Development and loss of insecticide resistance in the houseflies, *Musca domestica*, at a waste disposal site in relation to insecticide application

Chobei IMAI*

*Osaka City Institute of Public Health and Environmental Sciences,
Tohjoh-cho, Tennohji-ku, Osaka 543, Japan*

(Received: August 22, 1983)

Key words: insecticide, resistance, housefly, *Musca domestica*, waste disposal site, insecticide application.

Abstract: The development and loss of insecticide resistance in a field population of the houseflies, *Musca domestica*, at Hokko, a waste disposal site in Osaka Bay was studied in relation to insecticide application and state of the resistance in the surrounding urban area. The resistance developed to trichlorfon, fenitrothion, fenthion, dichlorvos and diazinon after two years application of trichlorfon, and such resistance remained in an equilibrium for subsequent one year after the spray of calclofos and dichlorvos was substituted for that of trichlorfon. However, the resistance was not observed to two pyrethroids, namely allethrin and phthalthrins. After the insecticidal pressure was weakened in the fourth year through reducing the amount of chemicals sprayed, the resistance to organophosphorus compounds except for fenitrothion and diazinon was lowered a little. The prevalence of resistance could be explained by first, the accumulated amount of insecticides sprayed since the beginning of application to the collection date of each colony and second, the amount of chemicals sprayed during 7 days prior to the each collection date. The former and the latter may reflect the history of selection and the intensity of direct selection pressure in each generation of insects, respectively. Three urban colonies showed a low level of resistance as compared to Hokko population in the fourth year, but the diluting effect which was considered to be caused by immigrant houseflies from urban area to Hokko Site was not suggested to be so serious as compared to the effect of selection pressure.

INTRODUCTION

The insecticide resistance in field populations of the houseflies, *Musca domestica* has been reported from many sites in Japan

(Hayashi *et al.*, 1977, 1979 etc.) and from waste disposal sites called 'Yumenoshima' in Tokyo Bay (Yasutomi, 1966, 1973, 1975; Yasutomi and Shudo, 1978; Kudamatsu *et al.*, 1979). Therefore, we have much information on the state of the insecticide resistance in Japanese houseflies, but most of these results were reported without the quantitative information on the history of insecticide

* 今井長兵衛: 大阪市立環境科学研究所 (〒543 大阪市天王寺区東上町 8-34)

use at the sites where the housefly colonies tested were collected.

This paper deals with the prevalence of insecticide resistance in a housefly population inhabiting at Hokko Waste Disposal Site in Osaka Bay, in relation to the application of insecticides there and the state of the resistance in the surrounding urban area.

STUDY SITE AND METHODS

Study site: The study was carried out in 1974–1977 at Hokko which was a sea-filling waste disposal site located in North Osaka Port, about 1 km away from urban area across the sea. Approximately 1,000 ton of refuse collected from Osaka City has been daily disposed there since December 1973. As the control sites, the following three spots in Osaka City were chosen. Nishiyodo Refuse Incineration Plant where about 360 ton of refuse has been daily incinerated is located in 6 km north-east of Hokko Site. Central Market and East Market (the eastern branch of Central Market) are placed in 8 km east and 15 km east-south-east, respectively, of Hokko Site.

Housefly colony tested: To estimate the resistance level in field populations, the following housefly colonies were tested.

Hokko colonies: Houseflies were collected seven times, namely in May and November 1974, in October 1975, in June and October 1976, and in March and October 1977. In 1974, the collection was carried out by the member of Public Cleansing Bureau, Osaka City.

Three urban colonies

Houseflies were collected in October 1977 from Nishiyodo Incineration Plant, Central Market and East Market.

Hokko 75f colony

This colony was originated from Hokko colony collected in October 1975, of which adults were exposed to LD₈₀ of fenthion for 10 generations.

Takatsuki strain

This is a laboratory susceptible strain distributed by Dr. Buei.

Insecticide

Seven organophosphorus compounds, namely trichlorfon, fenitrothion, fenthion, cyanophos, calclofos, dichlorvos and diazinon, and two pyrethroids, namely allethrin and phthalthrin were used in this experiment.

