Accumulation of Phosvel in adipose tissue of hens

N. Konno and H. Kinebuchi[1]

Department of Public Health, Fukushima Medical College, Fukushima 960 (Japan), 20 February 1978

Summary. Phosvel, an organophosphorus pesticide, was stored in the adipose tissue of hens after they were given daily a single oral dose. The concentration of Phosvel in fat was related to the size of the daily dose.

Phosvel (Leptophos) is one of the organophosphorus compounds which produce delayed neurotoxic effects in hens after a single oral dose[2,3]. It has also been demonstrated that the repeated administration of small doses of Phosvel to hens causes delayed neurotoxicity[4]. From the chemical properties of Phosvel, Davies et al.[5] have suggested that the chemical might be deposited in the fat of animals. In our study, residues of crude Phosvel were found in the fat of hens until 21–28 days following a single oral dose of 250 mg/kg[6].

To our knowledge, the work reported here is the first designed to confirm the accumulation of Phosvel in the adipose tissue of hens after a daily single oral dose. This report also gives an account of an investigation into the clinical effects on the birds.

Materials and methods. Crystallized Phosvel [O-(4-bromo-2,5-dichlorophenyl) O-methyl phenylphosphonothioate] was obtained by incorporation of 4 parts methanol with the Phosvel emulsifiable concentrate (containing 34% active ingredient) at room temperature. Identification was checked by comparing the retention time of the prepared crystalline and standard Phosvel (supplied by manufacturer), using gas chromatography. The purity of the crystalline was 96%. Hens, 22 months of age, of the White Leghorn strain, weighing 1.95 kg (1.42–2.42 kg) were placed in separate cages and kept under observation for 1 week before treatment. 36 hens were randomly divided into 3 test groups. The birds in each test group were also randomly subdivided into 4 groups of 3 birds, each subgroup corresponding to test periods of 7, 14, 21 and 28 days. 3 test groups of birds were given daily, a single oral dose of Phosvel in salad oil solution, at rates of 5, 10 and 20 mg/kg. 2 hens were given daily doses of salad oil alone, and 2 other hens that were not given anything were killed just before the experiment. These served as a control (table 1). The surviving birds were examined every day for any abnormalities in gait or behavior. Adipose tissue was removed mainly from the abdominal cavity of the hens which were sacrificed 24 h after the final dose. The fat samples were stored at −20 °C until analysis. For analysis, Phosvel was extracted into petroleum ether and the solvent was removed by evaporation. The fatty residue was weighed and recorded as the weight of sample so that the analysis could be based on the petroleum ether extractable fat. The extract was cleaned by n-hexane-acetonitrile partitioning and passage through a Florisil column. Reading was on a Hitachi model 163 FPD gas chromatograph with a column (3 mm by 2 m) packed with 5% OV-1 on Chromosorb WAW. Flow rates (ml/min) were: hydrogen, 140; oxygen, 20; and nitrogen carrier gas, 80. Temperatures were: column, 250 °C; injector, 300 °C; and detector, notch 7.

Results and discussion. No birds killed within 15 days of beginning the treatment showed sign of ataxia and paralysis (table 1). In the case of a single oral dose, the latent period before the onset of signs was between 8 and 14 days[2,3]. Therefore, if the observation of clinical conditions was made more than 8–14 days after the final dose, some of these birds might have developed neurotoxic symptoms. 3 birds given 10 and 20 mg/kg/day of Phosvel showed signs of ataxia after 17–19 days and paralysis after 20–22 days and died on day 25. These results are practically in accordance with the finding that the severity of the clinical condition depended on the size of the daily dose[4].

The level of Phosvel in the fat of hens (group A, B) increased accumulatively during the first 21 days, but fell slightly by the termination of the experiment at day 28. Concerning group C, Phosvel storage was relatively stable until 14 days of administration but increased thereafter. At day 25 (group C), Phosvel concentration in fat reached its maximum level (table 1).

Table 1. Concentration of Phosvel in adipose tissue and degree of neurotoxic signs in hens following the daily administration of a single oral dose of the pesticide

Group	Dose (mg/kg/day)	Duration of dose (days)	No. of hens	Phosvel concentration in fat (ppm)*		Degree of neurotoxicity and No. of hens		
				Mean	Range	Negative	Ataxia	Paralysis
A	5	7	3	0.94	0.73–1.30	3		
	5	14	3	0.85	0.73–0.92	3		
	5	21	3	2.05	1.39–2.39	3		
	5	28	3	1.22	0.73–1.58	3		
B	10	7	3	2.12	1.63–2.38	3		
	10	14	3	2.37	1.91–2.67	3		
	10	21	3	4.17	2.86–6.00	1	1	1
	10	25	1	n.e.				1**
		28	2	3.51	2.29–4.72		1	1
C	20	7	3	3.22	1.44–5.20	3		
	20	14	3	3.53	2.30–4.61	3		
	20	21	3	7.38	5.00–8.70			3
	20	25	2	11.03	9.77–12.29			2**
		28	1	n.e.				1
Control	0	0	2	n.d.		2		
Control	Salad oil	28	2	n.d.		2		

*The values, petroleum ether extractable fat basis. **The birds died on day 25. n.e., not estimated; n.d., not detected.

Table 2. Accumulation ratio of Phosvel in adipose tissue of hens*

Duration of dose (days)	Dose (mg/kg/day) 5	10	20
7	1.00	2.26	3.43
14	1.00	2.79	4.15
21	1.00	2.03	3.60
28	1.00	2.88	9.04**

* Each value was calculated as the ratio of mean concentration of Phosvel found in fat in the same test period. ** The samples were taken at 26 days after the begining the test.

