

## Insecticide Tolerance Level of Fleas from Southeast Asia

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**OBJECTIVE:** To determine resistance in populations of fleas from Southeast Asia to commonly used insecticides.

**DESCRIPTION:** Testing procedures were conducted in accordance with the recommendations of the World Health Organization. Ten fleas were exposed for 1 hour to papers impregnated with varying concentrations of the insecticides and then permitted to rest on clean paper in test tubes for 23 hours. With replicates, up to 100 fleas were tested in each exposure. One major exception to the above involved exposure to DDT. Many colonies were so resistant to this chemical that no mortality occurred after 1 hour exposure to paper impregnated with 4 per cent DDT (the highest concentration provided by WHO). Rather than increase the concentration, exposure time to 4 per cent DDT was increased to 2, 4, 8, 16 and 32 hrs.

Pesticides tested during this period included DDT, lindane, dieldrin, malathion, fenthion and carbaryl. Impregnated papers were prepared according to WHO standard techniques and were supplied by WHO and US Army Environmental and Hygiene Agency. Papers impregnated with lindane and carbaryl were prepared by SEATO Medical Research Laboratory in accordance with WHO standard techniques.

**PROGRESS:** Preliminary tests in Bangkok and Nha Trang indicated existence of high resistance of the Xenopsylla cheopis populations in those two cities to chlorinated hydrocarbons, especially DDT. Colonies of Xenopsylla cheopis from the Vietnamese provinces of Darlac (Pleiku) Khan Hoa (Nha Trang), Tuyen Duc (Dalat), and Binh Dinh (Qui Nhon) and from the provinces of Phranakhon (Bangkok), Surat Thani (Koh Samui), Nakhon Ratchasima (Korat) and Chiang Mai in Thailand were established in the SMRL insectary. In addition, a second species, Stivalius klossi, from Pak Thong Chai, Nakhon Ratchasima province, was tested for resistance/susceptibility to DDT, lindane, malathion, fenthion, and carbaryl. A strain of Xenopsylla cheopis from Florida (Gainesville), considered by USDA and the USPHS Technical Development Laboratories to be susceptible to DDT, was used in these tests as a standard for comparison.

Separate colonies of X. cheopis, from the same site in Bangkok, were initiated from different hosts (Rattus norvegicus and R. exulans). Extensive tests (at least 15 each) were carried out with lindane and carbaryl. The  $LC_{50}$  and the mortality at different concentrations were so similar that the colonies were combined without regard to host of origin. Comparisons of deaths of X. cheopis from exposure to all insecticides were made on the basis of original collections from different hosts in Vietnam also, but not necessarily from the same site, and no significant differences were observed. The hosts were house shrew (Suncus murinus), house rat (Rattus exulans), roof rat (R. rattus) and Norway rat (R. norvegicus).

Of particular interest is the fact that no change in resistance was evident on comparison of colonies of X. cheopis initiated before and after a massive plague control dusting program was conducted in Vietnam during 1968 and 1969 in which the principal insecticide used was 10 per cent DDT dust. Fleas were collected from Pleiku, Dalat and Qui Nhon and tested both before and after the program, and no difference in mortality to DDT or any other pesticide tested was noted in pre-and post-control program samples. It is possible that a change in level of resistance takes place over a period of time, though this could not be demonstrated with Vietnam colonies collected a few weeks apart following the plague control program.

**DDT:** All X. cheopis from Vietnam tested were so resistant to DDT that the standard 1 hour exposure to DDT-impregnated paper was inadequate to demonstrate any significant mortality. In hopes of demonstrating mortality and variation between collection sites, exposure times to 4 per cent DDT were increased to 1, 2,

4, 8, 16 and 32 hours. A few deaths (4 per cent—16 per cent mortality), resulted following 4 hours exposure in 3 of 10 colonies and at 32 hours exposure some deaths (12—54 per cent of the fleas) occurred among all colonies. The  $LC_{50}$  could not be calculated since exposure to all other pesticides was for 1 hour. However 54 per cent of fleas from a colony established from Binh Dinh province (Qui Nhon, Tuy Hoa) died after 32 hours exposure to 4 per cent DDT. The mortality rate, following this exposure time, was less than 50 per cent of fleas for the other Vietnamese strains of X. cheopis tested.

