Acute Oral Toxicity and Delayed Neurotoxicity of 5 Organophosphorus Compounds, Salithion, Cyanox, Surecide, Sumithion and Sumioxon in Adult Hens. Tadaomi KADOTA, Yasuyoshi OKUNO and Junshi MIYAMOTO (Research Department, Pesticides Division, Sumitomo Chemical Co., Ltd., Takarazuka, Hyogo, Japan) Received Jan. 16, 1975. *Botyo-Kagaku* 40, 49, 1975.

8. サリチオン，サイアノックス，シュアサイド，スミチオンおよびスミオキソンのニワトリにおける急性経口毒性ならびに遅延性神経毒性

門田忠由，奥野泰由，宮本純之（住友化学工業株式会社農薬事業部研究部） 50. 1. 16. 受理

4種の有機リン殺虫剤サリチオン，サイアノックス，シュアサイド，スミチオンおよびスミチオンの活性代謝産物たるスミオキソンのニワトリにおける急性毒性ならびに遅延性神経毒性を経口投与により試験した．またスミチオン，シュアサイドの亜急性経口投与による遅延性神経毒性を検索した．遅延性神経毒性は各化合物の急性 LD_{50} 値に近い量を3週間隔で2回経口投与し，投与直後にみられる急性中毒症状をアトロピンおよび 2-piridine aldoxime methiodide (2-PAM) で寛解させつつ試験した．スミチオン，シュアサイドの亜急性経口投与による遅延性神経毒性試験では各化合物の急性 LD_{50} 値の1/15および1/30量を毎日継続4週間投与した．各化合物は急性，亜急性実験いずれの場合も投与中および投与後3週間の観察期間においても脚部麻痺その他の不可逆的な遅延性神経症状をひきおこさず，病理組織学的にも座骨神経の異常は認められなかった．これに対し対照として用いた Tri-ortho tolyl phosphate (TOTP) では投与10～14日後に著明な脚部麻痺症状を認め起立不能を呈し，病理組織学的にも座骨神経の変性，脱髄現象を認めた．

Introduction

In addition to the direct toxic actions observed immediately after administration, some organophosphorus compounds are known to cause delayed neurotoxic effects in mammals including humans and in hens, which are characterized clinically by a permanent ataxia of legs or hind limbs (paralysis) and histopathologically by demyelination and degeneration of the peripheral nerves, especially of the sciatic nerve.[1-3] Since the delayed neurotoxic effect is an irreversible lesion on the nerve tissues, it is essential to confirm whether or not an organophosphorus compound has such toxic actions before it is practically used. Actually most of the existing organophosphorus insecticides such as parathion, diazinon, malathion, dimethoate, ethion, fenchlorphos, fenthion and dioxathion have been already demonstrated not to cause paralysis in hens.[4-8]

In this paper are presented the results of delayed neurotoxicity tests as well as acute toxicity in hens of 4 organophosphorus insecticides, Salithion®, Cyanox®, Surecide®, Sumithion® and also of Sumioxon, the active metabolite of Sumithion.

Materials and Methods

White leghorn hens (1-1.5 year old, weighing 1.3-2.3 kg) were purchased from Nihon Dobutsu Co., Ltd. They were kept at 25±1°C with relative humidity of 60±10% and had free access to diet (Osaka Shinkoh Shiryo). Test materials were; Sumithion, *O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate (lot No. 417, purity 97.2%), Salithion, 2-methoxy-4H-1,3,2-benzodioxa-phosphorin-2-sulfide (lot No. 193-4, purity 95.1%), Cyanox, *O,O*-dimethyl *O*-(4-cyanophenyl) phosphorothioate (lot No. 158, purity 95.0%) Surecide, *O*-ethyl, *O*-(4-cyanophenyl) phenylphosphonothioate (lot No. E 199, purity 92.0%), which were all technical products of Sumitomo Chem Co.. Sumioxon, *O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorate (purity 98% up) and TOTP, tri-ortho-tolyl phosphate (purity 99%) were prepared in this laboratory.

