

原 著

Screening of Phosphorus Amide and *s*-Triazine Chemosterilants in House Flies, Fruit Flies, and Azuki Bean Weevils. Sumio NAGASAWA, Isamu NAKAYAMA (Life Science Research Institute, Kumiai Chemical Industry Co., Ltd. Kikugawa, Shizuoka Pref., 439) Japan Alexej B. BORKOVEC, Paul H. TERRY, Albert B. DeMILO (Insect Chemosterilants Laboratory, Agr. Res. Serv., USDA, Beltsville, Maryland 20705, U.S.A.). Received June 12, 1974. *Botyu-Kagaku* 39, 105 (1974).

22. Phosphorus amide および *s*-triazine 系化合物のイエバエ、ショウジョウバエおよびアズキゾウムシに対する不妊性 長沢純夫・中山 勇 (クミアイ化学工業株式会社 生物科学研究所) Alexej B. BORKOVEC, Paul H. TERRY, Albert B. DeMILO (米国農務省昆虫化学不妊剤研究室)

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Phosphorus amide 系化合物30種と *s*-triazine 系化合物8種のイエバエ、ショウジョウバエに対する不妊性を、毒餌投与法により、アズキゾウムシに対するそれを滴下処理法によって評価した。前者の化合物群のうちでは、hempa, thiohempa と、hempa の水酸化物が有効であった。後者ではメチル化のすんだ化合物に不妊性がみられた。3種の供試昆虫のうち、イエバエが選抜試験のためには最も適しているようである。

The biological activity of chemosterilants varies in different species of insects. The first study of such variability was conducted by Gouck *et al.* (1963) who searched for a general screening technique applicable to house flies, *Musca domestica* L., and screwworms, *Cochliomyia hominivorax* (Coquerel). Similar comparative studies with other insects were conducted by Chamberlain and Barrett (1964), Dame and Ford (1964), Nakayama *et al.* (1969), and Terry and Borkovec (1973). Differences in susceptibility can be related to variations in the screening technique, i.e., oral, topical, or other administration of the sterilant, to inherent differences in the species, or to a combination of these and other factors. When the test compound is administered in the insect's diet or when it is applied topically, the quantitative aspects are always variable because the amount of the compound reaching the hemolymph, and ultimately the reproductive organs, cannot be predicted. Consequently, additional comparative studies are needed, particularly with nonalkylating compounds that tend to be more specific than the alkylating agents. Herein we report chemosterilant screening results with 30 nonalkylating phosphorus amides and 8 *s*-triazines that were added to the diets of house flies and fruit flies, *Drosophila melanogaster* Meigen, or applied topically to azuki bean weevils,

Callosobruchus chinensis L.

Materials and Methods

House flies Separate groups of 50 newly emerged males and females were fed dried milk containing various proportions of the candidate compound. After 3 days, the flies were transferred to one cage for mating and the medicated diet was replaced by a milk-soaked cotton pad. Mortality of the females and the number of eggs laid were recorded daily for 11 days. Each group of eggs was placed on a larval medium and their development was allowed to continue until adults emerged. The average reproductive performance of the treated females was assessed from the formula:

$$x = A_1/F_1 + A_2/F_2 + \dots A_{11}/F_{11}$$

where x is the average number of adults produced by a treated female in 11 days, F_1 - F_{11} are the numbers of surviving females on day 1-11, and A_1 - A_{11} are the numbers of adults which emerged from eggs deposited on day 1-11. In most instances, the concentration of the test compound in the diet was 2% (w/w) but when excessive mortality was encountered, lower concentrations were evaluated. For highly active compounds, EC_{50} (effective concentration causing 50% sterility) was calculated by the probit estimation method of the parameters of tolerance distribution

(Wadley 1949, Finney 1949).

Fruit flies In a procedure similar to that described for house flies, separate groups of 20 newly emerged males and females were fed medicated diet for 3 days but individual pairs were allowed to mate in 50-ml bottles containing culture medium. After 5 days, the flies were removed, deposited eggs were kept in the bottle, and the number of offspring that emerged was determined on the 20th day. Details of the procedure were described by Nagasawa and Nakayama (1972).

Azuki bean weevil Newly emerged adults of both sexes were treated on their dorsal side with 1 μ l of acetone containing various concentrations of the test compound. Acetone-insoluble materials were not tested. Treated weevils were paired individually and the eggs laid by the mated females were scored for adult emergence. Five pairs were used for each dosage. Details of the treatment were described by Bořkovec *et al.* (1968).

