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The Toxicity of Some Organophosphorus Compounds to Adult *Anopheles stephensi* *

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The authors have evaluated a number of organophosphorus compounds for residual contact toxicity to adult Anopheles stephensi. *Fenthion and malathion were the most promising of the compounds, and wettable powder deposits at a dosage of 1 g/m² on plywood remained effective for five months. There was, however, a rapid loss of effectiveness on dried mud bricks stored at 25° C and 50 %-55 % relative humidity.*

Diazinon and ronnel were less persistent on plywood. Guthion and coumaphos, although highly toxic by topical application, were both ineffective as contact insecticides when applied as solids in suspension. Trithion and methyl trithion were relatively low in toxicity both by topical application and as contact insecticides.

Investigations have been carried out in recent years on the toxicity to adult mosquitos and the residual properties on different building materials of numerous new insecticides. It is now proposed to record the biological results obtained with a number of organophosphorus compounds in this report, those with a series of carbamates in a second, and then to deal in turn with factors such as evaporation, sorption and decomposition that influence their residual action.

MATERIALS AND METHODS

Test insects

Anopheles stephensi and *Aëdes aegypti* were reared by standard methods at 25°C and 70%-80% relative humidity.

Toxicity by topical application

A w/v solution of each compound in di-isobutyl ketone was applied to the dorsal surface of the thorax of 1-2-day-old, unfed, female mosquitos from a microburette similar to that described by Kerr.[3] The dosage of insecticide applied was varied by varying the volume from 0.010 μl to 0.025 μl per mosquito. Two batches of 20 mosquitos were treated at each dosage and after treatment each batch was placed in an unwaxed paper cup with cotton gauze top, provided with sugar solution, and stored at 25°C and 70% relative humidity for 24 hours before a mortality count was made. The median lethal dose was derived from the dosage-mortality data.

Contact and residual toxicity

Surfaces used. The main purpose of the tests was to assess the residual properties of candidate compounds on surfaces representative of those likely to be found in sprayed houses. The materials occurring in houses are very varied in type but from past experience it is possible to divide them into two main groups. On the one hand, there are the thatches and walls of leaves, stems or various sawn woods, on which the main loss of insecticide is by evaporation, possibly accompanied by some decomposition. They tend to be of low porosity compared with the second group, which consists of the very porous dried soils used in house construction in many tropical countries. On these, changes in effectiveness result largely from loss of available insecticide owing to sorption. Sorption is accompanied by a reduction in evaporation rates, which can be beneficial, but sometimes also by increased decomposition due to the more intimate contact of the sorbed insecticide with the soil constituents. Therefore the surfaces

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[3] Kerr, R. W. (1954) *Bull. ent. Res.*, **45**, 323.

used in laboratory tests should illustrate the likely behaviour of the candidate compounds on at least these two main groups.

In addition the structural materials themselves may be coated with limewash, distemper or paint. Of these, limewash occurs frequently and must be considered because of its alkaline reaction and consequent degrading effect on many insecticides.

Plywood was taken as being an example of the group of relatively non-porous materials. It has no obvious catalytic action on decomposition processes, does not sorb insecticides and is an easily available standard material. The plywood used was " Invictus " board, resin-bonded plywood, gaboon-faced, to British Standard 1455 (1956), from the British Plywood Manufacturing Co. Ltd.

Dried soils constitute the second main group of building materials. It is not, however, possible to choose one as a representative of the group. Different soils show a wide variation in activity in their sorption and decomposition effects on insecticides. Three soils of high, medium and low sorptive capacities were therefore chosen. There is the additional advantage that two of them are also used in the hut trials carried out at the Tropical Pesticides Research Institute, Arusha, Tanganyika, as examples of high and low sorptive soils. This means that laboratory results can more easily be compared with those from the field.

The soils are identified as follows in decreasing order of sorptive powers for insecticides: *Babati*, a laterized red earth from about 100 miles south-west of Arusha; *Taveta*, a brown loam from about 70 miles east of Arusha; *Magugu*, a " black cotton soil " from about 80 miles south-west of Arusha. Occasionally, a red, highly laterized earth from *Uganda* was included, as it has very high sorptive powers for insecticides and is also very active in catalysing their decomposition.

