Studies on the Insecticide Resistance in Rat Fleas, *Xenopsylla cheopis* (Roth.) R. L. KALRA[1] and G. C. JOSHI (National Institute of Communicable Diseases, Delhi, India) Received July 31, 1974. *Botyu-Kagaku* 39, 110, 1974.

23. ケオプスネズミノミ *Xenopsylla cheopis* (Roth.) の殺虫剤抵抗性に関する研究

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DDT 抵抗性とディールドリン抵抗性系統のケオプスネズミノミ *X. cheopis* に対する各種殺虫剤の交差抵抗性，*p, p'*-DDT の脱塩酸，リピッドの性状を，感受性ケオプスネズミノミのそれらと比較検討した．その結果，DDT 抵抗性は，DDT の脱塩酸によるものでないこと，cyclodiene 殺虫剤に対する抵抗性の程度は，γ-BHC に対する抵抗性とほぼ同じ位であることがわかった．また，抵抗性および感受性系統から抽出したリピッドはほぼ同じ量で，中性脂質，りん脂質，脂肪酸の性状も両系統間で差を認めなかった．

Introduction

Xenopsylla cheopis, the vector of plague, has been reported to be resistant to organochlorine insecticides in different parts of the world. However, there does not seem to be any information available on the mechanism of resistance and cross-resistance in this species[4]. In order to understand the nature of the mechanism of resistance in *X. cheopis*, studies were undertaken on cross-resistance, dehydrohalogenation of DDT and estimation and characterization of lipids in susceptible and resistant strains.

Material and methods

Insects

Susceptible, DDT-resistant and dieldrin-resistant strains of *X. cheopis* were used during this investigation. The susceptible strain was originally collected in 1962 from the rats trapped in areas of urban Delhi. *X. cheopis* previously collected from the area were susceptible to insecticides[24]. The strain has been under continuous rearing in the laboratory since its colonization without any intentional contamination of insecticides. The technique adopted for rearing was essentially the one described by Krishnamurthy[18].

The DDT-resistant strain was developed from the susceptible strain of *X. cheopis* by exposing the blood-fed adults of succeeding generations to DDT-impregnated papers. The strain has been under continuous selection for 60 generations.

The dieldrin-resistant strain was also developed from the susceptible strain of *X. cheopis* by exposing the blood-fed adults of succeeding generations to dieldrin-impregnated papers. The strain has been under continuous selection pressure for 45 generations.

Insecticides

The following insecticides were used:

p, p'-DDT, *o, p'*-DDT, prolan, bulan, methoxychlor, aldrin, heptachlor, dieldrin, endrin, lindane, fenthion, carbaryl and DMC.

Bioassay

The susceptibility of adult fleas to DDT and dieldrin was determined using a WHO test kit[26]. Blood-fed adult fleas were exposed to filter-papers impregnated with graded concentrations of the insecticide in test tubes for 24 hrs and the mortality was recorded at the end of exposure period.

Tests with other insecticides were done by impregnation of the filter-paper (Whatman No. 1, 11 cm diameter) with 1 m*l* of the different concentrations of insecticidal solutions in acetone. After impregnation, the papers were dried for 1 hr and then cut to the desired size to line the test tubes. The exposure was done according to the WHO method. At the end of one hour exposure, the fleas were transferred to clean test tubes lined with white paper and the mortality count was made after the observation period of 24 hrs. In case fleas were exposed for 24 hrs, the mortality was recorded at the end of exposure. Ten fleas were exposed in each tube. A minimum

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of 20 fleas were exposed to each concentration. The results obtained were analysed by probit analysis[10].

Estimation of DDT metabolism

Batches of 1000 unfed adult fleas (one to two days old) were exposed to strips of filter-paper impregnated with 4% DDT, as supplied in the WHO test kit, for 24 hrs. Both dead and live fleas were then pooled, ground with sodium sulfate, and extracted with acetone in a Soxhlet apparatus for six hours. The extract was then purified and analysed by GLC following the method already described by Kalra *et al*[16].

Extraction, estimation and characterization of lipids

Batches of unfed adult fleas, one to two days old, were weighed and homogenized with 20 volumes of chloroform-methanol (2 : 1, v/v) in a Potter Elvejhem glass homogenizer. The homogenate was allowed to stand for two hours and was then filtered. After filtration, the chloroform-methanol extract was purified following the method of Folch *et al*[11]. The total lipid content was determined gravimetrically and the phosphorous in the lipids was estimated by the method of Bartlett[2]. The lipids were then fractionated, identified and estimated by following the methods earlier described[15].

The total lipids fraction was transmethylated using 6% methanol-sulfuric acid[12]. The methyl esters of the fatty acids were extracted with distilled hexane and characterized by gas-liquid chromatography using the conditions already described by Kalra[13].

