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DISCUSSION

Anka Sitar and Danica Pantović.[1] *Laboratory studies on resistance of the body louse to malathion and carbaryl.*[2] Resistance to insecticides in general was noted among insects 80 years ago (*17*). Because insect resistance is manifested more rapidly and prominently in reaction to DDT and other chlorinated hydrocarbons, more attention has been paid to these insecticides in recent years than previously.

The problem of insect resistance to DDT was first described in Sweden, where it was noted that compounds based on chlorine rarely killed flies. The appearance of resistance in flies induced a series of studies in many laboratories around the world, and soon afterward other insects, were found to be resistant to DDT.

In the body louse, *Pediculus humanus humanus* L., resistance was first noted in Korea (*13*). Shortly afterward, the resistance of lice to DDT and lindane in other parts of the world was reported. Lice resistant to DDT and BHC were found in Japan (*15, 20*), to DDT in Egypt (14), and to lindane in France (*16*). In Yugoslavia, resistance to DDT powder was found among the louse population in nature (*10, 18, 19*) and as a consequence of laboratory selection (*11*) as well. In the beginning, when DDT was broadly used and insects developed resistance to it, this reaction was thought to be specific to DDT alone, but the use of other chlorinated hydrocarbons such as gamexan, octachlorine, and chlordan clearly indicated that resistance to DDT is less exclusive and that it also includes other insecticides based on chlorine.

For these reasons it was necessary to pass from earlier studies to organophosphorus (malathion, parathion, diazinon) and carbamate insecticides which were good substitutes for earlier insecticides.

One of the insecticides properly studied in the group of phosphorus esters is malathion. Its toxicity for humans and mammals (*2, 12*) and its high insecticidal effect against lice were studied in laboratory conditions (*4, 5*). Malathion in powder was successfully used in territories where lice were resistant to DDT (*1*).

The development of resistance to malathion as an organophosphorus insecticide is a slow process and the level of acquired resistance is not as high as in the case of organochlorine insecticides. No resistance to it has been found up to now among the louse population in nature. By selection in laboratory conditions, many strains have shown a high sensitivity or only a limited degree of tolerance (*8*). As will be seen from further exposition, our strains are very sensitive to this insecticide.

Although it is an effective insecticide, malathion does have its shortcomings—for example, an unpleasant smell and rapid dis-

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integration, especially if it is stored in small quantities.

Carbaryl has properties similar to those of organophosphates and it belongs to the group of carbamate compounds. It was one of the first insecticides to be used as a substitute for DDT and lindane, and is also one of the most widely known and most frequently used carbamates. As a consequence, it has been studied more than any of the other substitutes. Carbaryl acts as an inhibitor of cholinesterase in the same way as organophosphorus insecticides, and its activity accordingly depends on the presence of enzymes in the group of esterases (*9*). It has a long residual effect. It has been proved that carbaryl is highly synergistic with sulfoxide and piperonyl butoxide (*7*). By selection in laboratory conditions, strains of body lice resistant to this insecticide were obtained (*3*).

Material and methods

In our investigations of the resistance of body lice, *P. h. humanus* L., to malathion and carbaryl the following strains were used: L, S, and B.

The L strain was obtained in 1970 from Professor Gaon in Sarajevo, who had been keeping it since 1951. The lice had not been brought into any contact with insecticides and the attempt to obtain resistance to DDT through selection was unsuccessful (*11*).

The S and B strains originated in Sjenica and Brodarevo (the territory of Sandžak in Serbia), both formerly endemic regions of typhus. They were treated from time to time with 2 per cent DDT emulsion during the lice control campaign in 1965-67 (*21*). The S strain has been kept in our laboratory since June 1970 and the B strain since November of the same year.

All three strains have been fed on humans once a day.

Acetone solutions of malathion and carbaryl were used in the experiment. The pure substance of malathion (99.7 per cent) was obtained from the American Cyanamid Company and that of carbaryl (99.9 per cent) from the Union Carbide Company.

The beaker test was the method used (*21*). In brief, 20 lice of both sexes aged 10 to 12 days from a whole generation were placed on patches of woolen cloth soaked with 0.6 ml of acetone solution of insecticide and then dried. The exposure lasted 24 hours at 27°C, the relative humidity being 60 to 70 per cent.

Results and discussion

Malathion

Two strains of body lice were selected and each strain was exposed to nine different concentrations of malathion ranging from 0.0008 to 0.0025 per cent. The exposure was carried out over a two-year period, and it was found that by the 24th generation both strains had increased their resistance by about threefold. The total number of lice tested was 92,889 of the L strain and 76,535 of the S strain.

