TECHNICAL

Report

THE EFFECT OF ULTRA LOW VOLUME AERIAL DISPERSAL OF NALED ON AN AQUATIC HABITAT ROBINS AFB GA

EHL(K) 74-25

October 1974

USAF ENVIRONMENTAL
HEALTH LABORATORY
KELLY AFB, TEXAS

USAF ENVIRONMENTAL HEALTH LABORATORY (AFLC)
UNITED STATES AIR FORCE
KELLY AFB, TEXAS 78241

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NOTICE

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I. SUMMARY

Ultra low volume aerial application of naled (Dibrom 14) at 0.75 and 1.5 oz per acre was made at Robins AFB, GA on 31 Jul 74. Environmental monitoring utilizing caged fish, shoreline sampling, drift traps, drop traps, acetylcholinesterase monitoring and other observations indicated negligible effect on the environment. Acetylcholinesterase activity in fish brains was depressed in the treated area but no mortality was noted. No extensive monitoring activities are necessary for conducting routine spray missions at conventional rates. Closely spaced, multiple applications may require monitoring.

II. INTRODUCTION

A. At the request of AFLC/SGB letter of 26 Jun 74 (Appendix A) this laboratory assisted USAF SAM/EPE in evaluating an aerial dispersal of insecticide for mosquito and eye gnat control. The purpose of the Environmental Health Laboratory, Kelly portion of the survey was to evaluate the effects of naled (Dibrom 14) at rates of 0.75 and 1.5 oz/A on biological systems in aquatic habitats.

B. Aerial application of naled using ultra low volume technique (ULV) was performed at Robins AFB, GA on 31 Jul 74 for control of mosquitoes and eye gnats. Spraying was accomplished, using a C-123 aircraft equipped with ULV equipment, by the 355TAS/Spray from Rickenbacker AFB, OH. The aircraft flew at 150 ft altitude and at 150 mph giving a spray swath of approximately 1,000 ft. The entire area was treated with 0.75 oz naled/A and a portion was retreated with 0.75 oz/A to give a total treatment of 1.5 oz/A. The effectiveness of the spray for control of the target insects was evaluated by personnel from USAF SAM/EPE, Brooks AFB, TX (Biery, 1974).

C. The land around and on Robins AFB consists of low swampy areas on the north and east side of the base (Fig. 1). Permanent and temporary pools of water are abundant, the former more so. Two small creeks run through this area and empty into the Ocmulgee River. The overstory consists of hardwoods in the wet areas and pine in the higher regions. The center and west side of the base is higher with mostly clay soil. The base has three major ponds with two in the recreation areas and one along the golf course. During the time of the survey there was very little rain and water levels dropped. In some areas such as the check sites, the water level dropped about 12-18 inches. In the swampy areas the water level dropped about 3-6 inches. At the time of spray, the water levels were low.

D. Previous field and laboratory tests have indicated that naled has little effect on non-target organisms at low rates. Bearden (1967) reported little mortality in fish but high morality in shrimp when naled was applied by ground fogging equipment in an estuarine environment. Similar results were observed when aerial applications of one and two ounces active ingredient (AI) were made. Byrd and Oberheu (1967) concluded that 0.6 oz AI/A caused an increase in cholinesterase levels in fish brain but no mortality in fish, wildlife or aquatic arthropods. Terrestrial Diptera, however, were severely affected. The increase in cholinesterase level was unexpected, and Byrd and Oberheu speculated that it might be due to stimulation by a low dose of naled.

