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## Determination of Mosquito Chemosterilant Recovered from Air during Real and Simulated Use

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Many studies have documented volatilization of pesticides from water and treated surfaces, especially water-insoluble chlorinated hydrocarbons. We report on recovery of a water-soluble chemosterilant, bisazir [*P,P*-bis(1-aziridinyl)-*N*-methylphosphinothioic amide] from the air during simulated use. When samples were obtained by drawing air at 0.5 L/min through porous polymer sampling tubes suspended above pans containing 1% solutions of the material, analysis by GLC-FPD showed concentrations in air of 100-700 ng/L. When this sampling method was used at a remote work station, 9-54 ng/L of the material was recovered from the air during and after working hours in the work area. Installation of a second vent fan reduced quantities found by 50-80% in three tests. The vapor pressure of this chemosterilant was estimated by comparison of GLC retention times of *n*-paraffins to be 5  $\mu\text{m}$  ( $5 \times 10^{-3}$  mmHg) at 25 °C, which is considered highly volatile. Vapor pressures of hempa, tepa, and thiotepa were found to be 19.5, 3.5, and 1.5  $\mu\text{m}$ , respectively, at 25 °C by this same method.

Chemosterilants have been used as genetic alkylating agents for a number of years in laboratory and field demonstrations of the sterile male technique. Potential users of chemosterilants should be aware that the physical appearance of chemicals, whether white solid or viscous liquid, does not mean that they are nonvolatile at room temperature. Water solubility does not preclude the loss to the surroundings of a hydrophilic chemical from non-aqueous or aqueous solution. Male stable flies, *Stomoxys calcitrans* (LaBrecque and Meifert, 1975), house flies, *Musca domestica* (Meifert and LaBrecque, 1977), *Culex pipiens quinquefasciatus* mosquitoes (Patterson et al., 1970), and *Anopheles albimanus* (Bailey et al., 1979) mosquitoes have been reared in large numbers and sterilized, the last by immersing pupae for 1 h in a 1.0% solution of an appropriate chemosterilant. Gas chromatographic (GLC) analyses of treated pupae ensured that metabolism had occurred and that residues declined to a minimum level before release of adult insects in the field (Bowman and Beroza, 1966).

Jensen and Schall (1966) determined GLC retention times of 24 herbicides, all esters of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid. They used a nonpolar column of 3% SE-30 and found that calculated vapor pressures correlated well with experimentally determined vapor pressures. Since plots of the familiar equation  $\log P$  vs.  $1/T$  showed these data to be linear, extrapolation to 25 °C gave values close to those

determined by the Knudsen method of diffusion through a pinhole (Knudsen, 1909; Mullison and Hummer, 1949). Many studies of airborne pesticides and herbicides have been made by trapping vapors in cold traps, ethylene glycol filled impingers, or on various solid substrates including Tenax GC, Porapak Q, and Chromosorb 101 (Seiber et al., 1975) and Chromosorb 102 (Thomas and Seiber, 1974), but there are no previous reports of recovery of chemosterilants from the surroundings during their use.

Our preliminary experiments showed unexpectedly large recoveries of chemosterilant from the air in the laboratory and in a remote working area near aqueous solutions of bisazir. The desire to demonstrate reduced exposure of workers to bisazir prompted confirmation of this phenomenon. For that reason multiple replications for statistical purposes were not considered essential. We report on methods of trapping and quantitation of one such compound recovered from air and its loss to air from aqueous solution. Samples were taken from an actual work site for analysis. Finally, the vapor pressures of several chemosterilants were estimated by gas chromatography.

## MATERIALS AND METHODS

A 1% solution in water of a thioaziridine chemosterilant, *P,P*-bis(1-aziridinyl)-*N*-methylphosphinothioic amide (hereafter referred to as bisazir) was used for recovery studies.

Solvents used were reagent-grade chloroform and ethylene glycol (Fisher); *n*-hexane (Phillips) was washed with concentrated sulfuric acid and water and distilled from metallic sodium. Chromosorb 102 (Johns-Manville; 60-80 mesh) was used in glass traps.