In laboratory testing with small blocks it is essential that the blocks be uniform in surface texture. This could be achieved by sieving out the particles greater than about 1 mm, but this is very laborious when large supplies are needed. Each soil was therefore dried and passed through a hammer-mill fitted with a quarter-inch (6.3 mm) screen. Milled soil was mixed with water and the plastic mass pressed into moulds made from brass rings, 3.5 inches (8.9 cm) in diameter and 0.5 inch (1.3 cm) deep. After air-drying, the blocks measured about 3 inches (7.5 cm) in diameter by 0.4 inch (1 cm) deep.

Limewash deposits in the field can be very variable depending upon the lime used, the way in which they are applied and their age. These factors cannot be considered in a field trial and so we have chosen to use the most severe conditions for measurement of insecticide persistence—that is, pure lime, freshly prepared deposits, and no addition of sizing materials.

One part of calcium hydroxide was ground with two parts of water to a fine suspension and applied to plywood plaques by brushing. The average deposit was about 50 g per m^2.

Formulations. The formulations used in these tests were usually wettable powders made available by the manufacturers of the various compounds. The concentration of active ingredient, the inert carriers and wetting and dispersing agents varied considerably, and performances of the insecticides are not, therefore, strictly comparable.

Spraying. Test surfaces were sprayed singly in a Potter [1] tower with the chosen formulation of each compound. Normally a standard dosage of 1 g/m^2 was given, although higher or low dosages were sometimes given on a particular surface according to the effectiveness of the compound. With the tower in use 1 g/m^2 necessitated spraying suspensions or emulsions of about 3% of active ingredient and the ratio of liquid to insecticide to surface area was consequently very similar to that used in practical spraying in the field.

Storage. The sprayed test surfaces were stored on open shelves in a room kept at 25°C and 50%-55% relative humidity. Air could diffuse freely around the samples but there was no forced ventilation and the door was kept closed.

Exposure of insects. At intervals after treatment 2-3-day-old, blood-fed *Anopheles stephensi* were exposed in contact with the treated surfaces for known times. Batches of 10 mosquitos were introduced into Perspex chambers 2½ inches (6.4 cm) in diameter and 1 inch (2.5 cm) deep, and were allowed to alight and rest on the vertical treated surface. After the required contact time the mosquitos were knocked down with carbon dioxide and transferred to paper-cup cages and stored at 25°C and 70% relative humidity for 24 hours before mortality counts were made. Exposure times varied according to the effectiveness and age of the deposits.

[1] Potter, C. (1941) *Ann. appl. Biol.*, **28**, 142.

RESULTS

Toxicity by topical application

Median lethal doses, in μg per female, of the various compounds to the two test species were as follows:

Compound	*Anopheles stephensi*	*Aëdes aegypti*
Fenthion	0.002	0.003
Malathion	0.006	0.006
Chlorthion	0.007	0.003
Diazinon	0.006	0.016
Ronnel	0.016	0.021
Trithion	0.037	0.162
Coumaphos	0.002	0.012
Guthion	0.003	0.008
Dimethoate	0.003	0.005
DDT (for reference)	0.018	0.018

Residual contact toxicity

Fenthion (=Baytex): O,O-dimethyl O-3-methyl-4-methylthiophenyl phosphorothionate. A 40% wettable powder, supplied by Farbenfabriken Bayer A.G., was sprayed on to plywood panels at dosages of 1, 0.5 and 0.25 g/m². Deposits were highly toxic initially, high kills being obtained after only two minutes' contact on all three dosages. At a dosage of 1 g/m² deposits had a long residual life and were still very highly effective after 26 weeks (Table 1).

TABLE 1

CONTACT TOXICITY TO *A. STEPHENSI* OF FENTHION ON PLYWOOD

Dosage (g/m²)	Age of deposit (weeks)	Mean kill (%) after contact time shown					
		2 min.	5 min.	15 min.	30 min.	60 min.	120 min.
1	0	100					
	2	100					
	4	95					
	7	90					
	10	90	100				
	17	39	98				
	26		0	38	85		
0.5	0	98	100				
	3	98	100				
	6	68	100				
	10	0	12	90			
0.25	0	87	100				
	3	38	95				
	6		0	14	75		
	10				0	0	88

Deposits of the wettable powder at a dosage of 1 g/m² were initially very much less toxic to mosquitos on porous dried mud bricks (Table 2) than on plywood, and loss in effectiveness during the first few days after application was very marked. After one week, kills of mosquitos exposed on the treated surfaces for four hours were negligible.