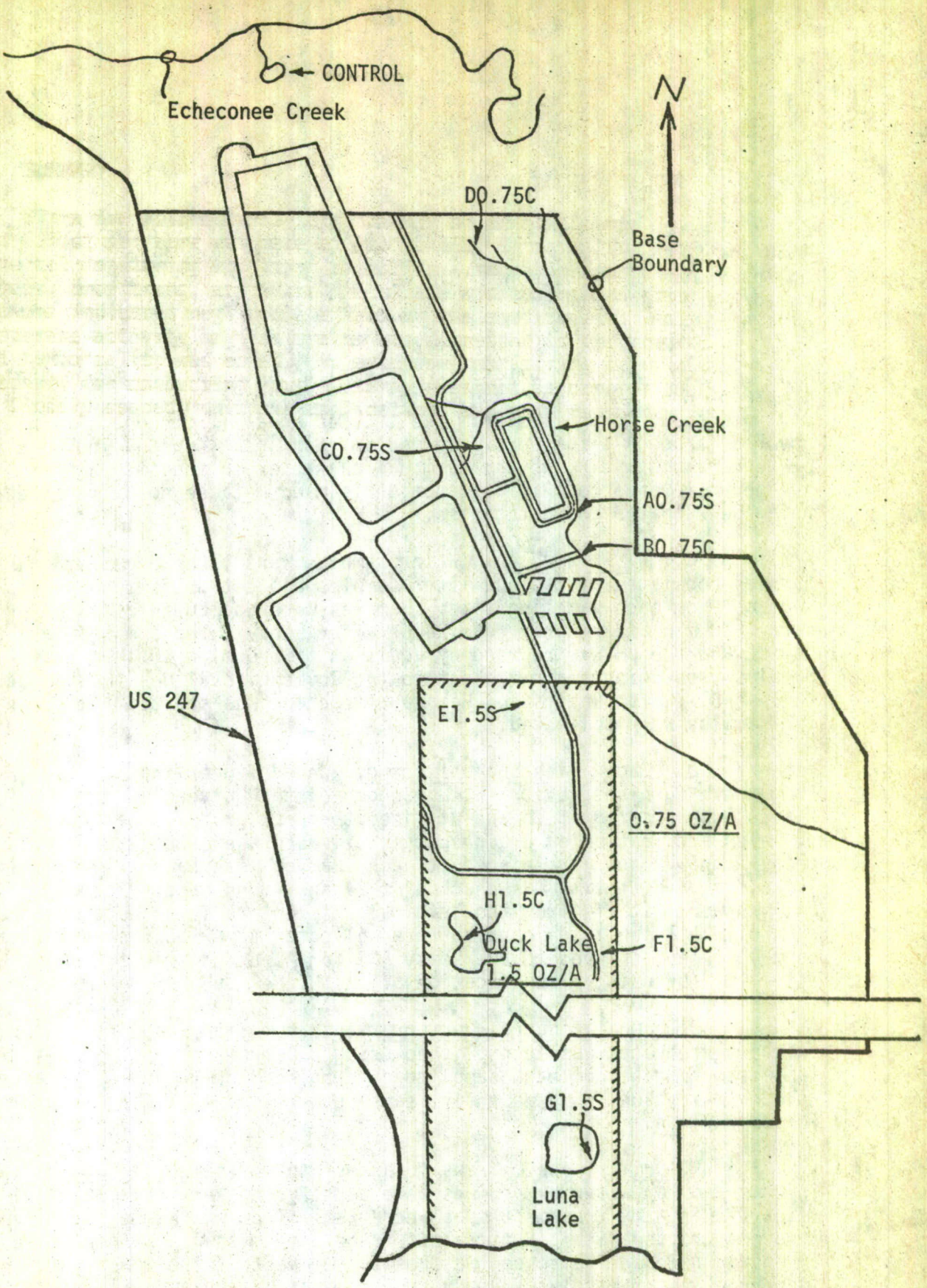

FIGURE 1. Map of Robins AFB, GA with sampling sites indicated. Area inside hatched lines received 1.5 oz naled/A and the other areas on the base received 0.75 oz naled/A.

III. MATERIALS AND METHODS

A. Application. Dibrom 14 was applied at Robins AFB, GA between 0630 and 0800 hrs on 31 Jul 74. Application was made by a C-123 aircraft equipped with ULV equipment. The entire test area was treated with 0.75 oz naled/A and a portion was re-treated with 0.75 oz/A to give a total treatment of 1.5 oz/A.

B. Sampling Sites. Four sampling sites were set up within each treatment area (0.75 oz/A and 1.5 oz/A areas), and two sites were set up as controls about 0.25 miles from the treated areas. Both open (sunny) and covered (shaded) sites were selected in each area (denoted in Table 1 by "S" or "C", respectively, as the terminal character in the site number). No further reference to this distinction is made because no differences were noted between the two categories.