**Loss Trials Using Ethylene Glycol Traps.** A 1-pt paper cup filled with 200 mL of a 1% solution of bisazir

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(2.0 g) was placed in a wire screen rearing cage (45 × 35 × 35 cm; 60-L volume). The cage was partially enclosed in a polyethylene bag to reduce drafts and was placed in a fume hood at room temperature for 1 h before start of sampling. Air sampling was conducted by drawing air from 10 cm above the surface of the solution (65-cm<sup>2</sup> area) at 500 mL/min through 400 mL of ethylene glycol held in a 700-mL gas washing bottle equipped with a fritted glass gas delivery tube. A standard vacuum pump with an air bleed was used to maintain a constant flow rate. At the conclusion of sampling, the ethylene glycol was washed out of the container and diluted with 1400 mL of distilled water before being extracted 3 times with a total of 400 mL of chloroform (Fisher ACS grade) that was concentrated with a rotary evaporator. A small volume of standard solution containing 50 mg of bisazir was diluted with proportionate amounts of ethylene glycol, extracted with chloroform (Seawright et al., 1973), and concentrated for analysis to determine recovery.

**Loss Trials Using Chromosorb 102.** Trials to determine losses during extraction, collection, and storage were conducted by placing hexane solutions of bisazir into Chromosorb 102 traps, made by fusing both ends of sections of Pyrex tubing (15 mm diameter × 50 mm) to smaller glass tubing that fits Swagelok 3/8-in. fittings. The tubes were packed with Chromosorb 102 porous polymer (3 mL), held with glass wool, and purged with helium at 200 °C overnight in a GLC oven. The traps were then washed with hexane and purged with nitrogen gas before use. Portions of a standard 0.01% solution of bisazir in hexane were unjected onto traps for extraction, collection, and storage loss trials as follows.

For determination of extraction losses, traps treated with 500 or 100 µg of bisazir were washed with 50-mL portions of hexane, either in the same direction as the flow of purge gas or in the opposite direction. The eluates were analyzed without concentration or further treatment to determine recovery.

For determination of storage losses, six traps treated with 50 µg of bisazir were capped and held at either 4 or 27 °C for 7 days before elution by back-flushing with hexane and analysis.

Collection losses during trapping on Chromosorb 102 were studied by injection of 100 µg of bisazir onto a trap. A total of 1620 L of air was drawn through the trap at 1500 mL/min for 18 h, before elution by back-flushing and analysis.

**Concentration Losses.** A solution containing 2 µg of bisazir in 200 mL of hexane (0.001%) was heated to reflux with a boiling stone, and aliquots were withdrawn for immediate analysis without concentration. In addition, solutions containing 2 µg of bisazir in 200 mL of hexane (0.001%) were concentrated with a rotary evaporator to just under 1 mL, transferred quantitatively, and made up to 1 mL for analysis.

**Recovery Trials from Air over Aqueous Solutions.** In one test, a semiclosed environment was created by placing a polyethylene cover loosely about an aluminum cake pan containing 1500 mL of 1% bisazir aqueous solution having a surface area of 600 cm<sup>2</sup>. A purged trap was placed 10 cm above the water surface, and room air was withdrawn from the trap by a mechanical pump at 350 mL/min. Traps were eluted with 50-mL portions of hexane in the reverse directions and analyzed without concentration.

In a second test, a closed environment was created by placing a cake pan containing 1500 mL of 1% solution in a polyethylene bag which was filled with compressed air

from a cylinder, sealed to a trap with rubber bands, and allowed to stand at room temperature (25 °C) for 20 h. Most of the air in the bag was then withdrawn through the trap at 540 mL/min for 132 min for a volume of 71.3 L. Water had condensed inside the bag in one corner during overnight equilibration due to cool room air blowing on the outside of the bag. After air was withdrawn from the bag, tissue was used with forceps to absorb about half of the water condensed inside the bag, and then the wet tissue was extracted with 5 mL of chloroform for analysis. Traps were eluted as before.

