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Toxicity of Non-substituted Benzyl Phosphates

Sumio NAGASAWA*

非置換ベンジル燐酸エステルの毒性

長　澤　純　夫

Since the great pioneer work of Dr. Gehard Schrader of Farbenfabriken Bayer, Germany, which began about 1934 and was first published in 1947, thousands of phosphorus compounds of many types have been synthesized and evaluated for biological, (particularly insecticidal) properties in many parts of the world. In the early 1960's at the Ihara Agricultural Chemicals Institute of the Ihara Agricultural Chemicals Co., Ltd. (now Kumiai Chemical Industry Co., Ltd.), the author and his co-workers devoted themselves to the screening tests of a group of benzyl phosphorus compounds to find promising chemicals of fine insecticidal properties and of low mammalian and fish toxicities. The living organisms used for screening tests were the house fly, German cockroach, rice stem borer, cabbage worm, army worm, diamond back moth, tea tortrix, green rice leafhopper, small brown planthopper, white backed planthopper, aphids, spider mite, grain mite, goldfish, dojo fish, carp fish, mouse and rat. In spite of their efforts, however, no chemicals have been brought to market so far as insecticides. Two chemicals, O,O-diethyl S-benzyl phosphorothioate (Kitazin®) and its diisopropylester, O,O-diisopropyl S-benzyl phosphorothioate (Kitazin-P®), had been developed by their fungicide research group as rice blast control agents which did not contain mercury. A few years after Kitazin was placed on the market, closely related compounds, O-methyl S-benzyl benzenephosphonothioate (Inejin F 254®), O-methyl O-cyclohexyl S-(4-chlorophenyl) phosphorothioate (Cerezin®), O-ethyl S,S-diphenyl phosphorodithioate (Hinosan®), and O-*n*-butyl S-ethyl S-benzyl phosphorodithioate (Conen®) were developed as rice fungicides by other industrial groups in Japan.

In those days, a large number of related benzyl phosphorus compounds were synthesized by the Ihara chemical group and refered to the biological section for various screening tests. These results have been covered by patents[1] and some of the work has been published in scientific journals.[2,3] The author and his co-worker[4-8] have also reported some results of their work. The relationship between the structure of some closely related derivatives of Kitazin and Kitazin-P and their toxicity in houseflies, mice and carp fish is reported in this paper.

Materials and Methods

Fifty-five non-substituted benzyl phosphorus compounds tested in the present screen-

* Laboratory of Chemical Contamination Biology. Former address : Ihara Agricultural Chemicals Institute, Shimizu, Japan

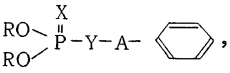
ing experiments were all synthesized in their chemical section. The chemical structures of the test samples are shown in the left side of Table 1. Malathion of 95% purity was used as the standard chemical for comparison.

The houseflies used to test the contact insecticidal properties were the offsprings of the so-called "Takatsuki" strain. This strain had been inbred in the laboratory for more than ten years. The "okara" culture medium was used for rearing larvae ; sugar and water were given to adult flies as diet. The environmental condition of the rearing room was kept at ca. 25°C and a relative humidity of ca. 60%. Male mice and carp fish were obtained from dealers. Mice of about 20 grams in weight and fish of about 3—5 cm in body length were selected for the test. Their strains and rearing conditions were not specified.

Flies, 4 to 5 days old, were lightly anaesthetized with carbon dioxide and sexed. Chemicals were diluted in acetone at several concentrations in logarithmic scale. One cublic microliter of test solution was applied topically to the mesonotum of each fly using a micrometer driven syringe. After treatment. the flies were kept in a glass vial (9 cm in diameter and 5 cm in height) with diluted milk under the same conditions as the rearing room. For the controls, acetone alone was applied. About 40 individuals were used for each concentration and control. A mortality count was made at 24 hours after application. A correction was made by Abbott's formula when dead individuals were found in the control. The median lethal dose of each test or standard chemiclas was calculated by the standard probit method. As it was impossible to evaluate the LD_{50}'s of test chemicals at one time, experiments were carried out dividing samples into several groups. The relative toxicity ρ of the LD_{50} of the test samples to that of the standard sample was used for the discussion.