Salithion, Cyanox, Surecide, Sumithion or Sumioxon were suspended in 10% Tween 80 aqueous solution and administered orally by stomach tube at the rate of 1 ml/kg of the suspension to 3 or 4 groups of hens, each consisting of 4 to 6 individuals. The animals were observed for 5 weeks. The LD_{50} value was

calculated by Litchfield & Wilcoxon method.[9]

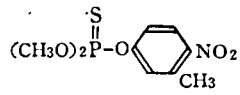
The delayed neurotoxicity study was carrid out according to the proposed guideline of Environmental Protection Agency of the United States.[10] The administered solution was prepared by adding 1/5 as much of Sorpol 355, an emulsifier, to Sumithion, and by diluting it in distilled water so that the test hens would be given 2.0 ml of the solution/kg body weight. Four other organophosphorus compounds were suspended in 10% Tween 80. The solution was administered orally to the specified numbers of hen at the dosage of around LD_{50} value of the compound (the actual number of hen and the dosage are shown in Table 1). To protect the animals from acute intoxication, both atropine (20 mg/kg by subcutaneous injection) and pyridine-2-aldoxime methiodide (2-PAM, 100 mg/kg by intraperitoneal injection) were administered several times depending on the symptoms, for example in the case of Sumithion 5 times, namely, immediately after and 6, 24, 48, 72 hours after administration.

Three weeks after the first administration the same treatment was repeated and the birds were observed for another three weeks.

During the whole six-week experimental period, paralysis in legs was checked as an indicator for the delayed neurotoxicity. TOTP was suspended in 10% Tween 80 aqueous solution and administered orally at the level of 300 to 500mg/kg to hens and the animals were observed for 4 weeks. After the observation period, all the treated birds excluding those dead of acute intoxication were sacrificed and their sciatic nerves were dissected out and examined histopathologically through formalin fixation, paraffin embedding and hematoxylin-eosin staining or Luxol-fast blue staining.

In subacute toxicity studies, Sumithion or Surecide was suspended in 10% Tween 80 aqueous solution and 1/15 and 1/30 of the acute LD_{50} values were administered orally to 8 hens per group everyday for consecutive four weeks. Thereafter, the birds were observed for three weeks on basal diet. During the whole period (seven weeks) the toxic symptoms and body weight in the hens were observed. At the termination of the observation period, the birds were sacrificed and their sciatic nerves were examined histopathologically.

Table 1. Acute oral toxicity of Sumithion in hens.

Compound	Dosage mg/kg	Daily mortality 1	2	10	Mortality %	Symptoms	LD_{50} mg/kg
Sumithion 	250	0/6		0/6	0	Decrease of spontaneous motor activity after 2-3 hrs. Motor ataxia and irregular respiration after 24 hrs. Recovered to normal in 4-6 days.	
	500	0/6	3/6	3/6	50.0	Decrease of spontaneous motor activity after 30-60 min. Motor ataxia after 3 hrs. Salivation, irregular respiration and dark red discoloration of the comb after 24 hrs. Motor ataxia became severe. Recovered to normal in 8-10 days.	500 (384-650)*
	1000	1/6	6/6	6/6	100.0	The same as above.	

* 95% confidence limit

Results and Discussions

Acute oral toxicity: The toxic symptoms and mortality caused by single administration of Sumithion are reproduced in Table I, which indicates that LD_{50} of Sumithion in birds was 500 mg/kg. Table 2 summarizes the test results with other 4 organophosphorus compounds. The toxic symptoms were more or less similar to those of Sumithion.

Delayed neurotoxicity: By oral administration of 500 mg/kg of Sumithion, motor ataxia, decrease of spontaneous motor activity, irregular respiration were observed and five out of sixteen hens died during 24-48 hrs. Symptoms of acute intoxication disappeared in 5-7 days and there appeared no further noteworthy changes. Almost the same symptoms were noted after the second administration, except that no death was recorded. During the second 21-day observation period, paralysis in legs was not observed at all after disappearance of acute intoxication. Histopathological findings of sciatic nerves in the birds treated with Sumithion were normal. The results are summarized in Table 3.

Four other organophosphorus compounds were tested similarly. None of them caused delayed paralysis in legs, as shown in Table 3. In every trial using TOTP (suspended in 10% Tween 80 aqueous solution), delayed neurotoxicity was observed well reproducibly; motor ataxia appeared on 10th to 12th day and all the hens showed paralysis in legs on 14th to 15th day (Table 3). Upon histopathological examinations, degeneration and outstanding demyelination of sciatic nerves were noted, as reproduced in Fig. 1.

Sumithion and Surecide were tested by consecutive 4 week oral administration at the dosage of 1/15 and 1/30 LD_{50} per day. As shown in Fig. 2, Sumithion at 16.7 mg/kg/day caused a slight body weight decrease which lasted during the administration period. The body weight recovered slowly on termination of the administ-

Table 2. Acute oral toxicity of Salithion, Cyanox, Surecide and Sumioxon in hens.