In a third test, saturation of air with bisazir was accomplished by connecting three gas washing bottles, each containing 400 mL of 1% solution, in a series with a purged trap. Room air was drawn through this system for 2 h at 350 mL/min, and traps were eluted as before.

**Analysis of Air Samples from a Work Area.** A pilot test was conducted in El Salvador, Central America, by the U.S. Department of Agriculture to study the feasibility of the sterile male technique for the control of *A. albimanus*, an important vector of malaria. The study involved the use of bisazir to sterilize more than one million pupae of this species per day for release (Bailey et al., 1979). The sterilization area within the rearing facility consisted of a closed room 4.5 × 2.2 × 2.2 m high, equipped with exhaust fans in the ceiling, and an adjacent work room 3.6 × 2.4 × 2.2 m high. The closed room where the sterilization was done contained shelves of stainless steel pans, each containing 2 L of a 1% solution of bisazir in water; the work area was used for packaging pupae, cleaning equipment, and recording data.

In March 1977, air from the adult colony room and from the sterilization room was tested for the presence of bisazir, the only chemosterilant used by the laboratory. At that time, air in the sterilization room was exhausted by a single shaded-pole blower (525 cfm) and the room contained 18 pans of chemosterilant solution. Air samples were taken during different periods of activity in the sterilization room (during sterilization; after work while pans were covered) and from the exhaust vent by drawing room air at the rate of 1 L/min through new glass traps containing previously unused Chromosorb 102 (Table IV, test 1). The samples were stored for no more than 2 weeks at 4 ± 0.5 °C before and after shipment to Gainesville, FL, before analysis.

In May 1977, a hood was constructed over the sterilant pans and two shaded-pole blowers (525 cfm each) were installed in the hood. At that time more air samples were taken from the sterilization room and the work area and sent to Gainesville, FL, for analysis (test 2). Later, the number of sterilant pans was increased to 24 so the two shaded-pole blowers were replaced with three larger blowers (1780 cfm each). Finally, in Jan and Sept 1978, more air samples were taken from the sterilization room and analyzed for bisazir (tests 3 and 4).

In all tests, the traps were extracted by slowly percolating through them three portions of 50 mL of hexane that were then combined and concentrated to 1 mL by using either a Kuderna-Danish apparatus (test 1) or a rotary flash evaporator (tests 2-4).

**Gas Chromatography (GLC).** Residue analyses were performed on a F & M Model 810 gas chromatograph fitted with a 1 m × 2 mm i.d. glass column packed with 3% Dexsil 410 on Gas-Chrom Q (100-120 mesh) and helium carrier gas at 20 mL/min. The column effluent was routed through 1/16 in. × 0.010 in. i.d. stainless steel tubing to a four-port Carle valve attached to the inside wall of the oven and then to a flame photometric detector (FPD; Tracor) operated in the phosphorus mode with a 526-nm

Table I. Bisazir Recovered from Air above a Cup<sup>a</sup> by Using Ethylene Glycol

sample	sampling time, h	bisazir found, $\mu\text{g}$	air sampled, L	concn in air, ng/L
1	3	2.5 <sup>b</sup>	60	42
2	3	16.0	60	260
3	3	4.3	60	72
2	8	49.0	160	306
3	8	24.0	160	150
1	68	66.0 <sup>b</sup>	2040	33
2	64	450.0	1920	231

<sup>a</sup> Cup of 1% solution had a surface area of 65 cm<sup>2</sup>.

<sup>b</sup> Sampling started immediately upon introduction of cup.

filter. The valve was attached to a metered source of helium to deliver column effluent or purge gas at identical flow rates with those of the detector. Solvent from the sample was vented to the oven which was held at 140 °C, with the injector and detector at 190 and 180 °C, respectively. The lower limit of detection of bisazir was 0.25 ng/injection, or 0.05 ng/ $\mu\text{L}$  in a 5- $\mu\text{L}$  injection. Thus, there was less than 0.5  $\mu\text{g}$  in a typical 1-mL sample labeled "none detected" (ND). Relative retention times of chemosterilants and authentic paraffin standards (Analabs) were obtained by GLC by using a 1.8 m  $\times$  2 mm i.d. glass column packed with 3% OV-1 on 120–140 mesh Chromosorb W AW DMCS at 140 °C with a flame ionization detector.