Deposits of wettable powder at a dosage of 1 g/m² on fresh limewash and calcium carbonate showed no significant loss of toxicity to mosquitos over a period of six weeks.

Malathion: O,O-dimethyl S-(di(ethoxycarbonyl) ethyl)phosphorodithioate. Preliminary tests with a number of malathion wettable powder formulations showed that a 30% wettable powder supplied by Ratsouris Ltd. was slightly more effective on plywood than the others. At a dosage of 1 g/m² deposits from this wettable powder on plywood panels initially gave 100% kills after 15 minutes' contact, and there was only a slow loss in toxicity over a period of 20 weeks (Table 3).

On the other hand, deposits from the wettable powder at a dosage of 1 g/m² were very much less effective on porous dried muds (Table 4) than on plywood, and only one day after application no kills were obtained when mosquitos were exposed for four hours on the treated surfaces.

Numerous other malathion wettable powders have all given poor results on dried mud bricks, even when the dosage has been increased to 2 g/m². For example, experimental 50%, 30% and 25% wettable powders were applied to dried mud bricks made from Babati soil at a dosage of 2 g/m² and one day later none of the mosquitos exposed on the treated surfaces for two hours was killed.

On fresh deposits of limewash malathion was rapidly decomposed, and one day after application at a dosage of 1 g/m² deposits of wettable powder killed none of the mosquitos exposed on them for four hours. On the other hand, there was no significant change in the contact toxicity of similar deposits on calcium carbonate over a period of six weeks.

Chlorthion: O,O-dimethyl O-(3-chloro-4-nitrophenyl)phosphorothioate. A 20% chlorthion wettable powder, supplied by Farbenfabriken Bayer A.G., was sprayed on to plywood panels at a dosage

TABLE 2

CONTACT TOXICITY TO *A. STEPHENSI* OF FENTHION ON DRIED MUD BRICKS

Soil	Dosage (g/m^2)	Age of deposit (days)	Mean kill (%) after contact time shown						
			2 min.	5 min.	15 min.	30 min.	60 min.	120 min.	240 min.
Uganda	1	0	0	10	100				
		1				0	0		
		7						0	
Babati	1	0		0	27				
		1						0	0
Taveta	1	0		21	92				
		1						0	26
Magugu	1	0		43	100				
		1				9	68	100	
		7					0	0	24
Taveta	2	0		96					
		1					0	35	
		7						0	0

of 1 g/m^2. Deposits were less toxic than those of fenthion and malathion to mosquitos, but there was very little loss in toxicity over a period of 20 weeks (Table 5).

Deposits of the wettable powder on dried mud bricks were relatively ineffective, however, and on the day of application kills of mosquitos exposed on the treated surfaces for two hours were negligible.

Diazinon : O,O-diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl)phosphorothioate. A 40% wettable powder, supplied by the Geigy Co. Ltd., was sprayed on to plywood panels and dried mud bricks made from Uganda soil. Deposits on plywood were initially highly toxic to mosquitos, and at a dosage of 0.25 g/m^2 a contact of two minutes was sufficient to give 100% kills. The deposits lost their toxicity

TABLE 3

CONTACT TOXICITY TO *A. STEPHENSI* OF MALATHION AT A DOSAGE OF 1 g/m^2 ON PLYWOOD

Age of deposit (weeks)	Mean kill (%) after contact time shown			
	2 min.	5 min.	15 min.	30 min.
0	20	71	100	
1		36	100	
2		21	100	
4		15	98	
7		5	80	100
10		0	77	100
16		0	30	93
20		0	15	85

TABLE 4

CONTACT TOXICITY TO *A. STEPHENSI* OF MALATHION AT A DOSAGE OF 1 g/m^2 ON DRIED MUD BRICKS

Soil	Age of deposit	Mean kill (%) after contact time shown		
		60 min.	120 min.	240 min.
Babati	1 hour	0		
	1 day		0	0
Taveta	1 hour	4		
	1 day		0	0
Magugu	1 hour	48		
	1 day		0	0

TABLE 5

CONTACT TOXICITY TO *A. STEPHENSI* OF CHLORTHION AT A DOSAGE OF 1 g/m² ON PLYWOOD

Age of deposit (weeks)	Mean kill (%) after contact time shown			
	5 min.	15 min.	30 min.	60 min.
0	5	58	85	
1	5	60	90	
4	0	45	75	
7	5	49	76	
10	0	26	66	
16		18	66	95
20		5	48	85

fairly rapidly, however, and a dosage of 1 g/m² was relatively ineffective after seven weeks (Table 6).

