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DEVELOPMENT AND VALIDATION OF A RAPID ANALYTICAL METHOD TO QUANTIFY NALED RESIDUE

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ABSTRACT. A rapid gas chromatographic method for detecting residues of the thio-organophosphate naled was developed and subsequently validated in laboratory and field studies. More than 90% of naled was recovered by a gas chromatograph when equipped with a DB-5 capillary column and a thermionic specific detector. The limit of detection was 0.01 μg/ml with direct injection. Stabilization of naled under a variety of storage conditions also was examined. Analysis of field data showed that naled broke down rapidly in the environment but was stable when stored in hexane solvent at 4°C and 23°C for at least 7 days. Range of percentage matrix spike recovery was 31–49% for filter paper samples exposed under field conditions for 14 h. A field study was also initiated that collected naled droplets trapped on 6.7-m acrylic mohair-look yarn strands in addition to residue on filter paper after aerial ultra–low-volume mosquito adulticide application. Spike recovery was 79% for filter paper samples and 93% for yarn samples. Average naled residue concentrations with these methods were 373 μg/m² and 11.28–73.77 μg/yarn, respectively.

KEY WORDS Organophosphate, naled, insecticide monitoring

INTRODUCTION

Naled (*O,O*-dimethyl *O*-(1,2-dibromo-2,2-dichloroethyl)phosphorate) is 1 of the mosquito adulticides used by public health pest control programs in the United States (Mount et al. 1968; Haile et al. 1982, 1984; Linley et al. 1987, 1988; Curtis and Mason 1988; Howard and Oliver 1997). This insecticide is a member of an increasingly popular class of pesticides for which little environmental chemistry data exist. As a result, ecological risk cannot be assessed without accurately measuring the potential exposure concentration for nontarget organisms in their habitat. The relatively high vapor pressures of naled (266 kPa) and its primary degradation product 2,2-dichlorovinyl dimethyl phosphate (DDVP; 1,600 kPa; Hall et al. 1997) make residue detection difficult after field application. Tietze et al. (1996) reported that the half-life of naled was 1.37 h in direct sunlight and 4.81 h and 8.17 h at 87.7 and 46.9% relative humidity, respectively, when stored in a dark chamber. Because of the rapid environmental degradation of this compound, minimizing residue loss after aerial ultra–low-volume (ULV) application becomes a challenge during the sample collection and handling process. Identification of a method to stabilize and prevent further degradation of this compound is vital to accurately determine naled residue deposition. Hennessey et al. (1992) reported using α-cellulose pads (10.16 × 10.16 × 0.2 cm) to collect naled deposits when monitoring mosquito adulticide drift into wildlife refuges of the Florida Keys. Pads were retrieved within 15–30 min after application, wrapped with aluminum foil, sealed in plastic bags, and stored at −40°C until extraction and analysis. Hall et al. (1997) developed an alternate analytical method for naled and DDVP in air that used XAD-4 resin trapping when measuring air quality.

To maintain sample collection quality and minimize naled residue loss due to lag times in sample collection, storage, and transportation, a rapid extraction procedure was designed to determine naled stability and to validate the method under different exposure and storage conditions.

MATERIALS AND METHODS

Naled standards: Analytical-grade standards of naled (93+% active ingredient [AI]) and DDVP (99.8% AI) (Ultra Scientific, North Kingstown, RI) were used in this study. Analytical-grade naled (99.7% AI) and technical-grade naled (87.4% AI) also were obtained from AMVAC Chemical Corporation (Los Angeles, CA). All standards were stored at −20°C until used. Stock solutions (10 ml each) for analysis were prepared by dissolving naled and DDVP separately in hexane (10 mg/ml) and these were kept at 4°C until used. Field spiking and analytical standards were prepared from these stock solutions. Naled residues were quantified by using a 5-point external standard curve with linear regression. The calibration standard range, 0.1–10 μg/ml, was prepared by serial dilution of analytical-grade naled (93+% AI). The concentration of this standard was verified by an analytical standard (99.7% AI) from the secondary source mentioned above. An intermediate stock solution (180 μg/ml), prepared by both technical-grade naled (87.4% AI) and analytical-grade naled (99.7% AI) in hexane, was used to spike blank filter papers to measure efficiency of extraction. Quality assurance and quality control samples also included instrument blanks, continuous calibrations, sample preparation blanks, solvent spikes, and field spikes.