## RESULTS

**Loss Trials with Ethylene Glycol Traps.** The study showed that appreciable quantities of bisazir, a water-soluble chemosterilant, were recovered from air sampled directly above a water solution of the compound. We found that up to 0.26  $\mu\text{g}$  of bisazir was recovered from each liter of air sampled by being drawn through a trap containing ethylene glycol (Table I). This figure can be revised upward, as studies showed only 70% recovery from ethylene glycol, when 50 mg of standard bisazir was used as described. Also, losses of up to 50% occurred during concentration of chloroform solution with a rotary evaporator. We also observed that even at a slow sampling rate of 333 mL/min, rather large bubbles passed upward from the glass frit through the ethylene glycol trapping medium, and intimate contact between the air and the glycol was not achieved. Thus

$$0.23 \mu\text{g/L experimental value} / (70\% \text{ recovery efficiency} \times 50\% \text{ loss on sample concn}) = 0.66 \mu\text{g of bisazir/L of air or } 660 \text{ ng of bisazir/L of air}$$

thus, estimated continuous transfer of sterilant into air was just over 660 ng/h above a small paper cup when the surface area of liquid was 65 cm<sup>2</sup>. The effects of air sampling speed, temperature, and constantly moving living organisms (mosquito pupae) in the paper cup are unknown. However, the use of porous polymer traps was more convenient, and Chromosorb 102 was used in all subsequent experiments.

**Loss Trials with Chromosorb 102 Traps.** GLC analysis showed that 95% of the bisazir was extracted on back-flushing of spiked Chromosorb 102 traps with one 50-mL portion of hexane. There were only traces in the second and third portions. All subsequent extractions then followed this method. In collection trials, 74.5% of two standard samples were recovered after passage of 1620 L of air through each spiked trap, and 0.015% of spiked bisazir was lost per L of air passed through the traps. This source of error was therefore ignored in calculations, since in all air sampling, a relatively low flow rate of less than 1500 mL/min was used. In storage trials, samples of bisazir held on Chromosorb 102 traps for 7 days showed

Table II. Bisazir Recovered from Chromosorb 102 Traps after 7 Days of Storage at Indicated Temperatures

test no.	bisazir recovered, %	
	4 °C	27 °C
1	80.5	82
2	91	62.5
3	68	57
4	77	74
av:	79	69

Table III. Bisazir Recovered from Air above a Pan by Using Chromosorb 102

sampling rate, L/min <sup>a</sup>	sampling time, h	bisazir found, $\mu\text{g}$	concn in air, ng/L
(a) 0.60	3	13.4	124
(b) 0.33	21	181	430
2nd trap <sup>b</sup>	21	ND <sup>e</sup>	
(c) 0.33	45	610	700
2nd trap <sup>b</sup>	45	1.2	1.3
(d) 0.35 <sup>c</sup>	3	32	510
(e) 0.42 <sup>c</sup>	3	37 <sup>d</sup>	490

<sup>a</sup> Samples a–c were collected sequentially after 1-h equilibration of the system. <sup>b</sup> Backup trap connected in series. <sup>c</sup> Chemosterilant pan contained live pupae. Samples d and e were collected sequentially after 1 h. <sup>d</sup> Estimated due to partial loss of sample. <sup>e</sup> ND, none detected.

average losses of 21 and 31% when the traps were stored at 4 and 27 °C, respectively (Table II). Losses of 25% were therefore assumed for each trapped and stored field sample because refrigerator storage was followed by air shipment and further cold storage preceded analysis.

**Concentration Losses.** Analysis of a boiled solution of bisazir in hexane at 1 ng/ $\mu\text{L}$  showed 49% and 77% loss at 30 and 60 min, respectively. Thus, loss during use of a Kuderna-Danish evaporator for 15 min was estimated at 25%. Determination of losses during concentration of known solutions to 1 mL on a rotary flash evaporator showed average loss of 11% of bisazir in three replications.

