Delayed Neurotoxicity Resulting from Administration of Leptophos to the Comb of Domestic Fowl

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YAMAUCHI, T., KONNO, N. and KINEBUCHI, H. Delayed Neurotoxicity Resulting from Administration of Leptophos to the Comb of Domestic Fowl. Tohoku J. exp. Med., 1982, 138 (2), 199-208 — We investigated the occurrence of delayed neurotoxicity in domestic fowl following percutaneous application of leptophos. Five groups of 5 adult hens received daily percutaneous doses of 1.0 ml/hen of leptophos emulsion (leptophos; 340 mg/hen/day) for 2, 5, 10, 15 or 20 days. There was no abnormal gait in the 2-day group. Two out of 5 hens in the 5-day group showed mild ataxia from about 2 weeks after the final administration, but did not develop severe neuropathy. On the contrary, 4 out of 5 birds in the 10-day group and all hens in the 15- and 20-day groups were affected by various stages of neurotoxicity. Some of them died from neurotoxicity. Ten of young male chickens were given the same dermal dose for 5 or 10 days. Although no abnormal chicken was observed in the 5-day group, all chickens in the 10-day group showed severe paralysis and two of them died. We studied the incidence rates of delayed neurotoxicity resulting from respective applications of the emulsion and the acetone solution of leptophos. No significant difference was observed between them. These results suggest that the daily dermal application of the relatively high dose of leptophos, even if for the short term, can cause the same delayed neurotoxic effects as by the oral administration in hens or chickens. ——— leptophos (Phosvel®); delayed neurotoxicity; topical administration; comb of hen

Leptophos (Phosvel®) [O-(4-bromo-2,5-dichlorophenyl)-O-methyl phenylphosphonothioate] is an organophosphorus pesticide that produces delayed neurotoxicity in hens after oral administration in various dosages (Abou-Donia et al. 1974; Kinebuchi et al. 1976; Yamauchi et al. 1980). The clinical condition of neurotoxic effects are characterized by ascending ataxia or paralysis which occurs 8 to 10 days after the administration of the compound. It is noteworthy that death due to development of the neurotoxic effects of leptophos can occur from a smaller dosage than acutely determined LD₅₀ (Abou-Donia et al. 1974) due to the cholinergic crisis (Kinebuchi et al. 1976).

It has been well known that some organophosphorus compounds such as TOCP (trioortho cresyl phosphate) (Smith and Elvove 1930) and mipafox (Bidstrup et al. 1953) have also the same neurotoxic effects, and it was recently demonstrated that

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CYP (cyanofenphos) produced delayed neurotoxicity in hens (Abou-Donia and Graham 1979; Abou-Donia and Komeil 1979; Kinebuchi et al. 1981) and in sheep (El-Sebae et al. 1979).

Thus, the delayed neurotoxicity from oral administration of some organophosphorus pesticides is widely known, but far less is known of effects of the dermal administration of these compounds. The results of repeated topical administration of leptophos (Abou-Donia and Graham 1978), TOCP (Glees and White 1960, 1961) and CYP (cyanofenphos) (Konno et al. 1982) to the comb of hens have been demonstrated. Repeated doses of a low level of leptophos (0.5–20 mg/kg, daily) to the comb of hens for a long period (maximal 324 days) could cause ataxia or paralysis in hens (Abou-Donia and Graham 1978).

In this study, the delayed neurotoxic effects after relatively high level administration of leptophos to the comb of hens for a short term were studied to investigate delayed neuropathy after acute or subacute exposure to the pesticide. At the same time, delayed neurotoxicity was compared in adult hens and young chickens.

**MATERIALS AND METHODS**

Twelve young male chickens, 6 months old and weighing 1.45±0.19 kg, and 40 adult hens, 24 months old and weighing 1.72±0.22 kg, of mixed breed white leghorn were used. Commercially available emulsion of leptophos containing 34% of leptophos and crystalline leptophos preparation were used. We obtained the crystallized compound by adding 4 parts of methanol to the commercial emulsion, allowing the mixture to stand overnight and used it as an acetone solution at the time of administration. Both preparations were painted on the comb with a micropipette.