Compound	Dosage mg/kg	Daily mortality 1	2	10	Mortality %	LD_{50} mg/kg
Salithion	20	0/3		0/3	0	
	40	0/3		0/3	0	
	80	0/5		0/5	0	
	100	0/5	2/5	2/5	40.0	110
	120	1/5	3/5	3/5	60.0	(88.6-136)*
	160	2/3	3/3	3/3	100.0	
	200	3/3		3/3	100.0	
Cyanox	10	0/5		0/5	0	
	25	1/5	2/5	2/5	40.0	37.5
	50	3/5		3/5	60.0	(23.4-60.0)*
	100	5/5		5/5	100.0	
Surecide	5	0/3		0/3	0	
	10	0/3		0/3	0	
	20	0/3		0/3	0	60.0
	40	1/3		1/3	33.3	(23.0-156)*
	80	1/3	2/3	2/3	66.7	
	160	3/3		3/3	100.0	
Sumioxon	10	0/4		0/4	0	
	25	1/4		1/4	25.0	35.0
	50	3/4		3/4	75.0	(21.0-57.0)*
	100	4/4		4/4	100.0	

* 95% confidence limits

Table 3. Delayed neurotoxic action of 5 organophosphorus compounds on hens.

Compound	Duplicate administrations			Consecutive administration		
	Dosage mg/kg	Mortality	Paralysis	Dosage mg/kg/day	Mortality	Paralysis
Salithion	100×2	3/10	0/7	—		
Cyanox	20×2	2/12	0/10	—		
Surecide	100×2	1/8	0/7	2.0	0/8	0/8
				4.0	1/8	0/7
Sumithion	500×2	5/16	0/11	16.7	0/8	0/8
				33.7	1/8	0/7
Sumioxon	35×2	5/12	0/7	—		
TOTP	300×1*	0/6	6/6	—		
	500×1*	1/6	5/5			

* single administration

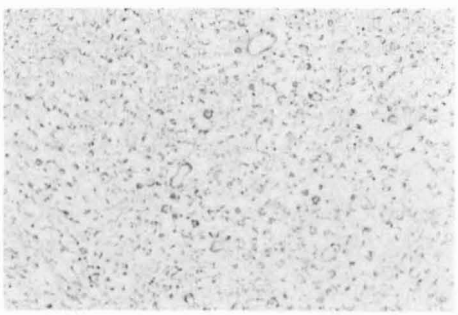


Fig. 1. Degeneration and demyelination of sciatic nerve in hen treated with TOTP, 500mg/kg.

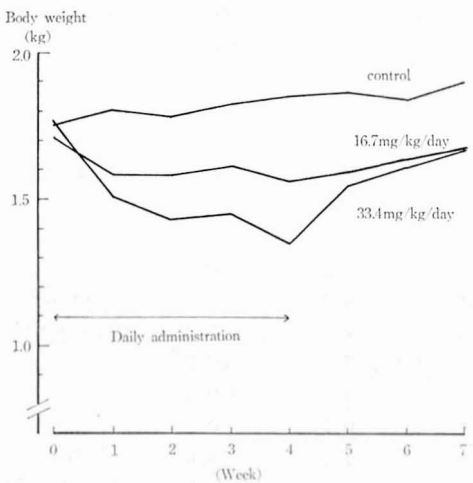


Fig. 2. Mean body weight curves of hens treated with Sumithion for 4 weeks.

ration. At the higher dosage level (33.4 mg/kg/day), the adverse effect on body weight was more marked. Such toxic symptoms were observed as decrease of spontaneous motor activity at the lower level and decrease of spontaneous motor activity, tremor, mortor ataxia as well as loss of appetite at the higher level. However, these symptoms gradually disappeared (approximately one week at the lower level and 14 days at the higher level). One hen in the higher level group died on the fifth day.

During the seven week investigation period, no paralysis in legs was observed at all. Histopathologically no abnormalities were found in sciatic nerves in the birds at any dosage group. Surecide at 2 and 4 mg/kg/day gave more or less similar toxic symptoms, but no delayed neurotoxic effects were observed.

The above results are included in Table 3. It can be concluded from the above results that these 5 organophosphorus compounds did not cause delayed neurotoxicity in hens, although the chemical structure is variable, that is, three are *O*, *O*-dialkyl phosphoro-thioates or -phosphorate, the fourth is a phosphonate and the fifth is a cyclic phosphorothioate.

Summary

The acute oral toxicity and the delayed neurotoxicity were tested in white leghorn hens of 5 organophosphorus compounds, Salithion, Cyanox, Surecide, Sumithion and Sumioxon.