**Recovery Trials from Air over Aqueous Solutions.** Analysis of bisazir recovered from air over a loosely enclosed cake pan is shown for four sampling periods of different lengths (Table III). Sampling at flow rates between 333 and 420 mL/min gave consistent results and a mean concentration of 550 ng/L of air (0.55 mg/m<sup>3</sup>) in air above the pan. A small amount of bisazir was found in only one backup trap. Sampling at a 600 mL/min flow rate in one short test gave a smaller value for bisazir of 120 ng/L, perhaps because of excessive flow rate. Calculated loss because of passage of air through the trap was only 0.3  $\mu\text{g}$ , which makes this source of error unimportant.

Results of a single test in a closed system (sealed bag) showed only 3.3  $\mu\text{g}$  of bisazir recovered from the first extraction of the trap and none from the second and third. However, analysis of chloroform used to extract 0.691 g of water found condensed inside the upper portion of the bag showed 467  $\mu\text{g}$  of bisazir present. This water was condensed, not splashed from the pan, and then apparently acted as a sink for the airborne chemical. Only about half of the condensed water was collected. If our interpretation is correct, 937.3  $\mu\text{g}$  of bisazir was displaced from the pan.

Results with the train of gas washing bottles showed recovery of 93.7  $\mu\text{g}$  of bisazir after only 42 L of air was drawn through this system at atmospheric pressure; the concentration of bisazir found (2200 ng/L) was 4 times that found over a pan as in the first trial. This higher value may reflect aerosol formation in the washing bottles.

**Analysis of Air Samples from a Work Area.** Five samples from test 1 (Table IV) showed the presence of an average of 29 ng/L bisazir in air sampled during and after work hours in the sterilant rooms. Covering of pans did

Table IV. Analysis of Air Samples Collected from a Work Area

length of sampling, h	time started	sample location	$\mu\text{g}$ found	$\mu\text{g}$ adjusted <sup>a</sup>	concn in air, ng/L
test 1					
16	p.m.	colony room	ND <sup>c</sup>		
24		colony room	ND		
4	a.m.	working, pans open <sup>b</sup>	6.23	13.0	54
4	a.m.	working, pans open <sup>b</sup>	2.3	4.8	20
4	p.m.	pans covered <sup>b</sup>	12.9	26.9	12
4	p.m.	pans covered <sup>b</sup>	3.1	6.5	27
16	p.m.	pans covered <sup>b</sup>	12.97	30.7	34
4	a.m.	in vent, working, pans open <sup>b</sup>	1.78	3.7	15
4	p.m.	in vent, pans covered <sup>b</sup>	1.99	4.1	17
16	p.m.	in vent, pans covered <sup>b</sup>	3.4	8.1	9
test 2					
5	a.m.	work area, near-sterilant room	ND		
24		work area, near-sterilant room	ND		
5	a.m.	working, pans open <sup>b</sup>	0.86	1.4	4
16	p.m.	pans covered <sup>b</sup>	2.07	3.8	4
24		pans open 7-12 a.m., closed remainder <sup>b</sup>	1.73	3.0	2
test 3					
20	p.m.	pans covered <sup>b</sup>	7.1	11.2	11
24		pans open 7-12 a.m., closed remainder <sup>b</sup>	10.0	15.8	13
4	a.m.	working, pans open <sup>b</sup>	1.5	2.4	10
test 4					
4	p.m.	pans covered, door closed <sup>b</sup>	3.20	4.6	19
4	a.m.	working, door open <sup>b</sup>	0.19	0.27	1
4	p.m.	pans covered, door closed <sup>b</sup>	1.01	1.45	6
4	a.m.	working, door open <sup>b</sup>	0	0	0
4	p.m.	pans covered, door closed <sup>b</sup>	0	0	0
4	a.m.	working, door open <sup>b</sup>	0	0	0

<sup>a</sup> Observed values were adjusted by using the following factors: (1) extraction loss, 5%; (2) collection loss, 0.016%/L of air passed through the trap; (3) storage loss, 25%/week applied to each sample; (4) concentration loss, 30% (Kuderna-Danish Concentrator, test 1 only); (5) rotary evaporator loss, 11% (test 2-4). <sup>b</sup> Samples taken subsequently from sterilant room. <sup>c</sup> ND, none detected.

not immediately stop trapping of chemosterilant from room air, while three samples taken in the air vents containing exhaust fans showed half as much bisazir, an average of 14 ng/L. None was detected in the adult (colony) room next door to the work room in any test.