Gas chromatography: A Varian 3400 gas chro-

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matograph (GC) equipped with a thermionic specific detector and 8200 autosampler (Varian Analytical Instruments, Sugar Land, TX) was used in this study. Data were collected by a Dell Computer Working Station (Dell Computer Corporation, One Dell Way, Round Rock, TX) equipped with data-handling software Star Chromatograph Version 4.51 (Varian Analytical Instruments). The injector was operated at 250°C in the splitless mode and the detector was operated at 300°C. A DB-5 capillary column (30 m × 0.25-mm inner diameter; 0.1-μm film thickness) bonded with fused silica was also used. The column oven temperature was held for 0.5 min at 120°C, ramped at 25°C/min to 260°C, and held for 1 min. A standard injection volume of 1 μl was used for all standards and samples. The retention time of naled was 3.65 min.

Naled stabilization was tested at the Public Health Entomology Research & Education Center (PHEREC), Florida A&M University at Panama City, FL. The test profiled naled degradation under several scenarios, including indoor or outdoor, sample dry or wet, room temperature (22–25°C) or refrigerator (4°C), and with or without hexane solvent. This study was conducted to identify the best sample storage and transportation method after residue samples were collected from the field. As part of an analytical quality control, 6 laboratory spike samples were prepared in hexane. Each sample consisted of 1 ml of spiking solution and 29 ml of hexane; the final dilution concentration was then equivalent to 6 μg/ml. The naled standard calibration consisted of 5-point calibration curve range from 0.1 to 10 μg/ml with $R^2 \geq 0.995$. Continuous calibration (CC) at the level of 2 μg/ml was conducted every 10 samples. The CC must pass the criteria of 100 ± 10% recovery each time, otherwise a new calibration curve must be generated. Laboratory and field spikes should pass the limit of 100 ± 20%. All blanks should be clean from contaminants.

Stabilization of naled in hexane: One milliliter of naled spike standard (180 μg/ml) was added directly into 29 ml of hexane contained in a 40-ml Pyrex® screw-capped culture tube (25 × 150 cm). This was called the solvent spike. The culture tube was shaken and inverted for 2 min to make sure the standard was completely mixed in the solvent. The solvent mix was ultrasonicated for 5 min, and shaken again for an additional 2 min. One milliliter of extract was then transferred into a 1.5-ml autosampler vial and subjected to GC analysis. The remaining naled extract in the culture was sealed and stored in a refrigerator (4°C). One milliliter of extracts was continually taken out of the culture tube daily for 4 days to analyze naled residue via GC.

Efficiency of naled extraction: Extraction efficiency and degradation of naled were tested on on filter paper (24 cm; Whatman International Ltd., Maidstone, England). Two filter papers were pinned on a styrofoam board adjacent to each other. One milliliter of the spike standard (180 μg/ml) made from technical-grade naled (87.4% AI) was added to each paper and evaporated to dryness under a hood. After this, the papers were immediately folded. Each paper was placed into a 40-ml Pyrex culture tube (25 × 150 cm) filled with 30 ml of hexane and topped with screw cap. Samples were shaken for 2 min, ultrasonicated for 5 min, and shaken again for an additional 2 min.