Test method

The second or third laboratory generation of each colony was utilized to evaluate LD₅₀ values for insecticides with topical application method. Aceton solution (0.5 μ l) of chemicals was applied to the dorsal thorax of 3–5 day old females. Four to six grades of dosage were prepared for each insecticide, and 60–80 individuals of females were tested for each dosage. Mortality rates were recorded after keeping the treated flies at 25°C for 24 hours with food and water. LD₅₀ values were computed on the basis of regression of probit mortality on log dosage.

Field application of insecticides

Suspension of insecticides was sprayed by landfill operators on the surface of waste at a high speed of 10 liters per second at Hokko Site. Daily records on the amount of chemicals sprayed were offered from Public Cleansing Bureau, Osaka City.

RESULTS

Table 1 shows LD₅₀ values of *Hokko colonies* for 9 insecticides, and Fig. 1 illustrates the prevalence of LD₅₀ values for four chemicals and the amount of insecticides applied at Hokko Site.

Trichlorfon 20% emulsifiable concentrate had been sprayed since May 1974, when LD₅₀ value for this chemical was 3.98 μ g/female and resistance ratio was 15 times of susceptible *Takatsuki strain*. A similar state was maintained till November of the year.

The failure in the chemical control of houseflies was firstly reported from Hokko in the autumn of 1975. The susceptibility test revealed the development of high resistance in Hokko housefly population against not only trichlorfon but fenitrothion, cyanophos and fenthion. LD₅₀ values, however, were not so high for calclofos, dichlorvos and diazinon. *Hokko 75f colony* which had been

Table 1 LD₅₀ values of several insecticides to seven colonies of houseflies collected from Hokko Waste Disposal Site during the period 1974-1977.

Insecticide	LD ₅₀ of Takatsuki strain	LD ₅₀ of Hokko colonies in the following collection dates;						
		May 1974	Nov. 1974	Oct. 1975	June 1976	Oct. 1976	Mar. 1977	Oct. 1977
Trichlorfon	0.270	3.98	4.93	58.8	>70	>70	>70	43.1
Fenitrothion	0.090	3.81	6.93	34.0	53.4	56.0	53.5	58.2
Cyanophos	0.066	—	—	57.0	57.0	74.3	—	—
Fenthion	0.099	0.525	1.52	6.22	6.87	6.20	4.74	3.85
Calclofos	0.051	—	—	2.47*	2.74	2.06	2.81	1.57
Dichlorvos	0.030	0.355	0.594	0.647*	0.930	1.02	0.897	0.757
Diazinon	0.086	1.78	0.605	2.12*	5.67	6.25	5.94	6.46
Allethrin	0.559	—	—	—	1.26	1.17	0.972	—
Phthalthrin	0.350	—	—	—	0.360	0.280	0.323	—

The values attached with the symbol * were obtained by using the sixth generation of the colony.

Fig. 1 Prevalence of insecticidal pressure and LD₅₀ values of houseflies for four organophosphorus compounds.
 (a) Monthly amount of the sprayed insecticides, oblique histogram: trichlorfon 20% emulsifiable concentrate, solid histogram: calclofos 5% water soluble concentrate, empty histogram: dichlorvos 5% emulsifiable concentrate, (b) Monthly frequency of applicaiton (solid line) and monthly average amount of insecticides sprayed in one time application (broken line), (c) Fenitrothion, (d) Fenthion (solid circle) and diazinon (empty circle), (e) Dichlorvos.

selected with fenthion pressure showed the following LD₅₀ values; fenthion, 71.4 µg; fenitrothion, 178 µg; calclofos, 5.32 µg; dichlorvos, 2.24 µg.
 Basing on the results in 1975, calclofos 5% water soluble concentrate and dichlorvos 5%

emulsifiable concentrate were substituted for trichlorfon in the spring of 1976. In June of the year, LD₅₀ values were found to be higher for fenitrothion, dichlorvos and diazinon. But the values for pyrethroids were similar to those of *Takatsuki strain*. In

Table 2 Values of coefficients; a , b_1 , b_2 and multiple correlation coefficient; r in multiple regression $Y=a+b_1X_1+b_2X_2$, where X_1 , X_2 and Y were accumulated amount of sprayed insecticides, amount of sprayed insecticides during 7 days prior to the sampling dates of houseflies and LD_{50} values, respectively.