C. Water Samples. Water samples were taken on three ocassions. Samples consisted of two one-liter samples collected two inches below the surface from each sampling site. Samples were collected 24 hr prior to spray, 30 min after spray, and 24 hr after spray. The samples were cooled on ice and the pH was reduced by addition of 7.0 ml of 0.1N HCl per liter. Samples were shipped air freight to the Environmental Health Laboratory, Kelly AFB, TX for analysis. Hexane extraction was started six hours after the samples were collected.

D. Caged Fish Biomonitoring. Caged fingerling catfish (2½ - 3 in long) were furnished by Dr. E. L. Snoddy of the University of Georgia Coastal Plains Experiment Station and utilized to check gross mortality and the effect of naled application on brain acetylcholinesterase (AChE) activity. Three metal minnow traps were placed at each station with each trap containing 10 catfish. Mortality was checked at 24 and 48 hr post-treatment. Twenty catfish were witheld from the original shipment to serve as a pre-treatment control. All surviving catfish were collected 48 hr post-spray, frozen on dry ice and shipped to EHL/Kelly AFB for AChE determination. AChE was determined by using a modification of the Michel ΔpH method (Michel, 1949).

E. Aquatic Macroinvertebrates. Shoreline sampling for aquatic macroinvertebrates was accomplished using a D-frame net. Two 20-ft linear samples were taken at each sampling site both prior to and 48 hr after naled application. Subsequently, samples were identified and enumerated for comparison.

F. Drift Traps. Traps to assess the effect of naled on flying insects and top water arthropods were constructed and placed at various sites within each treatment area where water flow was adequate. The traps were constructed with a 2 ft X 2 ft frame with mesh window screen supported by wooden poles. The screen was positioned so that the lower portion was submerged approximately six inches. Pre and post-spray evaluations were made.

TABLE 1. Description of sampling sites used for environmental monitoring at Robins AFB, GA 24 Jul-2 Aug 74

SITE NUMBER	SITE DESCRIPTION
	0.75 oz/A
A0.75S	Open, slow flowing creek with clay bottom, heavy bank vegetation.
B0.75C	On same creek as A; similar except heavy shade from canopy consisting mainly of hardwood.
C0.75S	Open, slow flowing creek with poor water quality and highly organic bottom; heavy bank vegetation.
D0.75C	Slow flowing drainage ditch rich in algae and with sandy bottom; heavy bank vegetation; shaded by canopy consisting mainly of hardwood.
	1.5 oz/A
E1.5S	Open swampy area with poor water quality and a bottom rich in organic matter; heavy bank vegetation.
F1.5C	Swampy area with clay bottom; little bank vegetation; shaded by canopy. For invertebrate sampling, a sandy, shaded drainage ditch with overhanging bank vegetation was used.
G1.5S	Open area of manmade pond with sandy bottom; no bank vegetation; moderate algal growth in water.
H1.5C	Partially shaded area of a manmade pond; bottom rich in organic matter; no bank vegetation.
	Control
ICKC	Small boat landing area of manmade pond with channel to nearby creek; moderate bank vegetation; gravel bottom, partially covered.
JCKS	Open fast flowing creek with clay bottom; moderate bank vegetation.

G. Drop Traps. Traps to evaluate the effect on non-target, flying, terrestrial insects were placed at several sampling sites. These traps were constructed by covering $1m^2$ of cardboard with aluminum foil and painting the exposed surface with "stickum". Four of these traps were placed at each sampling site.

H. Native Fish. Pre-treatment seining was carried out at several sampling sites to determine the fish species indigenous to the treatment area.

I. Visual Observation. Visual observation of naturally occuring arthropods and wildlife were made by all personnel involved with this study.

IV. RESULTS

A. Water Samples. At the time of this writing, analysis of water samples for naled is incomplete. A supplemental report will provide these results.

B. Caged Fish Biomonitoring. Only five test sites were utilized in the fish biomonitoring because of poor water quality at some stations. The fish in those stations not listed in Figure 1 died prior to spray and were thus removed from the test. The results of caged fish mortality, as shown in Table 2, indicate that no mortality occurred due to application of naled at either 0.75 or 1.5 oz/A. High mortality in the control and G1.5S can be attributed to the amount of human activity around the sampling sites. In the control there was a relatively high amount of boat activity which probably caused the caged fish to swim against the wire mesh thus damaging themselves and resulting in death. Children throwing rocks at the cages in G1.5S could have caused mortality in the same manner as the controls. In areas away from human activity, negligible mortality was noted.