Thirty adult hens were divided into 6 groups, each comprising 5 birds. Five groups of them received a dermal application of 0.5 ml of the commercial emulsion of leptophos (Nippon Noyaku, Tokyo) twice a day (leptophos, 340 mg/hen/day) for 2, 5, 10, 15 or 20 days. We called them “2 day group”, “5 day group” and so on. The sixth group remained without any treatment as control. At the same time, twelve young male chickens were divided into three groups, 2 groups of 5 and one group of 2 birds. The former 2 groups were given the same dermal administrations as adult hens for 5 or 10 days, and the latter group (2 birds) received no treatment as control.

The remaining adult hens were divided into two groups. One group was given dermally on the comb 1.0 ml of 17% acetone solution of leptophos twice a day (340 mg/day) for 10 days. The other group was treated with pure acetone alone (1.0 ml/hen) twice a day for 10 days. Although the commercial emulsion contained 34% leptophos, 17% acetone solution was used here because leptophos could not be dissolved in acetone to obtained a solution of 34%. To apply the same amount of leptophos to birds, a two-fold amount of the acetone solution was used.

The areas painted with emulsion or acetone solution were left exposed throughout the experimental period. The birds were fed with commercial feed, had free access to water, and were weighed regularly twice a week. All birds were observed daily for 28 days after the last application. We have used a grading system which grades the progress of clinical signs in four stages (Konno et al. 1980). ‘Mild ataxia (+)’ is slow, clumsy and unsteady gait. In ‘gross ataxia (+)’, gait is always abnormal and waddling, but bird is still active. Staggering and falling down occur very often. In ‘mild paralysis (+)’, the hen keeps typical posture, sitting on buttocks, extending its legs and ducking its head. Hen can move by wing flapping. In ‘severe paralysis (+)’, the bird is unable to keep its posture, to stand on its legs or to move at all.
Dose-response relationship in hens

Two out of 5 hens that received the dermal application for 5 days showed ataxia but did not develop paralysis. Four birds in the 10 day group were affected, i.e. 3 birds by ataxia and one by mild paralysis, but none died. Their clinical symptoms occurred from 9 to 21 days after the final leptophos application. All birds in both the 15 and 20 day groups were affected by various stages of delayed neurotoxicity. One bird of the 15 day group and two of the 20 day group developed severe paralysis and died due to respiratory disturbance. The birds of the 20 day group showed the neurotoxic effects before the completion of the treatment. The birds of the 2 day group and the control group were quite normal (Table 1). The mean total dosages per body-weight of each group were roughly evaluated from the group-mean-body-weight of hens and the total amounts of dosage, and given in Table 1.

Comparison of delayed neurotoxic effects in adult hens and young male chickens

It is difficult to obtain adult male fowls in the market. By fortunate chance, the twelve male chickens used in this study had grown up in our laboratory. The

### Table 1. Development of delayed neurotoxicity after daily dermal application of leptophos for several days in adult hens

<table>
<thead>
<tr>
<th>Group (Dose) (ml/bird × dose/day × days)</th>
<th>Estimated total dosage (mg/kg)</th>
<th>Number of abnormal birds</th>
<th>Number of birds tested</th>
<th>Bird No.</th>
<th>Number of days after final application</th>
<th>Death</th>
</tr>
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<tbody>
<tr>
<td>0.5 × 2 × 2</td>
<td>500</td>
<td>0/5</td>
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<td>0.5 × 2 × 5</td>
<td>970</td>
<td>2/5</td>
<td>313</td>
<td>14 16</td>
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<td>314</td>
<td>16</td>
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<tr>
<td>0.5 × 2 × 10</td>
<td>2,000</td>
<td>4/5</td>
<td>320</td>
<td>9 11 16</td>
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<td>5/5</td>
<td>304</td>
<td>1 6 21 22 23</td>
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<td>308</td>
<td>6 21</td>
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<td>0.5 × 2 × 20</td>
<td>3,900</td>
<td>5/5</td>
<td>433</td>
<td>-9* -5 0 4 7</td>
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<td>0/5</td>
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* The minus figure means the number of days before the end of the final administration.