Fourteen-hour overnight field exposure of naled: Extracts with filter paper and solvent were stored at 4°C for further quantification of naled residue levels. A 1-ml sample was then placed into a 1.5-ml autosampler vial and subjected to GC analysis. The other 8 filter papers were placed outdoors at 5:00 p.m. for overnight exposure. All filter papers were collected at 7:00 a.m. the next day. Each filter sample was stored in a Pyrex culture tube filled with 30 ml of hexane at room temperature (23°C). Two samples were extracted immediately. Each sample consisted of 30 ml of extract with filter paper inside. The extracts with hexane solvent were stored at 4°C. This study was duplicated in a 2nd week. A different procedure adopted in the 2nd week was that only 2 filter papers were collected from each field per day. The rest of the filter papers were continually exposed in the field throughout the week.

Comparison of naled degradation on 2 scenarios: Naled stability in hexane was compared at 23°C and 4°C. Extracts of naled samples exposed overnight (14 h) were stored with hexane in 40-ml Pyrex culture tubes at each temperature. Sample extracts were then analyzed daily for naled residue with a GC. Data shown in the tables were based on 6 replicates (2 samples × 3 replicate batches) except samples from day 5, which were based on 4 replicates (2 samples × 2 replicate batches). Values were mean ± standard deviation of 6 replicates except the last day (day 4), which had 4 replicates.

Validation of the method by determination of naled from field sample: To validate the naled extraction and analytical protocol, we developed a field aerial ULV trial at Palmetto, FL. Aerial ULV naled application was conducted by Manatee County Mosquito Control District with a Hughes-500D 1097C helicopter. Naled was applied on June 23, 1999, at a rate of 49.25 ml/ha (0.667 oz/acre). Wind direction was northeast and wind speed about 4.6 km/h at 3-m altitude. Field samples were collected from an open area, forest edge, and within a forest area. One control area was set up more than 24 km away from the spray zone. Residue that drifted through the air was trapped on 6.7-m acrylic mohair-look yarn (Lion Brand Yarn Co., Hoboken, NJ). The yarn was folded on a 61 × 61-cm polyvinyl chloride pipe frame (2.54-cm diameter × 60-cm length). Ground deposit of naled residue was collected by filter paper (24 cm, Whatman). Two filter papers were placed side by side on a 40 × 80-cm styrofoam board covered with aluminum

foil. Filter papers were pinned on top of the aluminum foil and replaced after each test. Residue samples were collected 2 h after the aerial spray application to allow spray droplets to settle. Each yarn sample was then taken off the frame with forceps and carefully placed into a 40-ml Pyrex culture tube, which was filled with hexane, before placement into a refrigerator at 4°C. Each filter paper was removed from the styrofoam board, folded with 2 forceps, and placed into a 40-ml Pyrex culture tube. Each tube was filled with 30 ml of hexane immediately after sample collection. All samples were placed in a cooler with ice substitute packs and held at 4°C. Quality control of residue recovery in field samples was conducted by spiking 50-μl naled standards (1 mg/ml) to clean yarn and filter papers at the time of aerial spray. Hexane was added to all field and spiked samples and transported to the laboratory at PHEREC for naled residue analysis.

Statistical analysis: Mean values of naled residues were analyzed by analysis of variance (PROC GLM, SAS Institute 1997). All data were subjected to a Student–Newman–Keuls test to determine significant ($P < 0.05$) differences among treatment means (Sokal and Rohlf 1981).

RESULTS

Stabilization of naled in hexane

Our minimum detection limit of naled in this method was 0.001 μg/ml and the quantifiable amount of naled was 0.01 μg/ml. At 4°C, naled residues on day 0 were significantly greater than on day 1 (Table 1). However, at 1 day of storage time, no significant difference occurred in residue recovery. The expected recovery of the naled spike standard was 6 μg/ml. The average percentage residue recovery during the first 3 days was 106% at day 0, 97% at day 1, 102% at day 2, and 101% at day 3, respectively, all within the recovery criteria of 100 ± 10%. The last day's residue (day 4) was 111%, which was 1% over the recovery limit. These results showed that naled was stable in hexane solvent.