The selection of the L strain with malathion started with three concentrations: 0.0020, 0.0015, and 0.0010 per cent. In the very beginning we noticed an abnormality in the parent generation: mortality recorded at a lower concentration was higher than at two higher ones, although the experiment was carried out under exactly the same conditions and using the same materials. In the course of selection this phenomenon was repeated in generations 11, 12, and 18. Various combinations occurred and there seemed to be no definite relationship between the degree of concentration and the number of surviving lice. We therefore simultaneously exposed the lice of one generation to several different concentrations, most often three but sometimes as many as six. We give the results for the same generation and the same concentration in average values. Normally, higher death rates occurred with the use of higher concentrations. Every time the death rate approached 95 per cent, we were forced to return to lower concentrations in the next generation. The highest concentration at which the lice were exposed, from the first to the fifth generation, was 0.0020 per cent, but between the sixth and 12th it was reduced to 0.0015 per cent. Between the 13th and 17th it was increased again to 0.0017 per cent, and in the 18th generation we were obliged to use 0.0010 per cent again. In the 22nd generation we came up to 0.0025 per cent, and from the 23rd we used 0.0020 per cent.

Table 1 shows the results of the exposure of strain L to malathion with the LC_{50} and LC_{90} values for several generations.

Table 1. Resistance of body lice, strain L, to malathion (beaker test) exposing the whole generation.

Generation	No. tested	LC_{50} %	LC_{90} %
F_3	7,391	0.0008	0.0020
F_4	4,423	0.0010	0.0015
F_{14}	4,236	0.0014	0.0017
F_{18}	2,550	0.0009	0.0010
F_{22}	4,837	0.0016	0.0023
F_{23}	5,258	0.0009	0.0020

The selection of the S strain was carried out simultaneously under the same conditions and using the same method as for strain L. The parental generation selection started with concentrations of 0.0008, 0.0015, and 0.0020 per cent. Because 95 per cent mortality was obtained in the fourth generation at the concentration of 0.0020 per cent, selection from generations 5 to 11 was carried out at lower concentrations (0.0008, 0.0009, and 0.0012 per cent). From the 12th to 23rd generations concentration was increased up to 0.0025 per cent. In the 24th generation we again reduced the concentration to 0.0010 per cent. The changeability of the selection course can better be seen in Table 2.

As can be seen from the results obtained, both strains of lice are very sensitive to malathion. Selection passed very unevenly and slowly, with frequent returns to lower concentrations of insecticides. Through 24 generations we scarcely succeeded in getting threefold tolerance when the lowest and the highest concentrations at which the lice survived were compared. Comparing the LC_{50} or LC_{90} values, we can observe an approximate twofold increase in louse tolerance to malathion.

Carbaryl

Selection with carbaryl was surer and more rapid. There was no need to return to lower concentrations, except at the beginning of the experiment when we were forced to return to lower concentrations in parental and some of the beginning generations of the L and S strains because selection was begun at too high concentrations (0.020 and 0.010 per cent). One to three concentrations were usually used for each generation except in two cases, in which six or seven concentrations were used. Lice that survived after a 24-hour exposure to carbaryl were immediately capable of taking blood. This was not the case with malathion, when sometimes the lice would not eat even after five hours.

Table 2. Resistance of body lice, strain S, to malathion (beaker test) exposing the whole generation.

Generation	No. tested	LC_{50} %	LC_{90} %
P	1,159	0.0008	0.0014
F_4	5,774	0.0009	0.0017
F_8	5,461	0.0008	0.0012
F_{12}	9,457	0.0010	0.0015
F_{13}	3,488	0.0015	0.0017
F_{18}	2,341	0.0008	0.0015
F_{21}	2,015	0.0011	0.0017
F_{22}	1,830	0.0014	0.0023

The lice of L strain were exposed to 14 concentrations of carbaryl ranging from 0.008 to 0.3 per cent. A total of 69,691 individuals up to the 23rd generation were tested. In most generations the mortality ranged from 60 to 70 per cent regardless of successive concentration increases. Exceptions were found between the fifth and ninth generations and in the 14th, in which the mortality went either above or below these limits. The course of selection is presented in Figure 1.

As can be seen from this figure, the mortality of 60 to 70 per cent in the first four generations was found at concentrations from 0.008 to 0.01 per cent. There were exceptions between generations 5 and 9. In generations 10 to 13, a lethal dose of 60 to 70 per cent was reached at the concentration of 0.04 per cent, but in the 14th generation the lethal dose surpassed 70 per cent. From the 16th generation onward it was necessary to increase the concentration greatly because resistance built up so rapidly that in the 23rd generation it was about 37 times greater than in the initial generation.

