THE RESISTANCE OF THE ONCOMELANIA SNAIL TO MOLLUSCICIDES

I. SODIUM PENTACHLOROPHENATE

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Sodium pentachlorophenate (NaPCP) is now considered to be one of the most promising molluscicides. Since 1954, this compound has been used for the practical molluscicidal purpose in the major endemic areas of schistosomiasis in Japan. However, the resistance in *Oncomelania* snails to NaPCP came into question as pointed out by Okabe *et al.* (1956) and Ota *et al.* (1956). Afterwards, Gancarz (1958) and Walton *et al.* (1958) studied the resistance problem in *Oncomelania* snails and presented objections against the development of PCP-resistance. The aim of the present study was: (1) to reexamine whether PCP-resistance has been developed in the treated snail population, (2) to know whether acquired resistance would develop, (3) to ascertain whether a strain of higher resistance exists, and if present (4) to investigate whether its resistance will develop with higher selection pressure of NaPCP.

MATERIALS AND METHODS

Snails used: *Oncomelania nosophora* used in this study was collected from the endemic areas in Yamanashi Prefecture. Only snails which were actively moving when placed in dechlorinated water were selected for the experiment.

Chemical used: NaPCP (contains at least 94% of the pure compound) furnished through the courtesy of the Asahi Denka Company was used in this study. This product has been used for the practice of molluscicidal work in Yamanashi Prefecture for several years. Stock solutions were prepared by dissolving 0.1 g of NaPCP in 100 cc of distilled water, and then a series of several twofold dilutions, *i. e.* 2.0, 1.0, 0.5, 0.25, 0.125, 0.0625 and 0.03125 ppm, was made.

Technic to bring snails in contact with the compound: Ten snails were put into a Petri dish, 12 cm in diameter and 2.5 cm in height, containing 100 cc of the stock solution. The surface of the solution was covered with a vinyl net of fine mesh with a wooden frame to prevent snails from creeping out of water. In each concentration of the chemical the 3 dishes (30 snails) were used. All dishes, except the case of an experiment in Chapter 1, were kept in an incubator at 25°C. After 48 hours' exposure to the chemical, snails were rinsed several times with running water and placed in dechlorinated water for 48 hours to examine their survival or death.

Criterion of death: The snails were judged as being dead when they showed no movement after their transfer into water. Those which showed no movement were crushed afterwards to confirm their death. For the determination of the LD₅₀ and LD₉₀ Behren's or Fisher's methods were adopted. The other procedures will be mentioned in each paragraph.

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RESULTS

1) *An Experiment to Examine Whether PCP-Resistance Has Been Developed in the Treated Snail Population.*

Snails were collected from Hatta and Shirane which had been treated with NaPCP twice a year for a period of four years. Control-group snails were collected from two areas, Ichikawa and Minobu which had never been treated with molluscicides. All snails used were collected on the same day and tested at the same time under identical conditions. The room temperature during the experiment ranged between 26°C and 31°C.

Fig. 1. Comparison of dosage-mortality curve of snails from treated and untreated areas.

The results are illustrated graphically in Fig. 1. It may be seen from the results that snails from the areas of Hatta and Shirane (treated areas) show less mortality in the same concentration of compounds than those from Ichikawa and Minobu (untreated areas). The M. L. D. of snails was as follows :

Snails from treated areas	
Those from Hatta	0.30 ppm
Those from Shirane	0.32 ppm
Snails from untreated areas	
Those from Ichikawa	0.23 ppm
Those from Minobu	0.25 ppm

As seen from the foregoing, the resistance of the snails from areas having previously been treated with NaPCP appeared to be slightly higher than that of the snails from untreated areas. However, the statistical analysis of the mortality curve suggested that there is no significant difference between the two.

During the course of this experiment it became of interest to know whether geographical difference exists in the susceptibility to chemicals among snails from separate areas that had been treated for approximately the same period, and the following experiment was carried out.

Fig. 2. Comparison of dosage-mortality curve of snails collected from three separate areas which have been treated with NaPCP for 7 years.

Snails were collected from Tamahata, Tatsuoka and Shirane which had been treated with NaPCP twice a year for seven years. All snails used were collected on the same day and tested at the same times under identical conditions. The dosage-mortality curves obtained are shown in Fig. 2, from which the LD_{50} of snails was calculated as follows :

Shirane	0.20 ppm
Tatsuoka	0.32 ppm
Tamahata	0.28 ppm

Thus the fact that susceptibility to NaPCP was slightly different even among the above three groups suggests that such a slight difference in susceptibility between snails from treated areas and those from untreated areas as seen in Fig. 1 may be ascribed

either to the geographical difference between the areas where snails were collected, or to a technical error in our immersion method.

Fig. 3. Dosage-mortality curve of snails given sublethal dose of NaPCP three times on every third week.

2) *An Experiment to Know Whether Acquired Resistance Would Develop.*

Since the LD_{50} of snails was 0.325 ppm in a preliminary test, snails were submerged into a 0.0625 ppm solution of NaPCP (a sublethal dose) for 48 hours after every 3 weeks. Those in the control group were submerged into water only. After three times submerging, the dosage-mortality curves of both experimental and control groups were examined.