C. Acetylcholinesterase Monitoring. AChE activity is a good indicator of exposure of fish to sublethal concentrations of organophosphate pesticides. Table 3 shows the depressed AChE activity in those fish exposed to the naled spray. The unexpected results exhibited in the control can be partially explained by the spray activities of the aircraft and the lack of wind at the particular time of spray. When the 0.75 oz/A application was being made on the north end of the base, the winds were calm. The spray aircraft could have "dragged" the spray in its vortex over the control area when it was making its turns. This activity could account for naled inadvertently being applied in the control area. Naled sensitive dye cards also indicated drift or accidental spray in the control area, (Biery, 1974). There was no significant difference ($p=.05$) in AChE activity between the control, 0.75 oz/A, and the 1.5 oz/A treatments. The 1.5 oz/A treatment, however, had a significant decrease in activity from the pre-treatment control. The control also was significantly different from the pre-treatment control indicating a substantial amount of naled was deposited in that area.

Table 2. Results of caged fish biomonitoring. Ten fish were placed in each cage and the results are given as number of dead fish at the end of the 24 and 48 hr.

	SAMPLING SITES																	
	Control*			A0.75S			B0.75C			F1.5C			G1.5S*			H1.5C		
Interval Post Treatment	Cage Number			Cage Number			Cage Number			Cage Number			Cage Number			Cage Number		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
24 hr	2	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
48 hr	3	5	2	0	1	0	1	1	0	0	0	0	3	3	1	0	0	0
TOTAL	5	6	4	0	2	0	1	2	0	0	0	0	3	3	1	0	0	0

*The control area and the G1.5S area were subject to disruption by human activity.

Table 3. Acetylcholinesterase activity in fish brains as measured by the Michel ΔpH method. Each value is the mean of two determinations. Means followed by the same letter are not significantly different at p=.05 (Duncan's multiple range test).

Replications	Treatments			
	Pretreatment ΔpH/hr	Control ΔpH/hr	0.75 oz/A ΔpH/hr	1.5 oz/A ΔpH/hr
1A	.73	.58	.79	.56
B	.73	.58	.67	.61
C	.73	.57	.76	.61
2A	.68	.56	.59	.56
B	.68	.62	.57	.54
C	.68	.67	.55	.52
x	.71a	.60b	.66ab	.57b

D. Aquatic Macroinvertebrates. Total aquatic macroinvertebrates are shown in Table 4. Each value in the table is a composite of two samples. As can be seen there was no apparent decrease in the numbers of macroinvertebrates in the samples after spraying naled. The discrepancy between the pre and post-spray counts of midge larvae in G1.5S and H1.5S is probably due to slight differences in the sampling location and sampling technique since the samples making up the pre-spray total values contained midges too numerous to count from one location and no midge larvae from the other. These samples were taken only a few feet apart at each sampling site.

E. Drift Traps. No dead organisms were found in any of the drift traps.

F. Drop Traps. Very few organisms were found on any of the drop traps. Primarily the ones found were carpenter bees, mosquitoes, gnats, deerflies, and some dragonflies. These were found in very low numbers and with the exception of mosquitoes and gnats, none exceeded 2 per trap.

G. Native Fish. Table 5 shows the pre-spray catch of fish seined at various sampling sites. The purpose of the seining was to determine what species of fish were present and would be exposed to naled. Mosquito fish were present in all areas and were the predominant species. No mortality of native fish was observed in any treatment area.

H. Visual Observation. During the post-spray period of this survey no decline in activity of the normal non-target fauna was noted. Numerous butterflies, dragonflies, and damselflies were noted. Honey bees continued to work. Many reptiles and amphibians were observed at several sampling sites in both treatment areas. Bird activity remained at the observed pre-spray level.

Table 4. Aquatic macroinvertebrates taken in pre and post-spray drag samples. Each number is the sum of two samples, each taken along 20 ft of shoreline. Pre-spray samples were taken 29 Jul and post-spray samples were taken 1 Aug.