Xenopsylla cheopis from Thailand were highly variable in response to exposure to DDT. Those from Koh Samui were as resistant as any from Vietnam; after 4 hours exposure to 4 per cent DDT only 2.1 per cent of the fleas died, and after 32 hours exposure only 27 per cent died. The only colonies of X. cheopis susceptible enough to calculate an  $LC_{50}$  (based on 1 hour exposure) came from Klong Toey in Bangkok and from Gainesville, Florida. The  $LC_{50}$  for the Gainesville strain was 3.4 per cent. The WHO has established that the  $LC_{50}$  for a susceptible strain of X. cheopis, unsexed, ranges from 0.35—0.7 per cent and the  $LC_{100}$  from 2.0—3.0 per cent. On longer exposure, Gainesville fleas are clearly more susceptible than any from Vietnam or Thailand. For these fleas the mortality was 51 per cent after 1 hour, 54 per cent after 2 hrs., 92 per cent after 4 hrs., 98 per cent after 8 hrs and 100 per cent after 16 and 32 hours, respectively, exposed to paper impregnated with 4 per cent DDT. A sylvan flea, Stivalius klossi, which occurs in Thailand in dry evergreen forest on Rattus surifer, was included in these for comparison since this species has probably never been exposed to DDT. It was completely susceptible to DDT, for the  $LC_{50}$  for this species was 1.4 per cent. The mortality of S. klossi exposed for 1 hour to 2 per cent DDT was 67 per cent (90 fleas tested). After a 1 hour exposure to 4 per cent DDT mortality in S. klossi was 97 per cent, and a complete kill was obtained following more than an hour's exposure.

**LINDANE AND DIELDRIN:** These insecticides were quite effective in the concentrations tested. The  $LC_{50}$  for lindane with X. cheopis from Vietnam ranged between 0.3 and 0.9 per cent and a mortality rate of 77—100 per cent was obtained after a 1 hour exposure to papers impregnated with 2 per cent lindane. Fleas from Thailand were a little more susceptible. The  $LC_{50}$  was between 0.2 per cent and 1.0 per cent when they were exposed to lindane impregnated papers. Mortalities of 94—100 per cent occurred following a 1 hour exposure to paper impregnated with 2 per cent lindane, while 75—100 per cent of X. cheopis died after a 1 hour exposure to 1.6 per cent dieldrin. The  $LC_{50}$  for dieldrin ranged between 0.4 and 1.4 per cent.

There appears to be some correlation between resistance of X. cheopis to DDT and resistance to lindane and dieldrin. This was evident in colonies from Pleiku and Nha Trang, Vietnam and from Koh Samui, Thailand (Table 1). Dieldrin was tested against as many colonies as lindane. Stivalius klossi was not resistant to lindane impregnated papers ( $LC_{50}$  0.3 per cent lindane).

**MALATHION AND FENTHION:** The organophosphates malathion and fenthion appear to be satisfactory pesticides for controlling X. cheopis. Results were quite uniform among the various colonies of X. cheopis tested. The  $LC_{50}$  for malathion varied between 2 per cent and almost 5 per cent. When X. cheopis was exposed to paper impregnated with 5 per cent malathion, mortality was about 60—100 per cent. One hour's exposure of X. cheopis to paper impregnated with 2.5 per cent fenthion was highly lethal (between 84 and 100 per cent mortality). Less than 8 per cent of 40 Stivalius klossi exposed to paper impregnated with 2.5 per cent fenthion died. The mortality rate of 80 S. klossi exposed to paper impregnated with 5 per cent malathion was only 30 per cent. Thus, S. klossi is apparently highly resistant to these two pesticides.

**CARBARYL:** Mortality from exposure to papers impregnated with varying concentrations of Carbaryl (Sevin) was erratic and sometimes difficult to correlate with a series of concentrations for a given colony. The  $LC_{50}$  calculated for various strains of fleas varied from about 0.4 per cent to more than 4 per cent. Since a regular progression of mortality was not always obtained, estimates of the  $LC_{50}$  were not considered reliable. Furthermore, it was observed that impregnated papers deteriorated rapidly during testing procedures. Thus further variation was thrown into the accumulating results. When the above difficulties with the bioassay were encountered a chemical assay of our test papers was undertaken on request of the USPHS

Technical Development Laboratories in Savannah. They determined that concentrations in our freshly prepared test papers were very close to stated values.

Another unexpected but interesting problem was that of quick knock down. Fleas which appeared dead on removal from impregnated papers often recovered after the 23 hour observation period. An even more confusing fact was that often at higher concentrations different fleas were dead after the 23 hours period than were first knocked down. Table 2 shows percent knock down and mortality of fleas from all colonies from all areas combined. The knock-down effect (first column) appears to be negligible below a concentration of 0.25 per cent carbaryl. In many individual tests involving 10 fleas the initial knock down from higher concentrations exceeded final mortality. In the third column the values are really averages so these individuals are not revealed. The knock down effect is not increased beyond a concentration of 1 per cent carbaryl.