Insecticide	a	b_1	b_2	b_1/b_2	r
Fenitrothion	2.71	5.98×10^{-4}	6.44×10^{-3}	0.0929	0.9892
Fenthion	1.04	3.18×10^{-5}	2.19×10^{-3}	0.0145	0.8609
Dichlorvos	0.391	4.23×10^{-6}	1.84×10^{-4}	0.0230	0.9753
Diazinon	0.583	6.30×10^{-5}	4.32×10^{-4}	0.1458	0.9557

Table 3 LD_{50} values of several insecticides to three housefly colonies of Osaka City in the autumn of 1977.

Collection site	LD_{50} of the following insecticides;					
	Trichlorfon	Fenitrothion	Fenthion	Calclofos	Dichlorvos	Diazinon
Nishiyodo Incineration Plant	38.0	8.90	1.35	1.36	0.464	0.990
Central Market	21.6	5.79	1.26	1.03	0.340	1.01
East Market	27.4	12.8	1.64	1.32	0.374	2.18

October, the resistance level stayed at a similar state to all compounds except for cyanophos.

In 1977, the amount of insecticides used at Hokko decreased remarkably because of the success in environmental control with soil-covering at the site. LD_{50} values of *Hokko colonies* lowered a little for fenthion, calclofos and dichlorvos, but they remained a similar level for fenitrothion and diazinon.

Correlation coefficients were calculated between LD_{50} values for fenitrothion, fenthion, dichlorvos or diazinon, and several factors relating to insecticidal selection pressure. Factors examined were 1) total accumulated amount of chemicals sprayed since the beginning of application in May 1974 to the sampling date of each colony, 2) total amount of chemicals sprayed during 7, 15, 30, 60 or 90 days prior to the sampling date of each colony, 3) the frequency of application during 7–90 days prior to the sampling date of each colony, and 4) average amount per one spray during 7–90 days prior to the sampling date of each colony. Significant relation was detected between LD_{50} values for fenitrothion, dichlorvos or diazinon, and accumulated amount of all insecticides since May 1974. The correlation

coefficients were relatively high between LD_{50} values and amount of all compounds sprayed during 7 days prior to the sampling dates though it was not significant. Multiple regression of LD_{50} values (Y) was computed on accumulated amount of insecticides (X_1) and amount of insecticides during 7 days prior to the sampling date (X_2). The equations obtained were expressed as

$$Y = a + b_1X_1 + b_2X_2$$

and each coefficient is shown in Table 2.

Table 3 shows LD_{50} values of three housefly colonies of Osaka City in the autumn of 1977. *Three urban colonies* showed a common tendency that LD_{50} values of trichlorfon, fenitrothion and dichlorvos were the highest, the second highest and the lowest, respectively, among those for six chemicals tested. The above tendency was also observed in *Hokko colonies*. *Three urban colonies* showed higher LD_{50} values than *Hokko colonies* collected in 1974, but these values were lower as compared to those of *Hokko colonies* collected since 1975.

DISCUSSION

Dilution effect of urban housefly population

on resistance of Hokko population

It is considered that Hokko housefly population was originated from populations in Osaka City according to the refuse collection system. Therefore the resistance level of populations in Osaka City might be similar to that of Hokko population in May 1974. Higher LD₅₀ values of *three urban colonies* in 1977 compared to the state in 1974 suggests the development of resistance in urban populations of houseflies during the period from 1974 to 1977. High resistance to trichlorfon and fenitrothion corresponds to the large amount of trichlorfon sprayed for the control of *Hyphantria cunea* on garden and street trees, and that of fenitrothion used for communicable disease control in Osaka City. The lower level of resistance of three urban populations compared to Hokko population in 1977 suggests that immigrant houseflies which have been brought to Hokko Site from urban area with collection of refuse have had a dilution effect against the development of resistance in the Hokko population. However the significant relation between accumulated amount of insecticides and LD₅₀ values implies continuity of Hokko population since 1974 to 1977 concerning to insecticide resistance, suggesting that the above dilution effect had not been so serious compared to selection pressure.

