INCREASED TOXICITY OF MALATHION DILUTED WITH HEAVY AROMATIC NAPHTHA ON ADULT *AEDES TAENIORHYNCHUS*[1]

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ABSTRACT. Field tests of ground ULV application of Cythion® diluted with heavy aromatic naphtha (HAN), 1:4 ratio, at 0.45 oz AI/acre resulted in a 96% and 49% mortality of caged adult *Aedes taeniorhynchus* located 150 and 300 ft downwind. Cythion, 0.45 oz AI/acre produced a 33% mortality at 150 ft and a 21% mortality at 300 ft. This compares with an 8% mortality at 150 ft on a plot treated with HAN only (0.45 oz/acre), and a 6% mortality in an untreated area. The study was repeated on four successive nights, reversing test plots, and the data pooled. Statistical analysis shows that the toxicity of the active ingredient, malathion, is significantly increased with the addition of the diluent, HAN.

INTRODUCTION

Heavy aromatic naphtha (HAN) has been used as a diluent for various insecticides (Sutherland et al. 1978). In the past it has been used as a carrier for the active ingredient (Rathburn 1985), and as a low viscosity diluent to decrease droplet size (World Health Organization 1985). In an effort to reduce application rates and costs, a number of studies were initiated to determine if ultra low volume (ULV) application of insecticide could maintain effective mosquito control when diluted with HAN and extended over a larger area (Haile et al. 1982). Some of these studies indicated better control with a mixture of insecticide diluted with HAN than can be obtained with the insecticide alone (Haile et al. 1982, Mount and Pierce 1972). This increased effectiveness cannot be ascribed simply to the increase in volume as documented by Glancey et al. (1965) and others in studies on the efficacy of ULV application. Smaller droplet size (Haile et al. 1982) and an increase in the total number of droplets over a given area (Roberts 1984) have been suggested as possible explanations for this phenomenon. Metcalf (1967) found that in virtually all instances where there is a substantially more than additive toxic action of any substance used together with an insecticide, the increase in toxicity is caused by the additive interfering with insect detoxification. Thus, this action by an additive/diluent can be used in restoring the activity of an insecticide against resistant strains of insects.

The purpose of this study was to determine if ULV application of HAN combined with malathion (MAL) increases mosquito mortality over MAL alone; and if so, to determine if the increases were statistically significant.

MATERIALS AND METHODS

One test was conducted on each of four successive evenings between 1900–2000 hr. in densely wooded test sites at Fort Eustis, VA, beginning on September 26, 1983. The test sites for the MAL and the MAL/HAN treatments were 1½ miles apart and were reversed each day. The area supported sparse undergrowth resulting from the thick canopy of mixed hardwood and conifers ranging up to 60 feet in height. A HAN control plot was located on a similar site between the two test areas.

Each formulation was applied at 0.45 oz AI/acre with a truck mounted Curtis Dyna-Fog Model 2740 ULV aerosol generator at a blower pressure of 8 psi to three test plots consisting of Cythion®, technical grade (91%) MAL, a mixture of HAN (Exxon® Aromatic 150) and MAL (4:1), and one control plot of HAN alone. Droplet size measurements of the aerosols were made with an Optical Array Cloud Droplet Spectrometer Probe (PMS) Model OAP-200X. Data were recorded and graphed on a Particle Data System Model 300 (PMS Incorporated, Boulder, CO).

A laboratory-reared strain of *Aedes taeniorhynchus* (Wiedemann) was used as the test insect. Three to six day postemergence female mosquitoes were immobilized by refrigeration, counted, and transferred to standard (WHO) exposure tubes (20/tube). Mosquito netting (18 mesh) was used as a screen for the tubes. In each treatment plot, tubes were suspended on 3 ft stakes (2/stake) 150 and 300 ft downwind and perpendicular to the line of travel of the truck-mounted ULV aerosol generator. In the HAN control plot, three exposure tubes (20 mosquitoes/tube) were suspended on the 3 ft stakes. Exposure tubes in the HAN control plot were 150 ft downwind of the aerosol generator.

During the tests, the temperature range was 19–22°C ($\bar{x}$ 21°C); the relative humidity range was 58–75% ($\bar{x}$ 67%); and the wind speed was 3–8 knots. Droplet size and volume median di-

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Table 1. Percent mortality of adult female *Aedes taeniorhynchus*[a] after ULV treatment with malathion and HAN.[b]

		9/26/83	9/27/83	9/28/83	9/29/83	4-day pooled	
		Mortality (%)				Mortality (%)	
ULV Treatment	Distance (ft)	12 hour				1 hour	12 hour
Malathion/HAN	150	85	100	100	100	59	96
Malathion	150	26	10	77	19	8	33
Malathion/HAN	300	31	58	95	13	20	49
Malathion	300	16	5	49	15	6	21
HAN control	150	19	7	2	5	4	8
Untreated control	—	10	3	9	3	—	6

[a] Caged mosquitoes on 3 ft. stakes.
[b] Heavy aromatic naphtha.

ameter (VMD) determinations were calculated with the PMS immediately before each test by sampling each formulation 6 ft from the aerosol generator. The VMD ranged from 10.9 to 19.9 microns (μ).

After the aerosol had sufficient time (30 min) to drift through the test plot, the mosquitoes were transferred into clean cages. Cotton pads containing a 10% sucrose solution were provided as an energy source posttreatment. Mortality counts were recorded at 1 and 12 hours posttreatment. Untreated control mosquitoes were handled in the same manner.