All test compounds were synthesized in the USDA laboratory in Beltsville, Md. (U.S.A.); the screening was performed at Ihara Agricultural Chemicals Institute in Shimizu, Shizuoka Pref. (Japan).

Results

Table 1 shows the relative sterilizing effectiveness of 29 variously substituted amides of phosphoric or phosphorothioic acid, (compounds 1-29), and of 8 substituted *s*-triazines (compounds 31-38) in house flies, fruit flies, and azuki bean weevils. Also included was a dimethylamino analog of apholate (compound 30) because of its structural relationship to phosphoramides and triazines. Frequently, excessive toxicity interfered with evaluations of the highest doses of the compounds and lower, nontoxic concentrations had to be determined. Therefore, in the following structure-activity and species susceptibility correlations the concentration factor was always considered.

It was not surprising that in the phosphorus amide group the most effective compounds were hempa (compound 9), its hydroxy derivative hempol (compound 11), and thiohempa (com-

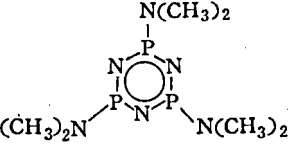
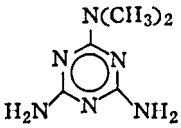
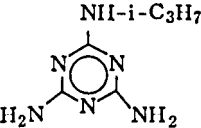
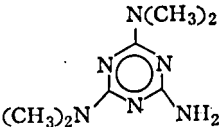
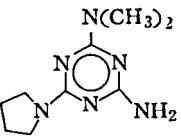
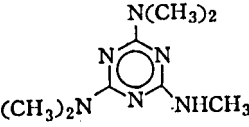
pound 10). The high activity of these chemosterilants is well established (Terry and Bořkovec, 1965, 1970). However, considerable activity was also exhibited by pentamethylphosphoric triamide (compound 6) that in previous house fly screening tests (Terry and Bořkovec, 1967) was reported less active than hempa. Though the results in Table 1 suggest that the pentamethyl compound is as active as hempa in the two species of flies, a reduced effectiveness is evident in the weevil. Phosphorus amides containing less than two dimethylamino groups (compounds 28, 29) or those with no dimethylamino substituents (compounds 1-4, 24, 25) had low activity in all test species with the possible exception of compound 4 in the house fly. In the *bis* (dimethylamino) compounds (compounds 5-23, 26, 27), however, the azetidiny (compounds 16, 17), pyrrolidiny (compound 18), and piperidino (compounds 19, 20) substituents were clearly most effective. The relationship between activities of PO and analogous PS compounds is irregular except for the high toxicity of the sulfur compounds in the fruit fly.

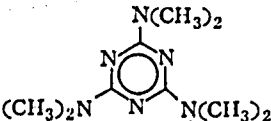
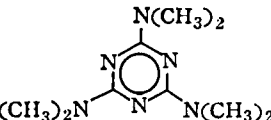
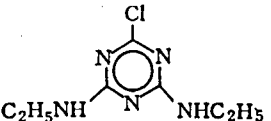
Because many of the *s*-triazines shown in Table 1 (compounds 31-38) were insoluble in acetone, the results of tests with weevils are incomplete. However, the data for flies indicate that highly methylated melamines (compounds 33, 35-37) were the most effective sterilants. This conclusion is in agreement with more extensive studies of *s*-triazinyl chemosterilants conducted by LaBrecque *et al.* (1968) and Bořkovec *et al.* (1972).

The present study indicates the advantage of simultaneous screening of chemosterilants in several species of insects. For example, the data in Table 1 would lose much of its meaning if the test insect were either the fruit fly or the weevil. At the same time, however, the wide elasticity of the house fly as a screening organism cannot be overlooked. Only in one instance did the toxicity of a compound interfere with its evaluation at the highest dose level in this insect. Thus, many of the conclusions concerning structure-activity correlations could be derived from the house fly tests alone, although with a somewhat lower degree of confidence.

Table 1. Sterilizing effects of compound fed to *Musca domestica* and *Drosophila melanogaster* or applied topically to *Callosobruchus chinensis*.