TAXON		SAMPLING SITES											
		A0.75S		B0.75C		D0.75C		F1.5C		G1.5S		H1.5S	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Ephemerellidae	(Mayflies)	3	1	2	2	0	0	0	0	0	0	13	12
Libellulidae	(Dragonflies)	13	7	1	7	4	7	2	2	0	0	1	1
Coenagrionidae	(Damselflies)	55	49	32	34	16	21	25	23	0	0	1	2
Notonectidae	(Back Swimmer)	21	19	3	6	1	1	0	0	0	0	1	0
Naucoridae	(Creeping Water Bugs)	0	0	0	0	0	0	0	0	0	0	0	0
Belostomatidae	(Giant Water Bugs)	0	1	1	0	0	0	1	0	0	0	0	0
Nepidae	(Water Scorpions)	0	0	2	0	0	0	0	0	0	0	0	0
Hydrometridae	(Marsh Treaders)	0	5	0	0	1	2	0	0	0	0	0	0
Veliidae	(Water Striders)	0	0	0	0	0	0	0	0	0	0	0	0
Hemiptera	(Misc. Bugs)	0	4	0	0	0	3	0	1	0	0	0	0
Leptoceridae	(Caddis Flies)	0	0	0	0	0	0	0	0	0	0	0	3
Pyralidae	(Moth Larvae)	0	0	0	1	1	0	2	1	0	0	0	0
Carabidae	(Ground Beetles)	0	0	0	0	0	0	0	0	0	0	0	0
Haliplidae	(Crawling Water Beetles)	0	0	1	0	4	2	0	0	0	0	0	0
Dytiscidae	(Water Scavenger Beetles)	0	0	0	9	1	1	0	0	0	1	0	1
Staphylinidae	(Rove Beetles)	0	0	2	0	0	0	0	0	0	0	0	0

TABLE 4 continued

TAXON		SAMPLING SITES											
		A0.75S		B0.75C		D0.75C		F1.5C		G1.5S		H1.5S	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Curculionidae	(Weevils)	0	0	0	0	0	0	1	0	0	0	0	0
Coleoptera	(Misc. Beetles)	0	1	0	3	0	0	0	0	0	0	0	0
Chironomidae	(Midges)	16	4	3	4	1	0	1	4	TNTC	0	TNTC	20
Culicidae	(Mosquitoes)	0	2	0	0	0	1	0	0	0	0	0	0
Ceratopogonidae	(Biting Midges)	0	11	0	0	0	0	0	0	0	0	0	0
Cecidomyiidae	(Gnats)	0	0	0	0	0	0	0	0	0	0	0	0
Amphipoda	(Scuds)	42	33	17	75	0	0	0	0	0	0	0	0
Cladocera	(Daphnia)	0	0	6	9	0	0	0	0	0	0	0	0
Decapoda	(Crayfish)	2	2	1	0	6	2	0	0	0	0	0	0
Araneida	(Spiders)	3	7	1	2	2	0	1	0	0	0	0	0
Oligochaeta	(Worms)	0	0	0	0	0	0	0	0	0	0	1	0
Hirudinea	(Leeches)	11	0	7	16	3	1	0	0	0	0	0	0
Pelecypoda	(Bivalves)	0	0	2	6	0	0	1	0	0	0	0	0
Gastropoda	(Snails)	0	0	0	2	0	0	0	3	0	0	3	0
Ectoprocta	(Bryozoan Colonies)	0	16	0	0	0	0	0	0	0	0	0	0
Total Organisms		171	151	92	167	42	43	35	39	TNTC	2	46*	39

Table 5. Native fish seined from various areas on Robins AFB, GA.

Sample Site	Species	Number
Control	Gambusia affinis (mosquito fish)	7
	Labidesthes sicculus (brook silverside)	3
	Esox americanus (grass pickerel)	2
AO.75S	G. affinis	8
	Cyprinidae-juveniles	2
DO.75	G. affinis	29
	Lepomis sp-juveniles	16
F1.5C	G. affinis	61
	Notemigonus crysoleucas (golden shiner)	2

V. DISCUSSION

A. The results of this study are generally consistent with those previously reported by Bearden (1967), Byrd and Oberheu (1967) and Favorite, et al. (1962). They found no significant hazard to wildlife and fish at low rates of naled. This study indicates there are negligible gross environmental hazards associated with the application of naled at rates of 0.75 and 1.5 oz/A. This is probably due to the rapid degradation of naled in an aquatic situation.

