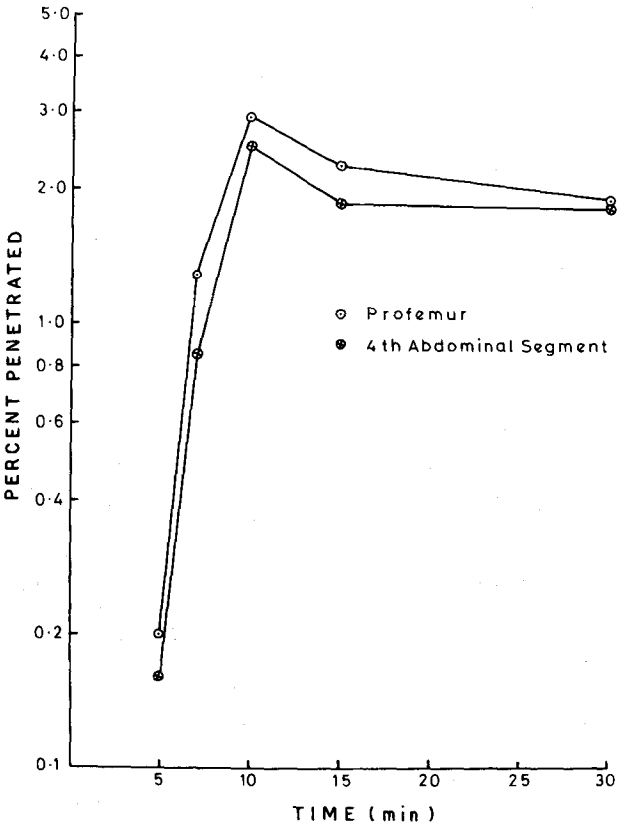

Fig. 1 Graph showing rate of penetration of malathion from Profemur and 4th abdominal segment.

Results and Discussion:

The amount of malathion that penetrated into the body at different time intervals, from two different sites of application, the 4th abdominal segment and profemur, are given in the table 1.

Table 1 - Amount (%) of malathion penetrating from Profemur and 4th abdominal segment

Site of application	Time after treatment (minutes)	Percent of * malathion penetrated
Profemur	5	0.20
	7	1.28
	10	2.90
	15	2.24
	30	1.90
4th Abdominal segment	5	0.16
	7	0.85
	10	2.50
	15	1.86
	30	1.82

* Mean of 20 different insects for each observation.

It was observed that the amount of malathion penetrating in the first 10 minutes was greater than that at 15 minutes and 30 minutes irrespective of loci. This shows that the initial penetration rate is more rapid.

A straight line was obtained by plotting the log of the amount of malathion that remained outside the body against time following treatment. Similarly, a straight line was observed with the application of 1% and 2% solution of malathion (Saxena Prabhu Narain, 1982). However, only data concerning 5% solution were reported. Our data on penetration rates thus follows Fick's law of diffusion ("The quantity diffusing across 1 cm^2 is the product of its diffusion coefficient, concentration gradient and time required for the flow").

The equation of Fick's law of diffusion has the form of first order reaction kinetics in which the rate of penetration at any time is proportional to the amount that remains outside the body at that time and is represented by the equation.

$$C = Coe^{-kt}$$

or

$$K = \left(\frac{2.303}{t_o - t}\right) X \log \left(\frac{No}{N}\right)$$

Where Co is initial concentration at t_o and K = proportionately constant. Our findings were similar to Buerger (1966) whose observations indicate that 1st order reaction kinetics are followed irrespective of the type of insecticide penetrating into an insect. Elliott et al. (1970) showed in experiments with mustard beetles Phaedon cochleariae that the plots of log percent remaining on surface vs time exhibit a linear relationship conforming to the equation $C = Coe^{-kt}$. It was also observed that the initial rate of penetration of malathion was higher irrespective of the locus of application i.e. the profemur or the 4th abdominal segment.