Mosquito mortality response to MAL and MAL/HAN at distances of 150 and 300 ft was analyzed using a linear weighted regression logistic (logit) model, 4 SAS PROC FUNCAT,[4] to test for treatment effect, day effect and interaction between treatment and day. The test statistic was Chi-square. Determinations of which treatments had significant effects were made using the above statistical procedure and test statistic for both distances. Comparisons between treatments at both distances were made using Chi-square with Yate's correction.

RESULTS AND DISCUSSION

Percent mortalities for ULV treatments with MAL and HAN at 1 and 12 hr posttreatment are presented in Table 1. The data show that at 150 ft the toxicity of the active ingredient MAL is greatly enhanced with the addition of the diluent HAN in every test downwind. Three hundred feet is at the outer limits of the aerosol range, and the droplets are quite dispersed at this point; however, the 4-day pooled data shows that even at this distance, MAL diluted with HAN is still considerably more effective than MAL alone. There is an overall interaction between MAL and HAN at both 150 ft ($p = 0.04$) and 300 feet ($p = 0.02$) which supports the hypothesis of increased toxicity. This is also supported by the 1 hr data which shows that a high percentage of the mortalities (4-day pooled 37% at 150 ft; 29% at 300 ft) did not occur immediately. As shown by the data from the HAN control site, the action of HAN alone is not toxic to the mosquitoes; mortality is approximately the same as the untreated controls. At 150 ft, $p = 0.09$ and at 300 ft, $p = 0.19$ for overall HAN effect.

Table 2. Chi-square tests for comparisons of treatments for 150 and 300 feet.[a]

Treatment	150 feet	300 feet
Malathion/HAN vs HAN[b]	278.24	76.59
Malathion/HAN vs malathion	131.85	22.05
Malathion/HAN vs untreated control	285.07	83.17
Malathion vs untreated control	40.44	16.28

[a] All <0.005.
[b] The 150 ft HAN control data was used for both the 150 ft and 300 ft Malathion/HAN comparison.

Table 2 shows that the MAL/HAN combination is significantly more toxic than MAL alone using Chi-square tests for comparison. This suggests an increase in the toxicity of the MAL/HAN combination. At 150 ft, comparisons of mortality for MAL/HAN and MAL alone are significantly different with their percentages of mortality being 96% and 33%, respectively. Also, at this distance, MAL at 33%, is more effective than the untreated control at 6%. For both 150 ft and 300 ft, there are significant differences between MAL/HAN and both control plots.

The problem of insect resistance is not new, and in fact, increases as time passes. The use of new pesticide combinations is one solution; however, this requires an extensive list of effective mixtures as insects begin to develop resistance to these new chemical combinations, and optimal efficacy may vary with the insect species (Liu et al. 1984).

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This study shows that the addition of HAN to MAL effectively increases the toxicity of MAL to control adult *Aedes taeniorhynchus*. Similar results obtained during treatment of *Aedes aegypti* (Linn.) with MAL alone and MAL plus HAN (1:2) in Puerto Rico in 1981,[5] suggest that HAN also acts to enhance the toxicity of MAL against *Aedes aegypti*. As HAN has been frequently used as a solvent/diluent in the past (Haile et al. 1984, Mount and Pierce 1972), it is already classified as an acceptable additive to pesticides (Rathburn 1985). Insecticide additives/diluents such as HAN are of practical importance in obtaining more effective control of insects, increasing the spectrum of activity of an insecticide, and restoring the activity of an insecticide against resistant strains of insects. Further study into the ability of HAN to magnify the insecticidal activity of other chemicals on different insect species is warranted.

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REFERENCES CITED

Glancey, B. M., C. S. Lofgren, J. Salmela and A. N. Davis. 1965. Low volume aerial spraying of malathion for control of adult salt-marsh mosquitoes. Mosq. News. 25:2:135–137.

Haile, D. G., T. L. Biery, J. F. Reinert and N. W. Pierce. 1982. Aerial applications of naled diluted in HAN with UC-123K aircraft for adult mosquito control. Mosq. News 42:1:41–48.

Haile, D. G., D. L. Kline, J. T. Reinert and T. L. Biery. 1984. Effects of aerial applications of naled on *Culicoides* biting midges, mosquitoes and tabanides on Parris Island, South Carolina. Mosq. News. 44:2:178–183.

Liu, M. Y., J. S. Chen and C. N. Sun. 1984. Synergism of pyrethroids by several compounds in larvae of the diamondback moth (*Lepidoptera:* Plutellidae). J. Econ. Entomol. 77:4:851–856.

Metcalf, R. L. 1967. Mode of action of insecticide synergists. Annu. Rev. Entomol. 12:229–256.

Mount, G. A. and N. W. Pierce. 1972. Adult mosquito kill and droplet size of ultra low volume ground aerosols of insecticides. Mosq. News. 32:3:354–357.

Rathburn, C. B., Jr. 1985. Insecticide formulations—Types and uses: A review. J. Am. Mosq. Control Assoc. 1:1:80–84.

Roberts, R. H. 1984. Evaluation of bendiocarb as an ULV cold aerosol against caged mosquitoes. Mosq. News. 44:4:457–460.

Sutherland, D. J., R. Kent and J. Downing. 1978. The effect of aerial ULV adulticiding with malathion and naled on field populations of *Aedes sollicitans*. Mosq. News. 38:4:488–491.

World Health Organization (WHO) 1985. Ultra-low-volume applications of insecticides for vector control. Unpublished document WHO/VBC/85.919, Geneva, 31 pp.