B. Aquatic insects, flying non-dipterous insects, fish, reptiles, and birds did not appear to be affected by naled at these two rates.

C. The depression of brain AChE activity found in this study at both rates is significant and in contrast to the findings of Byrd and Oberheu (1967) who reported an increase in cholinesterase levels after naled exposure of fish. Even though the findings of Byrd and Oberheu are reported in levels (amounts) of cholinesterase and our findings in AChE activity, the two are comparable as AChE activity is directly proportional to cholinesterase levels. The discrepancy between the two studies cannot be explained unless it resulted from the use of different fish species. However, studies of the effects of other organophosphates on fish indicate that AChE activity is depressed or inhibited following exposure. Benke and Murphy (1974) have shown that the onset of AChE inhibition in fish by some organophosphate insecticides occurs within 24 hr. Recovery requires an extended period of time (about 4 weeks) to approach normal activity again. The slow recovery could be significant if fish are subjected to multiple exposures over a short period of time since the inhibitory affects on AChE activity are additive.

D. Short term effects of naled on an aquatic habitat are negligible. However, frequent application of naled could have an adverse affect on both fish and aquatic arthropods.

VI. RECOMMENDATIONS

Large scale environmental monitoring is not recommended for routine intermittent application of naled by aerial spray, however, environmental monitoring should be performed in situations where multiple treatments at close intervals are employed. Additional testing of the sublethal effects of naled on aquatic organisms, especially fish, should be accomplished.

VII. REFERENCES CITED

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APPENDIX A

DEPARTMENT OF THE AIR FORCE
HEADQUARTERS AIR FORCE LOGISTICS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433

REPLY TO ATTN OF: SGB

26 June 1974

SUBJECT: Request for Entomological Support, Aerial Spray Project

TO: Epidemiology Division
USAFSAM/EP
Brooks AFB TX 78235

1. References:

a. AFR 161-12, USAF Epidemiology Services.

b. AFR 91-22, Aerial Dispersal of Pesticides.

c. USAFSAM/EP letter 6 Jun 74, "Entomological Evaluation, Robins AFB, GA."

d. AFLC/DEMM letter, 25 Jun 74, "Aerial Dispersal of Pesticides, Robins AFB, GA" (Atch 1).

2. This headquarters has approved a request by Robins AFB for aerial dispersal of insecticide for mosquito control. The request and supporting documents were forwarded on 25 Jun 74 to Hq AFRES/DOOT for approval and processing.

3. Request your organization provide pre-spray mission and post-spray mission entomological surveys to evaluate the effectiveness of the aerial dispersal project. As discussed 20 June 1974 with Col Melvin (USAFEHL Kelly) and Capt Clegern (Epidemiology Division, USAFSAM), the USAF Environmental Health Laboratory at Kelly AFB will provide a medical entomologist to assist in these surveys at Robins AFB. Anticipated period for the aerial spray mission is mid to late July; exact date will be provided when we are advised by AFRES/DOOT.

Atch 3

4. Project Officer at Robins AFB for the entomological surveys is Major M.G. Moody, USAF Hosp/SGB, Autovon 468-2248.

FOR THE COMMANDER

PHILLIP E. SMEAD, Maj, USAF, BSC
Staff Bioenvironmental Engineer
Office of the Surgeon

1 Atch
AFLC/DEMM ltr
25 Jul 74 w/atch

Cy to:
USAFEHL Kelly/CC w/atch
USAF Hosp Robins/SG w/atch
AFLC/DEMM w/o atch

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11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY

13. ABSTRACT Ultra low volume aerial application of naled (Dibrom 14) at 0.7 and 1.5 oz per acre were made at Robins AFB GA on 31 July 1974. The effects of the spray on non target aquatic organisms were monitored utilizing caged fish, shoreline sampling, drift traps, drop traps, acetylcholinesterase levels in fish brain and other observations.

Acetylcholinesterase activity in fish brains was depressed in the treated areas but no significant mortality of fish was noted. No gross effects on the normal fauna was observed.

From this test it is concluded that no extensive monitoring activities are required for routine spray missions using conventional rates of naled, but closely spaced, multiple applications may require monitoring.

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14. KEY WORDS	LINK A		LINK B		LINK C	
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Dibrom						
Fish						
Aquatic Macro Invertebrates						
Acetylcholinesterase						

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