Metabolism influences the rate of removal of insecticide from the inner layers of the integument. Thus an initial fairly rapid transfer of insecticide from the outer to the inner layers of the integument is followed by a reduction in the rate of penetration due to the saturation of the inner cuticular layers (Wilkinson, 1976). Further, we observed that the total amount of insecticide absorbed by an insect from an external dose within a given time, did not increase indefinitely with increasing dose, but approaches an upper limit. The result was similar to that reported by Hewlett (1958) who expressed the relationship between absorbance and externally applied doese by the expression

$$W = a(1-e^{-d/a})$$

Where W = amount of insecticide absorbed at a given time after topical application.

d = external dose

a = absorbance

We think an upper limit (on absorption of insecticide by an insect) is approached due to tissue saturation effects. This has been found to be true at all doses (1 & 2%) (Saxena,Prabhu Narain , 1982) and also at 5% of malathion used in the present investigation. It therefore appears that 3 phases are

involved in penetration of malathion (Table 2, Fig. 2) an initial phase of rapid penetration (10 minutes) during which the amount of external malathion decreases; a reduction in penetration rate to a level (15 minutes) approaching an equilibrium between externally remaining malathion and the malathion in the inner layers of integument and inner tissues and a third phase of loss according to first order kinetics (conforming to the equation $C = Coe^{-kt}$).

Table 2 - Amount (%) of malathion left at Profemur and 4th abdominal segment

Time in minutes	Amount left at 4th abdominal segment Expt. No. 1	Amount left at Profemur Expt. No. 2
5	99.2	98.56
7	94.0	91.00
10	75.0	71.00
15	72.00	66.00
30	66.09	59.00

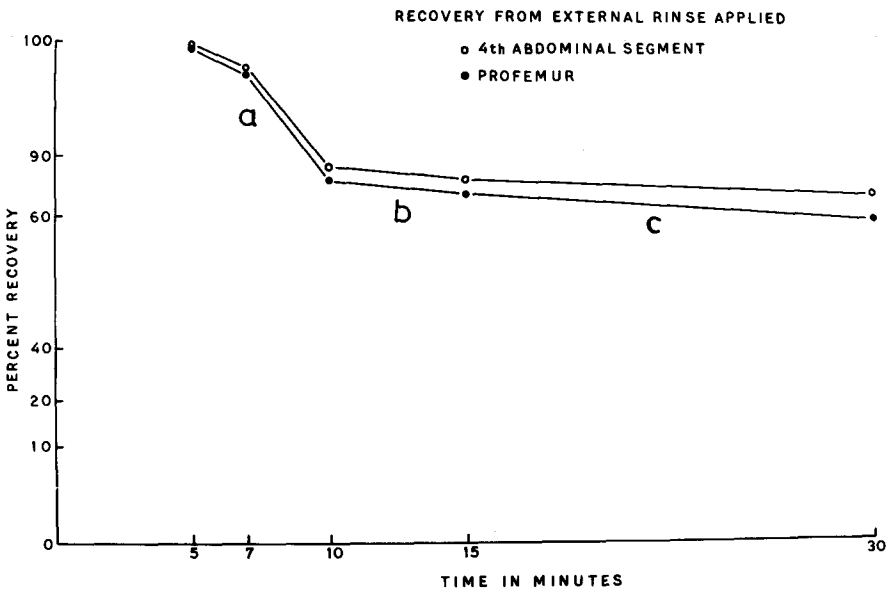

Fig. 2 Graph showing percent recovery of malathion after external rinsing where
a = initial phase; b = intermediate phase
c = third phase.

Brooks (1966) reported similar results with metabolically stable (they do not change into metabolic forms like malathion - malaoxon) chlorinated insecticide such as dieldrin and Heptachlor exposide in houseflies. The second phase of malathion penetration probably appears to correspond to the approach to the internal plateau level of toxicant because a metabolic process (Malathion — Malaoxon) is present, a steady state will first be achieved in which metabolic rate balances penetration rate. Then as metabolism of the internal toxicant continues the external toxicant remaining eventually becomes insufficient to maintain the steady state internal level, which therefore begins to decline from its maximum value. This is in agreement with Elliott et al. (1970).

The linear third phase of the penetration curve corresponds to the period of constant internal concentration and the slope of this portion is therefore a measure of both penetration and metabolic rate. From the curves (Table 2; Fig. 2) it is clear that overall malathion penetration is slow like other organophosphorus compounds, which finds support from Sun (1968) who also observed a slow penetration of most of the organophosphorus compounds viz., Parathion, Paraoxon and Dimethoate.

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