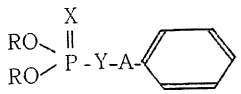
Rank classification (>300, $100-300$, $30-100$, <30 mg/kg) of oral acute toxicity to mice was determined by administration of a chemical diluted in sesame oil at the prescribed concentration. The quantity of administrated oil was 0.005 ml per 1 g of body weight. For the fish toxicity test, a 10% wettable powder of the chemical was prepared. Five carp fish were immersed in 2000 cc of the suspended solution of the rank classified concentration (0.2, 0.5, 2, 10 or 20%). The number of dead individuals was counted at 24 hours after administration or immersion.

Results and Discussion

Results of screening experiments are shown on the right side of Table 1. Concerning the contact insecticidal properties on houseflies, closely related series of compounds generally demonstrated satisfactory correlations between chemical structures and toxicities. The general structural formula,  , will be used for the discussion.

The toxicity of test compounds depends to a considerable extent upon the nature of the alkyl group (R). In the case of $A=CH_2$, the order of toxicity was $C_2H_5>CH_3>n$-$C_3H_7>iso$-$C_3H_7>n$-C_4H_9, in the case of $A=(CH_2)_3$, it was $C_2H_5>n$-$C_3H_7>CH_3>iso$-$C_3H_7>n$-C_4H_9. The order of toxicity was also clear between the nature of X and Y ; namely X=O, Y=O>X=S, Y=S>X=O, Y=S>X=S, Y=O. For example, in the case of $R=CH_3$, the order of toxicity was benzyl dimethyl phosphorate>S-benzyl O, O-

Table 1. Relative toxicity of non-substituted benzyl phosphates



Sample No.	Chemical structure				Toxicity		
	R	X	Y	A	House fly (ρ)	Mice (mg/kg)	Fish (%)
1	CH_3	O	S	CH_2	0.071	>300	2–20
2	〃	S	S	〃	0.117	>300	2–20
3	〃	S	O	〃	0.019	>300	0.5–10
4	〃	O	O	〃	0.160	>300	2–20
5	C_2H_5	O	S	〃	0.085	100–300	2–20
6	〃	S	S	〃	0.149	>300	2–20
7	〃	S	O	〃	0.053	>300	2–20
8	〃	O	O	〃	0.137	100–300	2–20
9	n-C_3H_7	O	S	〃	0.049	>300	2–20
10	〃	S	S	〃	0.064	>300	2–20
11	〃	S	O	〃	0.029	>300	0.2–2
12	〃	O	O	〃	0.143	100–300	2–20
13	*iso*-C_3H_7	O	S	〃	0.048	>300	2–20
14	〃	S	S	〃	0.051	>300	0.2–2
15	〃	S	O	〃	0.046	>300	2–20
16	〃	O	O	〃	0.775	100–300	2–20
17	n-C_4H_9	O	S	〃	0.017	>300	0.5–10
18	〃	S	S	〃	0.009	>300	0.5–10
19	〃	S	O	〃	0.007	>300	0.5–10
20	〃	O	O	〃	0.019	>300	0.5–10
21	CH_3	O	S	$(CH_2)_3$	0.034	>300	2–20
22	〃	S	S	〃	0.058	>300	0.2–2
23	〃	S	O	〃	0.017	100–300	2–20
24	〃	O	O	〃	0.096	100–300	2–20
25	C_2H_5	O	S	〃	0.111	>300	2–20
26	〃	S	S	〃	0.220	>300	0.2–2
27	〃	S	O	〃	0.067	>300	0.5–10
28	〃	O	O	〃	0.072	—	—
29	n-C_3H_7	O	S	〃	0.047	>300	2–20
30	〃	S	S	〃	0.061	>300	0.2–2
31	〃	S	O	〃	0.020	>300	0.5–10
32	〃	O	O	〃	0.352	100–300	2–20
33	*iso*-C_3H_7	O	S	〃	0.052	>300	2–20
34	〃	S	S	〃	0.035	>300	0.2–2
35	〃	S	O	〃	0.007	>300	0.5–10
36	〃	O	O	〃	0.588	100–300	2–20
37	n-C_4H_9	O	S	〃	0.016	—	—
38	〃	S	S	〃	0.007	>300	0.5–10
39	〃	S	O	〃	0.006	—	0.5–10
40	〃	O	O	〃	0.014	100–300	2–20
41	C_2H_5	O	S	CH_3CH	0.070	>300	2–20