Results

Cross resistance

A comparison of the susceptibility of DDT-selected, dieldrin-selected and the susceptible strains of *X. cheopis* to various insecticides is given in Table 1. The results indicated that the strain of *X. cheopis* selected with DDT showed a fairly high degree of resistance to *p, p'*-DDT. When adult fleas of the DDT selected strain were exposed for 24 hours to 4% DDT-impregnated papers, only 20% kill was observed. The LC_{50} value of *p, p'*-DDT for the susceptible strain tested under identical conditions was found to be 0.25%. The DDT-selected strain manifested high resistance to insecticides structurally related to *p, p'*-DDT, *i.e.* methoxychlor, prolan, bulan and *o, p'*-DDT. In addition, the strain also exhibited resistance varying from 5 to 32-fold to cyclodiene insecticides. The strain also showed a fairly high level of resistance to gamma-HCH and little increased tolerance to fenthion and carbaryl.

The dieldrin-selected strain showed a high level of resistance to dieldrin and other cyclodiene insecticides. The mortality of 80% was obtained in the adult fleas of the susceptible strain on their exposure for 24 hours to papers impregnated with 0.05% dieldrin whereas the mortality of only 20% was observed in the adults of the dieldrin-selected strain on their exposure to 4% dieldrin-impregnated papers. The dieldrin-selected rat fleas were found to be almost immune to endrin and heptachlor. The level of resistance to lindane was 43-fold and did not differ significantly from the level of resistance manifested by the DDT-selected strain to the insecticide. It was interesting to observe that the dieldrin-selected strain showed a moderate level of resistance to *p, p'*-DDT and other related insecticides. Nevertheless, the strain was found to be susceptible to carbaryl and manifested about 3-fold tolerance to fenthion.

Dehydrohalogenation of *p, p'*-DDT in susceptible and DDT-resistant strains

The results obtained indicated insignificant metabolism of *p, p'*-DDT to *p, p'*-DDE both in susceptible and resistant strains of *X. cheopis* as the following figures show :

	μg/1000 fleas		Percentage
	DDT	DDE	metabolized
Susceptible	8.0	0.6	7.0
DDT-selected	23.3	1.0	4.2

Fleas were exposed to filter-papers impregnated with acetone solutions of *p, p'*-DDT, DMC and their mixture (1 : 1) to find the synergistic effect of DMC, if any. The following results (given below) indicated that DMC did not enhance the toxicity of *p, p'*-DDT against the DDT-selected strain:

Table 1. Cross-resistance pattern of DDT- and dieldrin-resistant strains of *X. cheopis*

Insecticide	Exposure period (hours)	Susceptible		DDT-selected		RR	Dieldrin-selected		RR
		LC_{50}%	Slope ±SE	LC_{50}%	Slope ±SE		LC_{50}%	Slope ±SE	
p, p'-DDT	24	0.25	—	>4.0(20% kill)	—	>16.0	1.48(1.07-2.04)	1.5±0.32	5.9
o, p'-DDT	24	2.63(1.60-4.27)	1.7±0.5	>4.0(nil kill)	—	>1.5	>4.0(20% kill)	—	>1.5
Prolan	24	0.018(0.01-0.035)	2.5±0.5	>4.0(30% kill)	—	>222.0	—	—	—
Bulan	24	0.12(0.09-0.15)	3.6±0.7	>4.0(nil kill)	—	>33.3	—	—	—
Methoxychlor	24	1.09(0.79-1.46)	2.5±0.6	>4.0(5% kill)	—	>4.0	2.32(1.48-3.66)	2.0±0.7	2.1
Gamma-HCH	24	0.0044(0.0030-0.0065)	1.8±0.4	0.15(0.10-0.22)	1.8±0.4	34.1	0.19(0.17-0.21)	1.6±0.2	43.2
Dieldrin	24	<0.05(80% kill)	—	1.6(1.07-2.36)	1.9±0.5	>32.0	>4.0(20% kill)	—	>80.0
Aldrin	24	0.0081(0.0062-0.0105)	2.4±0.4	0.038(0.035-0.043)	2.2±0.4	4.75	>1.0(35% kill)	—	>125.0
Endrin	24	0.0006(0.0004-0.0009)	2.0±0.5	0.011(0.009-0.013)	3.8±0.8	18.5	>0.1(nil kill)	—	>83.3
Heptachlor	24	0.023(0.015-0.036)	1.5±0.4	0.11(0.09-0.14)	2.4±0.4	4.8	>4.0(nil kill)	—	>174
Fenthion	1	0.0038(0.0028-0.0050)	2.4±0.4	0.0073(0.0058-0.0092)	3.1±0.6	1.9	0.012(0.009-0.016)	2.8±0.7	3.2
Carbaryl	1	2.24(1.78-2.82)	2.4±0.4	4.83(3.98-5.80)	3.3±0.4	2.1	1.90(1.17-3.10)	1.8±0.8	1.0

Figures in parentheses indicate the fiducial range of LC_{50} value at $p=0.05$ level or the percentage kill observed at the indicated concentration.