In test 2, values recovered from the air were 1 order of magnitude less ( $\sim 4$  ng/L) probably because of installation of a second electrically powered vent. Collection losses exceeded the quantities found, and values were adjusted for sampling time. In tests 3 and 4, values were variable and somewhat higher than in test 2 but well below those in test 1. Samples taken during test 3 consistently averaged 11 ng/L, less than half of the original values; an average of 4 ng/L was found during test 4 if all the tests, half of which showed no detectable bisazir, are included. Experimental GLC retention times obtained on a nonpolar column (3% OV-1) showed that bisazir eluted just before *n*-tetradecane. Vapor pressure values for 13-17 carbon *n*-paraffins at 25 °C were obtained by the Antoine equation, using published values (Jensen and Schall, 1966) and are plotted in Figure 1. Retention times of other chemosterilants were compared with those of *n*-paraffin standards and then plotted together with log vapor pressures of the standards (Figure 1). Vapor pressures at 25 °C were determined to be as follows: bisazir, 5.02  $\mu\text{mHg}$ ; thiotepa, [tris(1-aziridinyl)phosphine sulfide, 1.55  $\mu\text{mHg}$ ; hempa (hexamethylphosphoric triamide), 19.5  $\mu\text{mHg}$ ; tepa [tris(1-aziridinyl)phosphine oxide], 3.04  $\mu\text{mHg}$ .

#### DISCUSSION

The use of Chromosorb 102 proved to be a convenient and satisfactory technique for trapping of chemosterilant from the air, especially as samples taken at a remote location could be stored for later analysis. Previous discussions have dealt with water-insoluble pesticides of low

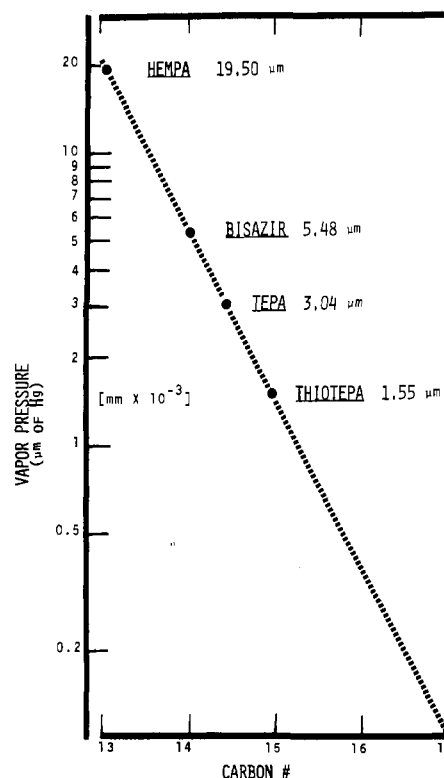


Figure 1. Vapor pressures of *n*-paraffins and chemosterilants at 25 °C estimated by using a plot of vapor pressures of *n*-paraffin standards calculated from the Antoine equation vs. GLC retention times for the *n*-paraffins upon which retention times of chemosterilants were plotted.

volatility, in which volatilization was shown to be a major pathway for loss and/or movement (Acree et al., 1963;

Hartley, 1969; Spencer and Cliath, 1970).

The transfer of relatively large quantities of bisazir to air can be ascribed to its relatively high vapor pressure. Jensen and Schall (1966) stated that "2,4-D and 2,4,5-T derivatives with a vapor pressure greater than  $0.15 \times 10^{-3}$  mm (0.15  $\mu$ m) of Hg at 25 °C should be classified as highly volatile". All of the chemosterilants studied here are similar in molecular weight and can be considered quite volatile at room temperature. Hempa, a popular, particularly useful polar solvent, is even more volatile than bisazir. We suggest handling these materials with appropriate caution and ventilation.

