Persistence of thiotepa and tepa in pupae and adults of *Culex pipiens fatigans* Wiedemann

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Abstract

Thiotepa and its oxygen analogue tepa, used to chemosterilize males of Culex pipiens fatigans *for genetic control purposes, are toxic and mutagenic. An investigation showed that adult mosquitos that had been treated as pupae showed no detectable chemosterilant in their tissue 24 hours after emergence from the pupal stage.*

In 1971, the WHO/Indian Council of Medical Research Unit on the Genetic Control of Mosquitos, New Delhi, India, began field studies to evaluate various sterilization and release techniques that might be used in the control or eradication of *Culex pipiens fatigans*. As chemosterilization with tris(1-aziridinyl)-phosphine sulfide (thiotepa) [5] was one of the sterility-inducing methods considered, it became necessary to determine the amount of residual thiotepa in mosquito tissue. Moreover, as thiotepa can be converted to tris(1-aziridinyl)phosphine oxide (tepa) (Parish & Arthur, 1965), it was also necessary to determine any residues of this chemical that might be present in the tissue. Bowman et al. (1966) devised a procedure for analysing subnanogram amounts of tepa by gas chromatography and the technique was subsequently modified for the analysis of thiotepa also (Seawright et al., 1971). These procedures were used in the tests described in this communication.

Mosquito pupae 2–24 hours old were chemosterilized by dipping them for 3 hours in a 0.6% aqueous solution of thiotepa buffered at a pH of 8.3. The pupae were then rinsed twice in fresh water. As the release techniques under study were envisaged for both pupae and adult mosquitos, the insects were kept for various periods of time after sterilization. Samples of pupae that had been exposed to the thiotepa solution 0, 6, 12, and 24 hours previously, and of adults taken 0–6, 24, and 48 hours after emergence, were quick-frozen with dry ice and kept under refrigeration until delivered to the Insects Affecting Man and Animals Research Laboratory, Gainesville, Fla., USA. Each sample contained 200 pupae or adults. Control samples of untreated pupae as well as some of the thiotepa solution used for the treatment were forwarded to the same laboratory under refrigeration.

Upon arrival in Gainesville, the insects were processed for analysis according to the technique described by Seawright et al. (1971). Preparation of the mosquito samples consisted essentially in extracting the chemosterilant by grinding each sample of mosquitos in methanol, centrifuging the mixture, and retaining the supernatant liquid. After repeating this procedure 3 times, the extracts were pooled and concentrated to the appropriate volume by using water-pump vacuum and a 60°C water bath; 1-ml samples of the concentrate were extracted twice with 5 ml of chloroform, and the water was supersaturated with sodium chloride and extracted twice more with chloroform. The 4 chloroform extracts were evaporated to dryness and the residue was reconstituted in methanol. The samples were then forwarded under refrigeration to M. C. Bowman at Tifton, Ga., for analysis of the thiotepa and tepa residues.

The analysis was performed by using a gas chromatograph equipped with a flame photometric detector operated in the phosphorus mode (526-nm filter). The 50-cm glass column (internal diameter: 4 mm) contained 5.0% OV-225 (a cyanopropyl phenyl methylsilicone) on gas Chrom Q and was operated isothermally at 145°C with a nitrogen carrier flow of 160 ml/min. Under the stated conditions 5 μl of the concentrated extract were injected into the column

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[5] Mention of a pesticide or proprietary product does not constitute recommendation or endorsement by the US Department of Agriculture.

Table 1. Residues of thiotepa and tepa found in *C. p. fatigans* at various stages and ages (average of 3 samples)

Stage analysed	Interval between treatment and analysis (hours)	Age of adults (hours after emergence)	Residue (ng per insect)	
			Thiotepa	Tepa
pupae	0	—	62.2	<0.5 [a]
	6	—	80.2	<0.5 [a]
	12	—	70.7	<0.5 [a]
	24	—	12.0	<0.5 [a]
	(control)	(no treatment)	<0.25 [a]	<0.5 [a]
adults	—	0–6	10.2	2.0
	—	24	<0.25 [a]	<0.5 [a]
	—	48	<0.25 [a]	<0.5 [a]

[a] No observable residue.

for analysis of the residues. Retention times for thiotepa and tepa were 2.10 and 2.80 minutes, respectively. Quantification was based on peak values. The results of the analysis are given in Table 1.

All pupae contained some residue of thiotepa and those held for 24 hours following chemosterilization contained on the average 12 ng of the chemosterilant. In order to accumulate 1 mg of thiotepa, it would take approximately 80 000 pupae of that stage, and 12 000 pupae when retention of the sterilant is at its maximum—i.e., 6 hours after treatment. No observable residue of tepa was found in the pupae. Residues in adult mosquitos were highest 0–6 hours after eclosion, when averages of 10 ng of thiotepa and 2 ng of tepa per insect were observed. Adults analysed 24 hours or more after emergence contained no observable residue of either chemosterilant in the tissues. Releases conducted at a later date indicated that the treated insects dispersed and survived best when they were released as adults 24 hours after eclosion. Analysis of the 3 samples of aqueous thiotepa prepared at a concentration of 0.6% and used to sterilize the pupae showed concentrations ranging from 0.52% to 0.61%, with an average of 0.56%. No tepa (<0.002%) was detected in the solution.

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