	% kill
p, p'-DDT (2.5%)	35
DMC (2.5%)	0
p, p'-DDT (2.5%)+DMC (2.5%)	34

Lipids of susceptible and resistant strains

The lipid content of the susceptible, the DDT-selected and the dieldrin-selected strain of *X. cheopis* did not reveal any significant difference between them (Table 2). The amount of the total lipids per g of fresh weight of unfed adult fleas was observed to be about 13-14mg, and phospholipids constituted about 30-40% of this amount.

Table 2. Lipids of susceptible, DDT-selected and dieldrin selected strains of *X. cheopis*

Strain	Total lipids mg/g wet weight	Phospholipids mg/g wet weight
Susceptible	13.4±1.6	4.5±0.8
DDT-selected	13.8±1.1	4.8±0.7
Dieldrin-selected	14.4±1.4	5.1±0.5

Thin-layer chromatography of the neutral lipid fraction, as obtained by elution with chloroform from a silicic acid column, revealed that triglycerides constituted the main component. Diglycerides, monoglycerides and free fatty acids were present only in traces. Cholesterol was found to constitute about 3-4% of the neutral lipids and it was present both in free and esterified form. There was not found to be any significant difference in the pattern of the neutral lipids between the susceptible and resistant strains.

The phospholipids in the adults of *X. cheopis* were found to be polyglycerol phosphatide, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, phosphatidyl choline and sphingomyelin. Lysophosphatidyl ethanolamine and lysophosphatidyl choline were detected in traces only. Table 3 presents data on the proportion of different phospholipids in susceptible and resistant strains of *X. cheopis*. The major phospholipids were found to be phosphatidyl ethanolamine and phosphatidyl choline. Both these phospholipids constituted about 75 to 80% of the total phospholipids of *X. cheopis*. The percentages of phosphatidyl serine, polyglycerol phosphatide and phosphatidyl inositol were about 9.5, 3.4 and 3.2 respectively in the susceptible strain. Sphingomyelin constituted about 6% of the total phospholipids. As is apparent from the data (Table 3), the susceptible, DDT-selected and dieldrin-selected strains of *X. cheopis* did not differ with regard to the pattern of their phospholipids.

The major fatty acids found in the adults of *X. cheopis* were palmitic acid, palmitoleic acid, stearic acid, oleic acid and linoleic acid. Oleic acid was the most predominant, having a proportion of 69.3% expressed on the basis of the major fatty acids only. The proportions of palmitic acid, palmitoleic acid, stearic acid and linoleic acid were 7.7, 1.7, 12.8 and 8.5 per cent respectively. The susceptible and resistant strains gave almost identical pattern of fatty acids.

Discussion

Cross-resistance arising from the type of DDT resistance in insects depending on the dehydrochlorination mechanism was found to extend only to a series of dehydrochlorinatable analogues of DDT[5]. However, in certain insect species like *Culex tarsalis*[22], *Culex p. fatigans*[14] and the Orlando-R and Dailan-R strain of the house-fly[23], DDT resistance extended both to dehydro-

Table 3. Percentage composition of the phospholipids in susceptible and resistant strains of *X. cheopis*

	Susceptible	DDT-selected	Dieldrin-selected
Polyglycerol phosphatide	3.2±0.2	4.3±1.0	3.7±1.2
Phosphatidyl ethanolamine	42.1±1.2	40.9±1.2	46.4±2.1
Phosphatidyl choline	35.3±0.3	35.8±1.5	32.2±0.2
Sphingomyelin	6.3±0.1	7.4±0.9	4.9±0.6
Phosphatidyl inositol	3.4±0.7	4.3±0.3	4.5±0.5
Phosphatidyl serine	9.5±0.3	8.5±0.7	9.2±0.4

chlorinatable and non-dehydrochlorinatable analogues of DDT. The resistance in these species/strains was considered due to the presence of defence mechanisms other than dehydrochlorination. In the case of *X. cheopis* also, the DDT-selected strain showed high resistance both to dehydrochlorinatable (*p, p'*-DDT, TDE) and non-dehydrochlorinatable (prolan, bulan, *o, p'*-DDT) analogues of DDT. These results, therefore, suggest that the dehydrohalogenation of DDT was not the defence mechanism in this species. Studies on the estimation of DDE in susceptible and resistant strains of *X. cheopis* further confirmed the absence of dehydrochlorination of *p, p'*-DDT to *p, p'*-DDE as a defence mechanism in this species.