The acute oral LD_{50} value of the above compounds were determined, respectively, to be 110, 37.5, 60, 500 and 35mg/kg. In the delayed neurotoxicity study, each compound at or near the acute oral LD_{50} value was orally administered and the treated birds were observed for 21 days (with concurrent uses of atropine and 2-PAM). Thereafter, the same treatment was carried out. No compound was demonstrated to cause the delayed neurotoxic effects of the survived hens. Four-week subacute oral administration of Sumithion and Surecide were also tested at two dosage levels, 1/15 and 1/30 of the acute oral LD_{50}/day. No delayed neurotoxicity was observed, either. On the other hand, the animals to which TOTP was singly administered as a positive control at the dosage of 300 to 500 mg/kg showed severe paralysis in legs. Degeneration and outstanding demyelination were observed in the sciatic nerves upon histopathological examinations.

Acknowledgement: The authors are very much grateful to Prof. N. Ito, Nagoya City University Medical School for carrying out the histopathological examinations. They wish to express their thanks to Mr. M. Kagoshima for his skilled technical assistances. They also thank Sumitomo Chemical Co., Ltd. for permission to publish this work.

References

1) Aldridge, W. N. and J. M. Barnes; *Proc. Eur. Soc. Study of Drug Toxicity*, 8, 162 (1967).
2) O'Brien, R. D.; Insecticides, p. 58 (Academic Press, New York) (1967).
3) Taylor, J. D.; *Toxicol. Appl. Pharmacol.*, 11, 538 (1967).
4) Aldridge, W. N., J. M. Barnes and M. K. Johnson; *Ann. N. Y. Acad. Sci.*, 160, 314 (1969).
5) O'Brien, R. D.; Toxic phosphorus esters, p. 190 (Academic Press, New York) (1960).
6) Anonymous; 1967 Evaluation of some pesticide residues in food (WHO/FAO) (1968).
7) Anonymous; 1968 Evaluation of some pesticide residues in food (WHO/FAO) (1969).
8) Anonymous; 1971 Evaluation of some pesticide residues in food (WHO/FAO) (1972).
9) Litchfield, J. T. and F. Wilcoxon ; *J. Pharmacol. Exptl. Therap.*, 96, 99 (1949).
10) Upholt, W. M.; *Federal Register*, 37, 19383 (1972).

抄　　録

ナシノヒメシンクイガの多成分性誘引物質系における各成分が行動に及ぼす役割

Behavioral Role of Individual Components of a Multichemical Attractant System in the Oriental Fruit Moth. R. T. Carde, T. C. Baker, W. L. Roelofs, *Nature*, 253, 348 (1975).

誘引剤として作用する性フェロモンに本来誘引性のない化合物を加え，誘引性を増加または減少させる物質を，その行動学的および神経生理学的な役割が不明のまま，それぞれ協力剤または阻害剤と呼んできた．ナシノヒメシンクイガ *Grapholitha molesta* (Busck) の主フェロモンは *cis*-8-dodecenyl acetate (c8-12 : Ac) と同定されているが，約8％のトランス異性体 (t8-12 : Ac) の混入が必要とされている．またこれに dodecyl alcohol (12 : OH) を加えると雄蛾の誘引率が2倍になるという．野外試験の結果，c8-12 : Ac (100μg) 単用の場合トラップへ向う雄はほとんどないが，これに6.8％の t8-12 : Acを加えるとトラップ率は56％となり，さらに12 : OH を加えると93％となった．トラップ率からいえば誘引協力剤12 : OH は着地頻度を高めるようである．直径60cm の円卓の中心から誘引剤を揮散させ詳しく12 : OH の役割を分析した．予備試験より 10μg の c8-12 : Ac に t8-12 : Ac を6.8％加えると好結果を与えることから，これに種々の量の12 : OH を加えて試験した．この結果明らかに雄の着地頻度を高め，はばたき歩き，誘引源への接近，そして hair pencil の提示頻度が高くなることが観察された．特有の化合物が hair pencil の提示を起すと判明した最初の例である．雌虫体からは c8-12 : Ac の存在しか確認されていないが，この種は今まで述べた如く多成分通信システムを利用しているように思われる．c8-及び t8-12 : Ac は誘引の必要条件であるが，以前協力剤と考えられていた 12 : OH を加えなければ前述の行動が起らないことから，12 : OH は交尾前行動の領域を刺激するものと考えられる．

（桑原保正）