At a dosage of 1 g/m², deposits on dried mud bricks were relatively ineffective on the day of application and a kill of only 16% was obtained when mosquitos were exposed on them for two hours.

Ronnel (=Fenchlorphos=Trolene=Dow Et-57): O,O-dimethyl O-2,4,5-trichlorophenyl phosphorothioate. A 25% wettable powder, supplied by Dow Agrochemicals Ltd., was sprayed on to plywood panels and dried mud bricks made from Uganda soil at a dosage of 1 g/m². Deposits on plywood were toxic to mosquitos for four weeks, but were relatively ineffective after seven weeks. Deposits on dried mud bricks lost their effectiveness even more rapidly (Table 7).

TABLE 6

CONTACT TOXICITY TO *A. STEPHENSI* OF DIAZINON ON PLYWOOD

Dosage (g/m²)	Age of deposit (weeks)	Mean kill (%) after contact time shown					
		2 min.	5 min.	15 min.	30 min.	60 min.	120 min.
1	0	100					
	1	100					
	2	25	100				
	4			0	30	98	
	7				0	0	13
0.5	0	100					
	1	35	80				
	2		15	95			
0.25	0	100					
	1	0	0	65			
	2			0	45		

TABLE 7

CONTACT TOXICITY TO *A. STEPHENSI* OF RONNEL AT DOSAGE OF 1 g/m²

Test surface	Age of deposit (days)	Mean kill (%) after contact time shown				
		2 min.	5 min.	15 min.	30 min.	60 min.
Plywood	0	0	50	100		
	7		28	100		
	14		16	100		
	28		18	90		
	49			5	3	10
Dried mud bricks	0		14	40		100
	1		0	0		35
	4			0		0

Trithion and methyl trithion: O,O-diethyl S-(*p*-chlorophenyl-thiomethyl)phosphorodithioate and O,O-dimethyl S-(*p*-chlorophenyl-thiomethyl)phosphorodithioate. A 50% trithion wettable powder and a 50% methyl trithion wettable powder, supplied by the Stauffer Chemical Company, were sprayed on to plywood panels at a dosage of 1 g/m². The contact toxicity of deposits to mosquitos was low; those of methyl trithion were slightly more toxic than those of trithion (Table 8).

Dimethoate (= Rogor): O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate. As no wettable powder was available, a 40% emulsion concentrate, supplied by Fisons Pest Control Ltd., was used. This was diluted with water to give a 3.2%

TABLE 8

CONTACT TOXICITY TO *A. STEPHENSI* OF TRITHION AND METHYL TRITHION AT A DOSAGE OF 1 g/m² ON PLYWOOD

Insecticide	Mean kill (%) after contact time shown			
	15 min.	30 min.	60 min.	120 min.
Trithion	0	0	13	90
Methyl trithion	0	5	38	98

emulsion which was sprayed on to glass plates, plywood panels and dried mud bricks made from Uganda soil at a dosage of 1 g/m². A few hours later the deposits on glass plates were highly toxic to mosquitos, but those on plywood panels and dried bricks were ineffective after a contact time of one hour because of penetration of the insecticide into the substrate (Table 9).

Coumaphos (=Co-Ral=Resitox=Bayer 21/199): O,O-diethyl O-(3-chloro-4-methyl-7-coumarinyl) phosphorothioate. A 30% wettable powder supplied by Farbenfabriken Bayer A.G. was sprayed on to plywood panels and dried bricks made from Uganda and Taveta soils at a dosage of 1 g/m². No kills were obtained when mosquitos were exposed on the treated surfaces for two hours.