The selection of *X. cheopis* with DDT resulted in a low level of resistance to cyclodiene insecticides also. This is unlike the observation made with a number of insect species. However, several exceptions to this generalization have already been recorded[4].

The results, further, indicated that the strains of *X. cheopis* selected with DDT and dieldrin showed almost the same level of resistance to lindane. These strains, however, differed much in their susceptibility to dieldrin and other cyclodiene insecticides. The results, therefore, suggested the presence of certain defence mechanisms of resistance for gamma-HCH which were not shared by dieldrin. Similarly there were indications of the presence of mechanism exclusive for dieldrin. It has generally been considered that gamma-HCH and dieldrin resistance in insects is a single entity[6]. Busvine & Townsend[7] later observed an additional mechanism of resistance for gamma-HCH based upon the metabolism of this insecticide which did not confer resistance to dieldrin in the house-fly. Oppenoorth & Nasarat[21], using genetical methods observed the presence of certain factors responsible for resistance to gamma-HCH only in the house-fly.

The results obtained did not indicate any correlation between the lipid content and the DDT and dieldrin resistance in *X. cheopis*. Wiesmann[25] and Nari *et al.*[20] observed that DDT-resistant strains of house-flies and mosquitoes contained significantly greater fat contents than the susceptible strains. Khan & Brown[17] found a higher amount of lipids in the dieldrin-resistant strains of *Aedes aegypti*. On the other hand, Ascher & Nari[1], Fast & Brown[9] and Kalra[13] did not find evidence of any correlation between the lipid content and resistance in house-flies, *Aedes aegypti* and *Culex fatigans* respectively.

Numerous attempts have been made to correlate the nature of fatty acids, iodine value, sterol content, phospholipids with the tolerance of insects to DDT and dieldrin[9,19,1,8,13,3]. The results of the present investigations did not indicate any consistent difference in the nature of neutral lipids, phospholipids and fatty acids between the susceptible and resistant strains.

Summary

Data on cross-resistance, dehydrohalogenation of *p, p'*-DDT, amount and nature of lipids in susceptible, DDT-resistant and dieldrin-resistant strains of *X. cheopis* were obtained. The cross-resistance pattern and estimation of DDE in susceptible and DDT-resistant strains suggested that the dehydrohalogenation is not a mechanism of DDT resistance in this species. DDT-resistant and dieldrin-resistant strains with varying levels of resistance to cyclodiene insecticides showed almost the same degree of resistance to gamma-HCH. Indications were thus obtained of the presence of defence mechanisms exclusively for gamma-HCH. The susceptible and resistant strains contained almost the same amount of lipids. The pattern of neutral lipids, phospholipids and fatty acids in the susceptible and resistant strains did not reveal any difference between them.

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The Resistant Level of the Houseflies to Several Insecticides in New Guinea. Akifumi HAYASHI[1], Masayoshi HATSUKADE[2], Satoshi SHINONAGA[1] and Rokuro KANO[1] (Department of Medical Zoology, Faculty of Medicine, Tokyo Medical and Dental University[1] & Laboratory of Applied Entomology, Taisho Pharmac., Ltd[2]., Tokyo.) Received August 1, 1974. *Botyu-Kagaku* 39, 115, 1974 (with English Summary 117)

24. ニューギニア産イエバエの殺虫剤に対する感受性について*.

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ニューギニア地域のイエバエの殺虫剤感受性について調査し，一般的に高緯系イエバエよりも各種の殺虫剤に対して高い感受性を示すことを明かにした．しかし，Malathion に対して Rabaul (90.848 μg) と Wau (3.344 μg) は強い抵抗性を持つ特殊なコロニーの存在することも明かになった．

最近における本邦産イエバエの殺虫剤感受性については林，ら (1973, 74)[1][2] によって報告されている．また，台湾，インドネシアについても報告された．しかし，ニューギニア方面の調査報告は全くないので調査を行い，知見を得たので報告する．

本文に入るに際し，供試虫の搬入に格別の御配慮をいただいた農林省横浜植物検疫所羽田支所川崎倫一支所長，国立予防衛生研究所衛生昆虫部長朝比奈正二郎博士に御礼申し上げる．また，実験に御協力いただいた研究室の各位に謝意を表する．

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実験材料および方法

供試昆虫 実験に用いたイエバエ*Musca domestica* Linné, 1758はニューギニアの次の地域で採集し，研究室に空輸して大量飼育した個体群である．なお，採集地と採集年月日は次の如くである．

Wau (New Guinea)……Wau Ecology Institute の室内，昭和49年1月13日採集．