Selection of the S strain was carried out at the same time and under the same conditions as for the L strain. The results of this selection are presented in Figure 2.

The lice were exposed to 17 concentrations of acetone solutions of carbaryl (0.008 to 0.5 per cent). Beginning with the 0.010 per cent concentration, the parental and the next three generations showed higher mortalities than the limits of 40 to 60 per cent shown in Figure 2. After the fourth generation at the 0.008 per cent concentration, the concentrations were gradually increased up to the 13th generation. In the 15th and 16th generations a certain reduction was made, and in the 17th a sudden increase took place that lasted to the 23rd, when a 40 to 60 per cent mortality was obtained at an 0.3 per

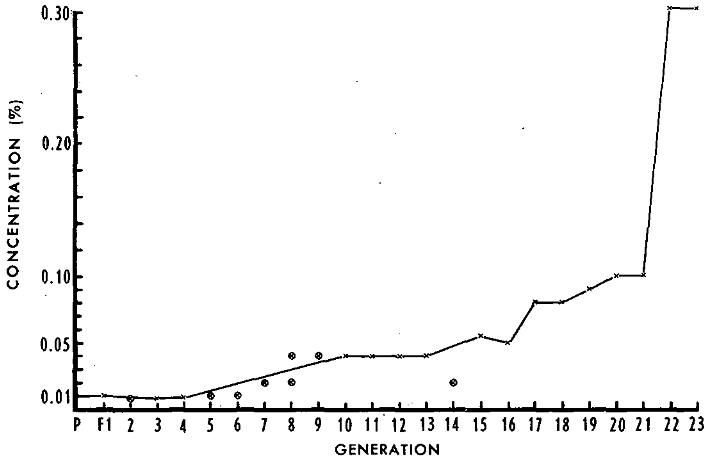

Figure 1. Mortality (60 to 70 per cent) of L strain body lice from carbaryl (beaker test).

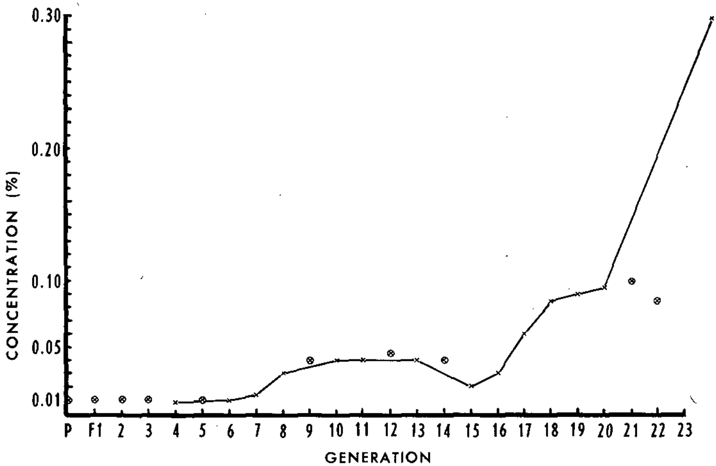

Figure 2. Mortality (40 to 60 per cent) of S strain body lice from carbaryl (beaker test).

cent concentration. During the selection of this strain there were exceptions to the 40 to 60 per cent mortality in generations 9, 12, 14, 21, and 22, in addition to the three generations mentioned above.

From the results obtained we can conclude that the S strain is somewhat more resistant than the L strain. A total of 122,117 individuals of the former strain were tested.

The selection of the B strain, as has already been mentioned, started later and 18 generations were obtained. A total of 29,651 individuals were exposed to concentrations ranging from 0.008 to 0.1 per cent. The results of the selection of this strain of lice are presented in Figure 3.

Selection started with low concentrations ranging from 0.008 to 0.010 per cent, but between the third and sixth generations the

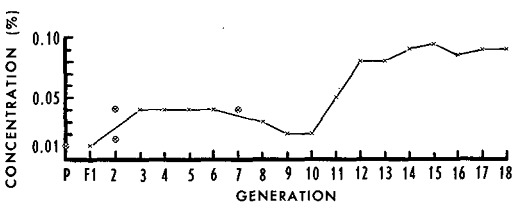

Figure 3. Mortality (50 to 70 per cent) of B strain body lice from carbaryl (beaker test).

concentration was increased to 0.04 per cent and the mortality was 50 to 70 per cent. In generations 8 to 10 the concentration was decreased to 0.02 per cent and in generations 11 to 18 it was increased to 0.09 per cent. This strain acquired about 12 times greater resistance by selection through 18 generations.

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