Fig. 3 shows the results, and the LD_{50} was as follows :

Experimental group	0.463 ppm
Control group	0.716 ppm

Thus the LD_{50} of snails submerged successively into 0.0625 ppm of NaPCP seems to be rather lower than that of the controls. Insofar as the above experiment is concerned, the exposure to small amounts of NaPCP appears to make snails rather more susceptible.

3) *An Experiment to Know Whether a Strain of Higher Resistance Exists.*

Snails collected on the same day were divided into two groups, experimental and control. Snails of the experimental group were submitted to such high selection pressures as LD_{50} and LD_{90} determined previously. The survivors after exposure in such selection doses were reared in the laboratory for 3 weeks. Then the mortality was

examined in comparison with that of the control.

The results are illustrated graphically in Fig. 4. The LD_{50} was as follows :

Snails selected with LD_{50}	0.224 ppm
Snails selected with LD_{90}	0.243 ppm
Control	0.216 ppm

Thus no marked difference was recognized among the three groups.

Fig. 4. Dosage-mortality curves of snails selected with LD_{50} and LD_{90} levels of NaPCP.

4) *An Experiment to Know Whether a Strain of Higher Resistance Will Develop with Higher Selection Pressure of NaPCP.*

In the preceding experiment snails selected with LD_{50} and LD_{90} levels of NaPCP were reared in the laboratory and produced their offsprings. Control-group snails were also reared under identical conditions. When the offsprings measured more than 6.0 mm in their shell-length, they were submitted to different doses of NaPCP to obtain their dosage-mortality curve.

Fig. 5 shows the results. The LD_{50} was as follows :

Offsprings of snails selected with LD_{50}	0.23 ppm
Offsprings of snails selected with LD_{90}	0.22 ppm
Offsprings of control	0.26 ppm

Thus there was no marked difference in susceptibility among the three groups, the results being similar to those obtained in the previous chapter.

Fig. 5. Dosage-mortality curves of offspring of snails selected with LD₅₀ and LD₉₀ levels of NaPCP.

DISCUSSION

A number of published reports are of interest in relation to the results presented here. Okabe *et al.* (1956) compared the LD₅₀ of snails collected from the area which had been treated with NaPCP with that of those from the area where this compound had never been applied. The results showed that LD₅₀ of snails from the treated area was found to be higher than that from the untreated one. In the same year, an experimental study on the resistance of snails to molluscicides was carried out by Ota *et al.* (1956). Here snails were exposed successively to 1/100,000, 1/90,000...1/20,000 and 1/10,000 dilutions of NaPCP for 1-6 hours at intervals of one week. As a results of this, they found that resistance to molluscicides develops in *O. nosophora* after repeated exposures to the increasing doses of NaPCP. Likewise, Gancarz (1958) studied the PCP-resistance in *O. hupensis* by almost the same procedure as was used by Ota *et al.* and found that the mortality of snails exposed successively at intervals of one week or one month to water solutions of NaPCP in the dosage of LD₃₀, LD₅₀, LD₇₀ and LD₉₀ as determined previously was slightly lower than that of the controls. Thus he stated that the small difference in mortality between the two groups of snails can hardly be attributed solely to the presence of resistance. A similar investigation was made by Walton *et al.* (1958), in which an immersion test was conducted with snails collected from areas having been treated for several years with NaPCP and from neighboring

control areas. As a result no resistance to NaPCP was demonstrated in snails from areas having been treated for 7 and 4 years, respectively.

As mentioned above there have been two different opinions concerning the development of resistance of snails to molluscicides, one supporting its existence (Okabe *et al.* and Ota *et al.*) and the other arguing against it (Walton *et al.* and Gancarz).

On the basis of the results from the present study, it is concluded that (1) no PCP-resistance has been developed in the treated snail population, (2) no snails acquire resistance to NaPCP during their life time; exposure to small amounts of NaPCP appears to make them rather more susceptible, (3) there is no snail-strain of a higher resistance to NaPCP, and (4) no resistance develops with higher selection pressure of NaPCP. As for the first and the second items, these findings are in accord with the statements of Walton *et al.* and of Gancarz, respectively. The wide difference from Okabe's results may be ascribed to the facts that in Okabe's work the plate method devised by McMullen (1949) was applied to test the susceptibility of snails to chemicals and that the tests on the two groups of snails were made at different times of the year. Although the plate method is considered to be suitable for testing water-insoluble molluscicides, it appeared unsuitable for testing quantitatively the susceptibility of snails to NaPCP (Komiya *et al.*, 1961). Therefore in the present experiment, the modified immersion method was adopted which appeared more satisfactory for this purpose. On the other hand, Tsuda (1952) indicated that the susceptibility of snails to molluscicides varies with the season of the year. To avoid the difference of such possibility, snails used in the present experiment were collected on the same day at each series of experiment.

The cause of the difference from Ota's result concerning the second item remains unexplained. It would seem desirable to investigate the resistance problem of schistosome-bearing snails not only in *Oncomelania* but also in other aquatic snails.

SUMMARY

An experimental study on the resistance of *Oncomelania nosophora* to sodium pentachlorophenate (NaPCP) has been made and the following conclusions reached:

- (1) No PCP-resistance has been developed in the treated snail population.
- (2) No snails acquired resistance to NaPCP during their life time; exposure to small amounts of NaPCP appears to make them rather more susceptible.
- (3) No snail-strain of a higher resistance to NaPCP was found.
- (4) No resistance develops with higher selection pressure of NaPCP.

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