Although the reason is obscure, this finding may reflect the effect of death. No contamination occurred in fat of the control hens. The concentration of Phosvel in fat of 2 hens given 10 and 20 mg/kg for 25 and 28 days respectively was not estimated, because the birds were depleted of body fat. Except for the case dosed 20 mg/kg for 25 days, the figures of the accumulation ratio show that the level of Phosvel in fat was proportional to the size of the daily dose (table 2). Although this study was of relatively short duration, it clearly indicates that the organophosphorus pesticide, Phosvel, accumulates in the adipose tissue of hens after daily oral administration of small doses.

1 Acknowledgment. The authors are grateful to Prof. Yoshito Tsuji for his valuable suggestions; to Mr Mitsuo Kaneda and Mr Kazuo Sasaki for their technical assistance.

2 M.B. Abou-Donia, M.A. Othman, G. Tantawy, A. Zaki Khalil and M.F. Shawer, Experientia *30*, 63 (1974).
3 H. Kinebuchi, N. Konno and T. Yamauchi, Jap. J. publ. Hlth *23*, 509 (1976) (in Japanese).
4 M.B. Abou-Donia and S.H. Preissig, Toxic. appl. Pharmac. *38*, 595 (1976).
5 J.E. Davies, A. Barquet, V.H. Freed, R. Haque, C. Morgade, R.E. Sonneborn and C. Vaclavek, Archs envir. Hlth *30*, 608 (1975).
6 N. Konno and H. Kinebuchi, Toxic. appl. Pharmac. *45*, 541 (1978).

Effect of 3 polyphenolic compounds against ear-rot of corn incited by *Fusarium moniliforme* Sheld*

S. Ghosal, D.K. Chakrabarti and R.S. Srivastava

Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Banaras Hindu University, Varanasi-5 (India), 28 February 1978

Summary. Anti-*F. moniliforme* activity of 3 non-toxic polyphenolic compounds, viz. mangiferin, 1,3,6,7-tetrahydroxyxanthone, and 2,2',4-trihydroxybenzophenone, was evaluated. The mycotoxin-producing fungus incited ear-rot in sweet corn in fields and during storage. The test compounds provided different degrees of protection to sweet corn from the ingress of the fungus. Additionally, noteworthy postinfectional curative action was observed in the case of the trihydroxybenzophenone.

We have recently reported[1] the incidence of *Fusarium moniliforme* Sheld. (CMI-IMI 204057)-induced ear-rot of Indian corn (*Zea mays* L., sweet corn, local variety) while growing in the valley of the Ganges, in Varanasi District of Uttar Pradesh. A market survey in the area also revealed that about 30% of the corn, collected at the preharvest stage for table use, were badly infested with the fungus. 3 mycotoxins, viz. zearalenone, diacetoxyscirpenol, and T-2 toxin, were isolated and characterized from the diseased corn[1]. The presence and prevalence of the mycotoxin-producing fungus in sweet corn in this area could be a recurring phenomenon and is, therefore, a cause for alarm from a public health view point. Application of the conventional fungicides to control the pathogen would be risky, because sweet corn is consumed in semi-baked form, or as such, in substantial amounts, in India. This fact necessitated the search for non-toxic agents to control this food-destructive mold.

Phenolic substances have been reported[2] to offer general resistance for higher plants towards bacteria and fungi. We have recently reported[3,4] significant antifungal actions of mangiferin, a naturally occurring glucosylxanthone (1,3,6,7-tetrahydroxy-C_2-β-D-glucosylxanthone) and of 2,2',4-trihydroxybenzophenone against *F. oxysporum* f.sp. *carthami*, the causal organism for the wilting of safflower. The toxicity of the 2 compounds, in animal testing, was found to be very low[3,5]. It was therefore thought worthwhile to evaluate the potential of the 2 compounds, and also of a simpler analogue of mangiferin, viz. 1,3,6,7-tetrahydroxyxanthone, against *F. moniliforme*-induced ear-rot of corn. The details of these findings constitute the subject of this communication.

Materials and methods. At the early milk-stage of sweet corn, growing in a field in the valley of the Ganges in Varanasi (July 1977, temperature 33±8 °C, relative humidity 46–78%), a dense spore suspension (about 5×10^5/ml) of *F. moniliforme* Sheld. (CMI-IMI 204057) was uniformly sprayed over the silk and husks of the developing ears. After 3 days, the 3 test compounds (1×10^{-4} M), in aqueous

Table 1. Effect of polyphenolic compounds against ear-rot of corn incited by *Fusarium moniliforme* Sheld. in the fields

Test compound	Symptoms
Mangiferin	[a] 4 kernels showed pinkish stain over the surface, bleached lesions (2–14 × 1–4 cm) upto 10th husk, grain formation normal. [b] Similar to those observed in the control.
1,3,6,7-tetrahydroxy-xanthone	[a] Brownish discolouration of epicarp of 3 kernels, bleached lesions (2–6 × 1–2 cm) upto 4th husk, grain formation normal. [b] Tip turned black, lesions (4–6 × 1–2 cm) upto 10th husk, grain formation observed only at the basal portion.
2,2',4-trihydroxy-benzophenone	[a] No sign of infection on grains, ears well developed. [b] Brownish discolouration of 3 kernels, lesions (2 × 1 cm) upto 4th husk, ears well developed.
Control	10 kernels from the tip turned brown and shrunken, bleached lesions (2–14 × 1–5 cm) upto 14th husk, grain formation irregular and only partial.

Test compound sprayed. [a] before inoculation; [b] after inoculation of the fungus.