Development of resistance after application of trichlorfon

The low level of resistance of Hokko population in May 1974 suggests the existence of resistant genes at low frequency before application of trichlorfon. In October 1975, after one and a half years application, Hokko population showed a high level of resistance to trichlorfon, fenitrothion and cyanophos and a moderate level of resistance to four other organophosphorus compounds tested.

The similar trichlorfon-resistance in the houseflies was found in Denmark after small scale farm treatments with residual spray of trichlorfon in 1966–1967 (Keiding, 1977). Trichlorfon-resistance was also reported on several housefly populations in Florida with a moderate cross-resistance to diazinon (LaBrecque *et al.*, 1958). On the other hand,

a housefly population at the third Yumenoshima, a waste diaposal site in Tokyo Bay showed a high level of resistance to trichlorfon, fenitrothion and cyanophos after ten years application of a mixture of fenthion and dichlorvos without selection pressure with trichlorfon (Kudamatsu *et al.*, 1979). A laboratory strain selected with fenitrothion for 17 generations showed a high level of cross-resistance to trichlorfon as well as diazinon and fenthion (Shono and Tsukamoto, 1983).

These results suggest a close relation among mechanisms regulating the resistance to trichlorfon, fenitrothion and cyanophos.

Equilibrium of resistance after switching insecticides

After calclofos and dichlorvos were substituted for trichlorfon, the resistance level of Hokko population seemed to be in an equilibrium in spite of large amount of insecticides applied. On the other hand, in the second- and the third-Yumenoshima population in Tokyo Bay, the development of resistance was found to all of five organophosphorus compounds tested after application of fenthion and dichlorvos (Yasutomi, 1973, 1975; Yasutomi and Shudo, 1978). The reason for the above difference between these populations is unknown, but it is worth noticing that the intensity of selection pressure to them was almost similar because daily application was carried out at the sites during the period concerned, and that the selection pressure with fenthion caused the development of resistance to organophosphorus compounds in *Hokko colony*. Therefore it is suspected that calclofos and dichlorvos caused only a low level of resistance to other groups of organophosphorus insecticides.

Effect of insecticidal pressure on prevalence of resistance

As Keiding (1975) points out, the persistence and intensity of insecticidal pressure is an important factor affecting the prevalence of insecticide resistance. This factor can be divided into the history of application of chemicals and the intensity of pressure to the existing population in a given time. It

is reasonable that the history of application and the intensity of pressure are represented by the accumulated amount of insecticides and the amount of chemicals sprayed during a given period prior to the date of examination, respectively, at least for a developing phase of resistance. Two coefficients b_1 and b_2 shown in Table 2 imply the degree of contribution of factor X_1 (the accumulated amount of chemicals) and factor X_2 (the amount of chemicals sprayed during 7 days prior to the sampling dates), respectively. The b_1/b_2 values were lower for fenthion and dichlorvos than for fenitrothion and diazinon. The results suggest that the contribution of the existing selection pressure was larger to fenthion or dichlorvos resistance in Hokko population than to fenitrothion or diazinon resistance in the same population. The above suggestion can explain the relatively rapid reversion of the resistance to fenthion and dichlorvos as compared to the relative stability of the resistance to fenitrothion and diazinon in Hokko population after the insecticidal pressure was weakened in 1977.

ACKNOWLEDGEMENTS

I wish to thank the members of Public Cleansing Bureau, Osaka City for their generous assistance in this study. I am very much indebted to Dr. K. Buei for his kind distribution of Takatsuki strain. Thanks are also due to Prof. emer. M. Ikuzawa, Prof. S. Takada and Dr. S. Yonemoto in Osaka City University, Medical School for their valuable advice, and Dr. A. Honda and Dr. T. Hotta in Osaka City Institute of Public Health and Environmental Sciences for their encouragement in the course of this study.