Guthion (= Gusathion): O,O-dimethyl S-(4-oxo-benzotriazino-3-methyl)phosphorodithioate. A 25% wettable powder supplied by Farbenfabriken Bayer A.G. was sprayed on to plywood panels and dried bricks made from Uganda and Taveta soils at a dosage of 1 g/m². No kills were obtained when mosquitos were exposed on the treated surfaces for two hours.

DISCUSSION

Of the organophosphorus compounds tested for residual contact toxicity to adult mosquitos, fenthion and malathion were the most effective. At a dosage of 1 g/m² on plywood panels stored at 25°C these compounds were still highly toxic after five months, and a similar long residual action could be expected on other relatively non-porous building materials such as bamboo, palm leaf and grass thatch. Malathion, however, although stable on calcium carbonate, decomposed rapidly on deposits of fresh limewash.

TABLE 9

CONTACT TOXICITY TO *A. STEPHENSI* OF DIMETHOATE AT A DOSAGE OF 1 g/m² ON VARIOUS SURFACES

Test surface	Mean kill (%) after contact time shown			
	2 min.	15 min.	30 min.	60 min.
Glass	100			
Plywood		0	0	7
Dried mud		0	0	0

The same formulations of these compounds were, however, much less effective when applied to porous dried mud bricks made from various soils and there was a very marked reduction in effectiveness after storage for only a few days at 25°C and 50%-55% relative humidity. These compounds, like chlorthion and diazinon, are liquids, and a proportion of the liquid insecticide usually separated from the inert carrier and formed an emulsion when the wettable powder was dispersed in water. This soaked into the porous substrate and gave a much smaller surface deposit than would be obtained from a dispersion of a solid insecticide. Measurements of the amounts of liquid insecticides left with the solid carrier of the wettable powder on a porous surface have shown that they may be as low as 2% of the nominal dosage sprayed. This can be compared with the high recoveries of more than 90% obtained with solid insecticides on the same surface.

Several malathion wettable powder formulations designed to increase dosages of the insecticide retained on the surface of porous materials have been tested, but, even when a higher proportion of malathion was retained on the carrier in the suspension or when the dosage remaining initially on the surface of dried mud was increased as a result of aggregation of carrier particles, sorption of the insecticide and diffusion into the substrate were rapid and resulted in a very low toxicity to mosquitos resting on the surface.

None of the other organosphosphorus compounds evaluated showed sufficient promise as residual contact insecticides for use against adult mosquitos. Trithion and methyl trithion were relatively low in toxicity both by topical application and as contact poisons. Diazinon and ronnel were too volatile and had insufficient persistence as deposits on plywood. Both Guthion and coumaphos were highly toxic by topical application in solution but were not effective as contact insecticides when applied to surfaces as solids in aqueous suspension. This may be related to their low solubility in paraffinic solvents and a low rate of penetration through the insect cuticle to the site of action. Solubilities of Guthion and coumaphos in *n*-hexadecane, for example, are 0.11 and 0.07 g per 100 ml respectively.

RÉSUMÉ

L'étude comparée de l'effet toxique de contact de divers organophosphorés sur *Anopheles stephensi* a montré que le fenthion et le malathion étaient les plus prometteurs. L'efficacité de la poudre mouillable, pulvérisée sur du contre-plaqué à raison de 1 g/m², s'est maintenue durant 5 mois. Sur les briques de boue séchée, cependant, à 25°C et sous une humidité relative de 50-55%, la substance perdait rapidement de son activité. Ce fait s'explique en grande partie par le fait que ces deux insecticides sont pulvérisés sous forme d'une émulsion — à la suite de la dispersion de la poudre mouillable dans l'eau — dont les particules se fixent dans les pores de la boue séchée, ne laissant en surface qu'une faible proportion, parfois 2% seulement, de la substance active (contre 90% lorsqu'il s'agit d'un insecticide sous forme solide).

Aucun des autres composés organophosphorés étudiés n'avait des qualités aussi satisfaisantes comme insecticide de contact. Le trithion et le méthyl-trithion sont relativement peu toxiques, soit en application locale soit en pulvérisation. Le diazinon et le ronnel sont trop volatils et leur effet rémanent trop fugace. Le Guthion et le coumaphos, fortement toxiques en applications locales, n'ont pas un effet rémanent suffisant.