When added together and averaged, the erratic results of each test become surprisingly uniform. When fleas were exposed to 4 per cent carbaryl (the highest concentration prepared) the mortality averaged 57 per cent for fleas from Vietnam and 60 per cent for fleas from Thailand. The  $LC_{50}$  averaged 1.2 per cent for Vietnamese strains and 1.7 per cent for Thai fleas. However, the mortality within individual colonies varied between 32 per cent and 90 per cent. In view of the difficulty in finding differences, and the apparent unreliability of data obtained because of reasons discussed above, existence of resistance or susceptibility to carbaryl remains undetected. Variation between areas cannot be demonstrated.

Values for X. cheopis from Gainesville, Florida and for Stivalius klossi (Table 1) are within the range of values discussed above.

Table 1. Preliminary Results of Pesticide Tests on Colonies of Xenopsylla cheopis and Sitivalius klassi Reared from Indicated Collection Sites, Vietnam

Pesticide Location	LC <sub>50</sub>	4% DDT				LINDANE		DIELDRIN		MALATHION		FENTH- ION		CARBARYL	
		Percent Mortality				LC <sub>50</sub>	P.M.* at 2.0%	LC <sub>50</sub>	P.M. at 1.6%	LC <sub>50</sub>	P.M. at 5.0%	P.M. at 2.5%	LC <sub>50</sub>	P.M. at 4%	
		Time Exposure													
		4	8	16	32										
Darlac, Pleiku (Post Dust)	*	2	6	10	12					(4.2)	59	85	(>4)	32	
Darlac, Pleiku (Pre Dust)	*	0	13	30	60	0.34	99	(1.0)	85	4.7	(60)		(0.5)	89	
Khanh Hoa, Nha Trang Xon Bon (Post)	*	16	14	35	21	0.4	100	0.8	84	3.7	78	97	0.96	57	
Khanh Hoa, Nha Trang Phuong Cui (Post)	*	0	0	17	20	0.34	100	0.4	100	3.0	(75)		0.38	100	
Tuyen Duc, Dalat Vo Thanh (Pre)	*	0	11	20		(0.6)	100	(0.6)	90	2.5	(75)		(0.5)	90	
Binh Dinh, Qui Nhon Nguyen Do (Pre)	*	0	0	10						2.0	(80)		(1.0)	74	
Binh Dinh, Qui Nhon Nguyen Do (Post)	*	7	4	29	54					1.2	100	100			
Binh Dinh, Qui Nhon Tuy Hoa (Post)	*	0	0	10	31	(0.9)	77			(4.2)	59				
Binh Dinh, Qui Nhon Cuong De (Post)	*	0	0	2.4	21.4	(0.7)	39								

Table 1. Continued; Thailand and U.S.A.

Pesticide Location	LC <sub>50</sub>	4% DDT				LINDANE		DIELDRIN		MALATHION		FENTHION		CARBARYL	
		Percent Mortality				LC <sub>50</sub>	P.M.* at 2.0%	LC <sub>50</sub>	P.M. at 1.6%	LC <sub>50</sub>	P.M. at 5.0%	ION P.M. at 2.5%	LC <sub>50</sub>	P.M. at 4%	
		Time Exposure													
		4	8	16	32										
Bangkok, Phayathai Din Dang Road	*	30	30	88	79	0.24	100			(1.6)	95	100	(1.0)	54	
Bangkok, Klong Toey 1969	3.1	42	66	73	81	(0.25)	100			(1.6)	100	100	> 4	48	
Bangkok, Klong Toey 1966	3.6	35	72	95	98	(0.21)	100	0.4	100	(2.9)	83		(0.4)	53	
Surat Thani Koh Samui	*	2	0	4	27	(1.0)	94	1.4	75	(0.96)	100	100	(1.5)	86	
Nakhon Ratchasima Korat Chiangmai	*	14	22	48	29					(0.8)	100	100	(1.5)	61	
Florida Gainesville	3.4	54	92	98	100	(0.2)	100	(0.6)	100	(3.0)	83	84	(1.3)	67	
<u>Stivalius klossi</u> Nakhon Ratchasima Pak Thongchai	1.4	100	100	100	100	0.3	100			(2.5)	99	100	(1.3)	50	
										7.6	31	8	4		

P.M. = Percent Mortality at concentration listed

\* LC<sub>50</sub> beyond measure

( ) = Estimated on probit scale—not calculated

LC<sub>50</sub> = Lethal concentration expressed as percent concentration of pesticide

Table 2. Percent Knock Down and Percent Mortality of 900 Xenopsylla cheopis Exposed for 1 Hour to Paper Impregnated with Each Indicated Concentration of Carbaryl.

CONCENTRATION %	Percent "down" of 900 fleas after 1 hour exposure	Percent Mortality of 900 fleas after 23 hours rest	Percent of Total: down/dead
4.0	47	54	47/53
2.0	42	47	47/53
1.0	41	45	48/52
0.5	20	31	39/61
0.25	20	30	41/59
0.125	6	15	27/73
0.0625	2	10	14/86
0.03125	0.1	5	2/98