Comparison of delayed neurotoxic effects in adult hens and young male chickens

It is difficult to obtain adult male fowls in the market. By fortunate chance, the twelve male chickens used in this study had grown up in our laboratory. The
young male chickens, who have a bigger comb than that of the young hen, are useful as young specimens for the topical application of the compound, since the comb of the young hen is too small to receive the compound.

Although there were no abnormal birds in the 5 day group of the young male chickens, all male chickens in the 10 day group showed abnormal gait from 4 to 10 days after their final administration. Two of them started mild ataxia on the 4th day, developed severe paralysis rapidly by the 13th day and died on the 20th and the 21st day. The other two birds developed severe paralysis, but survived by the end of the observation period. The remaining one of this group showed gross ataxia, but did not develop further effects. The control group was quite normal (Table 2).

The mean total dosages of both young chicken groups were estimated to be 1,150 mg/kg in the 5 day group and 2,400 mg/kg in the 10 day group. The young male chickens received a much greater dosage than the adult hens of "the corresponding group" did, since the mean body weight of the young male chickens was smaller than that of the adult hens.

Comparison between the emulsion and acetone solution of leptophos

Four out of 5 birds of the leptophos-acetone solution group showed delayed neurotoxicity from 8 to 14 days after the final application, and two of them developed severe paralysis and one died. Two remaining birds were affected by ataxia, but nothing more.

The incidence rate of delayed neurotoxicity in the leptophos-acetone solution group was equal to that of the leptophos-emulsion group, though the delayed neurotoxic effects seemed to appear slightly earlier and more strongly in the leptophos-acetone solution group than in the leptophos-emulsion group, but not to a significant extent. There were no abnormalities in hens which were treated with acetone alone (Table 3).
Change of the body-weights

Fig. 1 shows the changes of the mean body weight in each group where the adult hens were given the commercial emulsion of leptophos for 2, 5, 10, 15 and 20 days, in comparison with that of the control group.

The slight decrease of the mean body weight developed slowly in each group, except the 2 day group and the control group. The ratio of the body weights at the minimum point to the initial weights was as follows: 85% in the 5 day group, 82% in the 10 day group, 84% in the 15 day group and 72% in the 20 day group. In the case of the young male chickens, those in the 5 day group and control group...
gained weight slightly as the result of growth, but the chickens in the 10 day group lost weight slowly, the final weight being 83% of the initial body weight (Fig. 2). On the other hand, a remarkable decrease of body weight was observed in the group where hens received the 17% acetone solusion of leptophos. They lost finally...
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about 26% of their initial weight (Fig. 3). The reason for this remarkable decrease may be due to the acetone, because the group of hens receiving the pure acetone also lost weight, whereas they showed no delayed neurotoxic signs.

DISCUSSION

In this study, delayed neurotoxic effects of leptophos following its dermal application on the comb of the domestic fowls were investigated. Domestic fowl is a species which has high sensitivity to the neurotoxic effects of some organophosphorus compounds. The toxic effects of xenobiotics on an organism depend, among other things, upon entry into the organism and subsequent distribution to the site of action (Shah et al. 1981). In the case of percutaneous penetration, the entry of chemicals depends upon the physicochemical properties and the conditions of the treated area. The comb of a domestic fowl is a part of the head skin consisting of the epidermis without melanin, the corium and the subcutaneous tissue. The corium has three layers. The first layer, close to the epidermis, is a capillary area. So it may be possible to absorb the compound through the comb more efficiently than through the skin of other parts of the body. The lipophilic compounds, such as the emulsion or acetone solution of leptophos used in this study, can penetrate easily through the keratin layer and diffuse rapidly to the capillary area (Tsuruta 1981). These are the reasons why the comb of the domestic fowl is of great advantage in the dermal application of organophosphorus pesticides which cause delayed neurotoxicity.

Dermal toxicity is usually investigated by an occlusive technique (Draize at al. 1944), wherein the pesticide to be tested is applied under an occlusive dressing. In this study, however, applied areas of the comb were not protected, since the occlusive method does not resemble the usual condition of skin contamination (Abou-Donia and Graham 1978).