42	〃	S	S	〃	0.218	>300	2–20
43	CH_3	O	O	$(CH_2)_2$	0.001	>300	2–20
44	C_2H_5	O	S	〃	0.077	100–300	2–20
45	〃	S	S	〃	0.297	100–300	0.2–2
46	〃	O	O	〃	0.001	>300	2–20
47	*n*-C_3H_7	O	O	〃	0.001	>300	0.5–10
48	*iso*-C_3H_7	O	O	〃	0.001	>300	0.5–10
49	CH_3	O	O	CH_2–CH=CH	0.001	>300	2–20
50	C_2H_5	O	S	〃	0.163	30–100	0.2–2
51	〃	O	O	〃	0.085	30–100	2–20
52	*n*-C_3H_7	O	O	〃	0.185	>300	2–20
53	*iso*-C_3H_7	O	O	〃	0.001	30–100	2–20
54	C_2H_5	O	S	CH_2CHCl	0.432	<30	0.2–2
55	<H>	O	S	CH_2	0.001	>300	10

dimethyl phosphorodithioate>S-benzyl O,O-dimethyl phosphorothioate>O-benzyl O, O-dimethylphosphorothioate. Two samples (No. 8 and 28) were of questionable purity, and in general the phosphorate compounds were unstable. Nagasawa and Shiba[8] reported that in the case of O,O-diethyl nitro-substituted benzyl phosphorothioates and dithioates, the order of toxicity was X=O, Y=S>X=S, Y=S. The toxicity of these compounds also depends upon the nature of A. In the case of the dimethyl analogs (R=CH_3) of S-benzyl phosphorus dithioate compounds (X=S, Y=S), the toxicity was greater when A=CH_2 than when it was $(CH_2)_3$, with the diethyl analogs (R=C_2H_5) the influence of A on toxicity is $(CH_2)_2$>$(CH_2)_3$>CH_3CH>CH_2, and in the analogs where R=*n*-C_3H_7, *iso*-C_3H_7 or *n*-C_4H_9 the toxicity of the compounds was approximately equal when A was either CH_2 or $(CH_2)_3$. With the diethyl analogs (R=C_2H_5) of S-benzyl phosphorothioate compounds (X=O, Y=S) the influence of A on the toxicity was CH_3CH=CH>$(CH_2)_3$>$(CH_2)_2$>CH_3CH>CH_2. The insecticidal properties of the compounds R=C_2H_5, A=CH_2CHCl (No. 54) and R=*n*–C_3H_7, A=CH_2–CH=CH (No. 52) were unusually high. The compound R=cyclohexyl (No. 55) was not toxic.

In general, the acute oral toxicity to mice was low. Almost all phosphorodithioate compounds had a toxicity of >300 mg/kg while phosphorate compounds had toxicity of 100–300 mg/kg. The toxicities of the phosphorothioate compounds are intermediate between the phosphorodithioate and phosphorate compounds. The order of toxicity with regard to the nature of R can be generalized as C_2H_5>CH_3>*n*–C_3H_7=*iso*-C_3H_7> *n*- C_4H_9. The compound R=C_2H_5, A=CH_2CHCl, X=O, Y=S (No. 54) showed an extremely high toxicity to mice (<30 mg/kg). The compounds R=C_2H_5, A=CH_2–CH =CH, X=O, Y=S (No. 50) or X=O, Y=O (No. 51) and R=*iso*-C_3H_7, A=CH_2–CH= CH, X=O, Y=O (No. 53) were somewhat toxic to mice (30–100 mg/kg).

The toxicity of chemicals to carp fish did not show a definite relation between the classified ranks and the nature of R or A. However, in general, the fish toxicity of phosphorodithioate compounds is higher than that of phosphorate compounds. The toxicity of phosphorothioate compounds lies between phosphorodithioate and phosphorate compounds. The toxicity of the phosphorodithioate and phosphorate compounds to fish was the reverse of the acute oral toxicity of these compounds to mice.

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摘　　要

非置換ベンジル燐酸エステル55試料の化学構造と，イエバエに対する接触殺虫性との間には，比較的明瞭な規則性がみられたが，その有効度は，いずれもマラサイオンのそれより劣っていた．ハツカネズミ，コイに対しては，概して低毒性であった．