Compound		Percent sterility ^{a)}		
No.	Structure	<i>Musca</i>	<i>Drosophila</i>	<i>Callosobruchus</i>
1	$(H_2N)_3PO$	0 (2)	0 (2)	»
2	$(H_2N)_3PS$	24 (0.5)	25 (0.5)	»
3	$(CH_3NH)_3PO$	0 (2)	55 (2)	4 (100)
4	$(CH_3NH)_3PS$	80 (2)	0 (2)	36 (100)
5	$[(CH_3)_2N]_2P(O)NH_2$	51 (2)	6 (0.5)	31 (100)
6	$[(CH_3)_2N]_2P(O)NHCH_3$	100 (2)	94 (1)	59 (100)
7	$[(CH_3)_2N]_2P(S)NHCH_3$	93 (2)	60 (0.0625)	26 (25)
8	$[(CH_3)_2N]_2P(O)NHCH_2CH_2OCH_3$	57 (2)	62 (2)	57 (100)
9	$[(CH_3)_2N]_3PO$	100 (2)	100 (2)	100 (100)
10	$[(CH_3)_2N]_3PS$	100 (2)	12 (0.0625)	100 (100)
11	$[(CH_3)_2N]_2P(O)N(CH_3)CH_2OH$	100 (2)	99 (2)	79 (100)
12	$[(CH_3)_2N]_2P(O)NCH_3)_2CH_2$	0 (2)	0 (1)	21 (100)
13	$[(CH_3)_2N]_2P(S)NCH_3)_2CH_2$	76 (2)	29 (2)	74 (100)
14	$[(CH_3)_2N]_2P(O)N(CH_3)CHO$	0 (2)	37 (2)	20 (100)
15	$[(CH_3)_2N]_2P(O)N(OCH_3)CH_3$	47 (2)	17 (2)	51 (100)
16	$[(CH_3)_2N]_2P(O)N$ 	100 (2)	51 (1)	41 (12.5)
17	$[(CH_3)_2N]_2P(S)N$ 	0 (2)	0 (0.125)	91 (12.5)
18	$[(CH_3)_2N]_2P(O)N$ 	100 (2)	100 (2)	91 (25)
19	$[(CH_3)_2N]_2P(S)N$ 	68 (2)	0 (0.125)	64 (1.563)
20	$[(CH_3)_2N]_2P(O)N$ 	89 (2)	97 (1)	94 (100)
21	$[(CH_3)_2N]_2P(O)N$ 	0 (2)	1 (2)	29 (100)
22	$[(CH_3)_2N]_2P(O)N$  NCH_3	37 (2)	77 (2)	52 (25)
23	$[(CH_3)_2N]_2P(O)N$ 	40 (2)	45 (2)	34 (25)
24	$[(CH_3CH_2CH_2)_2N]_3PO$	0 (2)	36 (2)	6 (25)

25	$(\text{C}_6\text{H}_{11}\text{N})_3\text{PS}$	0 (2)	0 (2)	18 (50)
26	$[(\text{CH}_3)_2\text{N}]_2\text{P}(\text{O})\text{OCH}_3$	0 (2)	50 (2)	52 (50)
27	$[(\text{CH}_3)_2\text{N}]_2\text{P}(\text{O})$ 	85 (2)	0 (0.25)	25 (3.125)
28	$(\text{CH}_3)_2\text{NP}(\text{S})$  $_2$	0 (2)	43 (1)	17 (3.125)
29	$(\text{CH}_3)_2\text{NP}(\text{O})$  $_2$	0 (2)	0 (2)	7 (50)
30		e) 36 (2)	9 (0.25)	71 (100)
31		e) 56 (2)	5 (2)	b)
32		80 (2)	0.3 (2)	—
33		e) 80 (2)	84 (1)	b)
34		0 (2)	7 (2)	b)
35		e) 100 (2)	99 (2)	b)

36		50 (2)	99 (2)	83 (100)
37		c) 73 (2)	100 (2)	58 (100)
38		0 (2)	54 (2)	—

a) Numbers in parentheses are % concentrations (*Musca*, *Drosophila*) or µg/ul quantities (*Callosobruchus*) of the test compound.

b) Insoluble in acetone; not tested.

c) Monohydrochloride salt of structure shown.

Summary

Chemosterilizing activity of 30 phosphorus amides and 8 *s*-triazines was evaluated in adult *Musca domestica*, *Drosophila melanogaster*, and *Callosobruchus chinensis*. The compounds were administered to the flies as additives to their diet but the compounds were applied topically to the weevil. Effect of the compounds on reproduction of the insects indicated that the hexamethyl compounds hempa and thiohempa, and the hydroxy derivative of hempa were the most effective chemosterilants in the phosphorus amide series. In the *s*-triazine series, the highly methylated compounds were also the most effective. Differential susceptibility of the three test species was most clearly expressed by high toxicity of the sulfur compounds in *Drosophila* and by the uniformly low toxicity of all compounds in the house fly. Of the three species, the house fly appears best suited for broad screening of chemosterilants.

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