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## Larvicidal Effects of Substituted Diamino- and Triamino-s-triazines

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Larvicidal activity of 21 substituted 2,4-diamino-s-triazines and 14 substituted 2,4,6-triamino-s-triazines was determined in *Musca domestica* L., *Aedes aegypti* (L.), *Tribolium confusum* Jacquelin duVal, and *Plodia interpunctella* (Hübner). Some of the triazines examined in this study were previously reported to have insect chemosterilant activity. Larvicidal activity was highest in *M. domestica* and *A. aegypti*. Five of the compounds were more active than the larvicide 6-azido-*N*-cyclopropyl-*N'*-ethyl-1,3,5-triazine-2,4-diamine (CGA-19255) in *M. domestica*, and one was as active in *A. aegypti*.

The discovery of the insect sterilizing effects of diamino- and triamino-s-triazines (Borkovec and Terry, 1965; Borkovec and DeMilo, 1967; LaBrecque et al., 1968; Borkovec et al., 1972) presented a new type of inhibition of reproduction: when the female was treated, it mated and oviposited normally, and egg hatch was high, but the larvae died before reaching maturity. Apparently, the compounds or their metabolites disturbed the metabolism of the young larvae (Matolin and Landa, 1971). Recent reports on the larvicidal activity of 6-azido-*N*-cyclopropyl-*N'*-ethyl-1,3,5-triazine-2,4-diamine (AI3-70670; Ciba-Geigy CGA 19255) (Herzog and Brechbuehler, 1976; Christensen and Knapp, 1976; Miller et al., 1977) prompted us to determine the effects of 21 diamino- and 14 triamino-s-triazines on larvae of the yellow fever mosquito, *Aedes aegypti* (L.), housefly, *Musca domestica* L., Indianmeal moth, *Plodia interpunctella* (Hübner), and confused flour beetle, *Tribolium confusum* Jacquelin duVal. The results of these tests and the structure-activity correlations for the compounds are presented here.

#### EXPERIMENTAL SECTION

**Materials.** Except for the compound AI3-70670, which was supplied by the Ciba-Geigy Corp., all triazines shown in Table I were synthesized according to previously pub-

lished procedures (Smolin and Rapoport, 1959; Nestler and Fuerst, 1963; Borkovec and DeMilo, 1967; DeMilo and Borkovec, 1968; DeMilo, 1970). New compounds (AI3-60019, -60108, -60209, -61516, -62414, -62415, and -62525) were analyzed (Galbraith Laboratories, Inc., Knoxville, TN) for carbon, hydrogen, and nitrogen with acceptable results ( $\pm 0.3\%$  of theory). Table I contains melting points of these materials. Compounds AI3-62414, AI3-62415, and AI3-62525 were prepared from 6-hydrazino-*N,N,N',N'*-tetramethyl-1,3,5-triazine-2,4-diamine (DeMilo et al., 1973) and the appropriate aldehyde or isothiocyanate. Since many diamino and triamino-s-triazines are poorly soluble in water, several hydrochloride salts were tested; their activity was found to be comparable to that of the free bases. For convenience, some of the compounds listed in Table I were tested as hydrochlorides.

**Biological Testing.** All compounds were tested as additives to the diet of larvae of *A. aegypti* (A), *M. domestica* (M), *P. interpunctella* (P), and *T. confusum* (T). Testing procedures of Robbins et al. (1970) were used for A, M, and T, and the procedure of Cohen and Marks (1979) was used for P. Compounds that did not kill 75% or more of the test insects at their highest applied concentration (A, 10 ppm; M, 300 ppm; P, 1000 ppm; T, 3000 ppm) were considered inactive. All positive tests were replicated.

#### RESULTS AND DISCUSSION

Larvicidal activity of the s-triazines varied considerably among the four insect species, but the closest correlation could be made between the two Diptera: *M. domestica* and *A. aegypti* (Table I). In the series of 2-substituted-

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