Feldmann and Maibach (1974) had reported that out of 12 pesticides, the greatest absorption through the human skin was found with carbaryl, and that organophosphorus pesticides showed greater absorption rather than chlorinated hydrocarbons. Delayed neurotoxic compound, however, was not included in their report.

Dermal application of TOCP caused delayed neurotoxicity in hens (Glees and White 1960, 1961) and in slow loris (Ahmed and Glees 1971; Krishnamurti et al. 1972). An organophosphorus pesticide, cyanofenphos (Surecide®) brought on delayed neurotoxicity after its percutaneous administration (Konno et al. 1982). For leptophos, Abou-Donian and Graham (1978) have described that although hens given a daily dose of 0.1 mg/kg of leptophos showed no abnormalities, all hens given daily a single percutaneous dose of 0.5–20 mg/kg of the pesticide for 183–323 days developed ataxia, and that the dose of 0.5 mg/kg was the ‘threshold dose’ for ataxia. The long-term low level topical administration of the pesticide may have relevance to the chronic dermal exposure, for instance, which a number of workers may have to endure. The results of their study were valuable to point out the problem of
occupational safety. On the other hand, the present study showed that the relatively high-level of daily dose of leptophos for several days was also able to cause delayed neurotoxicity, and suggested that the method of the high dose of dermal administration for short-term is of great advantage, because we can determine, percutaneously, the delayed neurotoxicity of the pesticides within a limited period, especially in the case where the compounds have severe gastro-intestinal effects after oral administration.

All birds have received daily the same amount of leptophos (340 mg/hen/day) in this study. The group of hens which were treated for 2 days (680 mg leptophos/hen) showed no abnormal gait. Two hens in the 5 days group showed mild neuropathy (+ or ++). In the 10 day group, 4 out of 5 hens were affected by ataxia (+ or ++) or mild paralysis (++), but no hens died. On the other hand, all hens of the 15 and 20 day groups suffered various grades of neurotoxic effects, and one of the 15 day group and 2 of the 20 day group died after developing severe paralysis. From the point of view of the incidence rates, the grades of neuropathy and the mortality rates due to neurotoxicity, the greater the dose of the compound the hens received, the stronger were the delayed neurotoxic effects.

It is difficult in this study to estimate the absorption rate after dermal application. Abou-Donia (1979) reported that a total 35% of the applied dose was absorbed through the skin of the comb of hens. If this figure is accepted, about 175 mg/kg (500 mg/kg X 0.35 in the 2 day group) of “absorbed leptophos” cannot cause delayed neurotoxicity, but 340 mg/kg (970 mg/kg x 0.35 in the 5 day group) of “absorbed” pesticide can produce neuropathy. In the case of oral administration, a single dose of 180 mg/kg of leptophos cannot cause neuropathy, and 250 mg/kg of the compound can do so (Kinebuchi et al 1976).

The young male chickens received the same amount of leptophos per bird as the adult hens did. In both 5 and 10 day groups, however, it was estimated that young chickens were given much greater pesticide per body weight than adult hens in the corresponding group (Tables 1 and 2). For young male chickens, dermal application of 1,150 mg/kg of leptophos cannot cause delayed neurotoxicity and all birds given 2,500 mg/kg of the pesticide were affected with neuropathy. In the case of adult hens, 1,000 mg/kg of leptophos could cause neuropathy on 2 out of 5 hens, but with a dose of 2,000 mg/kg, 4 out of 5 birds were affected. It is possible to consider that delayed neurotoxicity by dermal administration of leptophos is also influenced by the age as in the case of oral administration (Konno et al. 1980).

There was no significant difference in the incidence rates between the group of hens which were given emulsion of leptophos for 10 days and the group of hens which received acetone solution of the pesticide for the same period. It is suggested that the unknown solvent in the commercial emulsion of the pesticide dose not give any influence on delayed neurotoxicity.

The opportunities for dermal exposure to the pesticides are often much greater than other routes of exposure, such as the respiratory route or the oral route. Even through the results of this study cannot be extended directly to the human
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beings, it is suggested that more caution should be exercised with regard to the so called 'low toxic organophosphorus pesticides' on behalf of the workers in pesticide factories as well as the farmers who use these chemicals.

Acknowledgment

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References