REFERENCES

- Hayashi, A. E., Funaki, M., Fuzimagari, R., Kano and K. Nomura (1977): The resistant level of the housefly to several synthetic insecticides in west of Kanto and Kyushu, Japan. *Botyu-Kagaku*, **42**: 198-203.
- Hayashi, A., E. Funaki, M. Fuzimagari and R. Kano (1979): The resistant level of the housefly to several synthetic insecticides in Tohoku, Hokuriku and San'in, Japan. *Jap. J. Sanit. Zool.*, **30**: 315-318.
- Keiding, J. (1975): Problems of housefly (*Musca domestica*) control due to multiresistance to insecticides. *J. Hyg. Epidemiol. Microbiol. Immunol.*, **19**: 340-355.
- Keiding, J. (1977): Resistance in the housefly in Denmark and elsewhere. In: *Pesticide management and insecticide resistance* (ed. Watson, D. L. and A. W. A. Brown), pp. 261-302, Academic Press, London and New York.
- Kudamatsu, A., T. Sato, A. Hayashi and R. Kano (1979): Cross resistance to various organophosphorus insecticides in a third Yumenoshima strain of the housefly, *Musca domestica* L. *Jap. J. Sanit. Zool.*, **30**: 255-261.
- LaBrecque, G. C., H. G. Wilson and J. B. Gahan (1958): Resistance of house flies in Florida to organophosphorus insecticides. *J. Econ. Entomol.*, **51**: 616-617.
- Shono, T. and M. Tsukamoto (1983): Linkage group analysis of fenitrothion resistance in the housefly, *Musca domestica* L. (Diptera: Muscidae). *Appl. Ent. Zool.*, **18**: 98-105.
- Yasutomi, K. (1966): Insecticide resistance of houseflies outbreaked at the dumping site, Yumenoshima-island, Tokyo. *Jap. J. Sanit. Zool.*, **17**: 71-73.
- Yasutomi, K. (1973): Insecticide resistance in the houseflies of New Yumenoshima, the 15h dumping-island of Tokyo. *Jap. J. Sanit. Zool.*, **23**: 255-256.
- Yasutomi, K. (1975): Insecticide resistance in the houseflies of the third Yumenoshima, a new dumping-island of Tokyo. *Jap. J. Sanit. Zool.*, **26**: 257-258.
- Yasutomi, K. and C. Shudo (1978): Insecticide resistance in the houseflies of the third Yumenoshima, a new dumping-island of Tokyo (II). *Jap. J. Sanit. Zool.*, **29**: 205-208.

摘 要

ごみ埋立地におけるイエバエの殺虫剤抵抗性の消長

大阪市北港廃棄物埋立処分地におけるイエバエの殺虫剤抵抗性の消長を殺虫剤散布との関連において調査した。trichlorfon 乳剤の散布開始2年後には、trichlorfon, fenitrothion に対する強度の抵抗性が発達し、他の有機リン剤に対する感受性も低下したが、pyrethroid 抵抗性は認められなかった。散布薬剤を calcofos 水溶剤と dichlorvos 乳剤に換えた後の1年間の抵抗性レベルは平衡状態にあったが、散布量が著しく減少した次の1年間には fenitrothion と diazinon を除く有機リン剤に対する抵抗性がやや弱まる傾向が認められた。大阪市内のイエバエの殺虫剤抵抗性のレベルは北港のイエバエより低く、これらがごみ収集時に北港へ移入して希釈効果を発揮したものと思われる。

が、その影響は殺虫剤の淘汰圧に比して小さかったものと推察された。北港のイエバエのLD₅₀値の消長は殺虫剤による淘汰圧と関連し、fenitrothion や diazinon 抵抗性は殺虫剤散布歴の影響を受けやすく、fenthion

や dichlorvos 抵抗性は前者に比して当該の世代における直接的な淘汰圧の影響を受けやすい傾向があり、これによって淘汰圧の弱まった後の両グループの抵抗性の消長の違いが説明できた。