In the 2nd experiment, recovery of naled residue was 5.95 ± 0.13 μg/ml on day 0 (equivalent to 99% recovery in room temperature without storage at 4°C; Table 2). No statistically significant differences were found between residue concentrations on days 1–4 when stored at 4°C (Table 2). The percentage average residue recovery at this temperature was 108, 100, 102, and 100% at days 1–4, respectively.

Efficiency of naled extraction

Naled recovered from the filter paper ranged from 82 to 94% and no significant differences were observed in residue recovery during the 5-day storage period (Table 1). Residue recovery of naled was 5.65 ± 0.15 μg immediately after extraction of the spike and filter paper (Table 1), equivalent to 94% recovery. Recovery was 82, 86, 83, and 92%, respectively, after 1–4 days of storage at 4°C. Naled recoveries from filter paper spike samples in the 2nd week's experiment were not significantly different from each other after being stored 1–5 days

Table 1. Comparative stabilization of naled residue during 5 days of exposure and storage, which includes outdoor exposure without hexane (day 0), indoor storage at room temperature with hexane (days 1–3 as indicated), and indoor storage at room temperature and at 4°C with hexane.

Treatments	*n*	Mean and standard error of naled residue after days stored at 4°C[1]				
		Day 0	Day 1	Day 2	Day 3	Day 4
Hexane spike	20	6.37 ± 0.06a	5.84 ± 0.16b	6.17 ± 0.09ab	6.02 ± 0.24ab	6.65 ± 0.26ab
Filter paper spike without exposure	20	5.65 ± 0.15a	4.95 ± 0.11a	5.09 ± 0.23a	4.91 ± 0.29a	5.53 ± 0.19a
Filter paper spike with 1 day field exposure	16	Outdoor exposure	2.96 ± 0.06b	2.95 ± 0.11b	3.06 ± 0.07b	3.44 ± 0.08a
Filter paper spike with one day of field exposure and one day indoors with hexane	12	Outdoor exposure	Indoors with hexane	2.97 ± 0.13a	3.06 ± 0.07a	3.34 ± 0.08a
Filter paper spike with 1 day of field exposure and 2 days indoors with hexane	8	Outdoor exposure	Indoors with hexane	Indoors with hexane	2.84 ± 0.06a	3.04 ± 0.06a
Filter paper spike with 1 day of field exposure and 3 days indoors with hexane	4	Outdoor exposure	Indoors with hexane	Indoors with hexane	Indoors with hexane	2.97 ± 0.02

[1] Means within each row followed by the same letter are not significantly different ($P > 0.05$) with Student–Newman–Keuls mean separation test (Sokal and Rohlf 1981).

Table 2. Comparative stabilization of naled residue with and without exposure. The samples were stored at 4°C with hexane after extraction.

Treatments	*n*	Mean and standard error of naled residue after days stored at 4°C[1]				
		Day 0	Day 1	Day 2	Day 3	Day 4
Hexane spike	20	5.95 ± 0.13a	6.47 ± 0.13a	6.01 ± 0.11a	6.14 ± 0.34a	6.02 ± 0.16a
Filter paper spike without exposure	20	4.81 ± 0.12a	5.38 ± 0.12a	5.01 ± 0.11a	5.26 ± 0.26a	5.30 ± 0.10a
Filter paper spike with 1 day of field exposure	16	Outdoor exposure	1.87 ± 0.07a	1.81 ± 0.06a	1.84 ± 0.08a	1.84 ± 0.05a
Filter paper spike with 2 days of field exposure	12	Outdoor exposure	Outdoor exposure	1.06 ± 0.04a	1.12 ± 0.05a	1.13 ± 0.03a
Filter paper spike with 3 days of field exposure	8	Outdoor exposure	Outdoor exposure	Outdoor exposure	0.71 ± 0.02a	0.70 ± 0.01a
Filter paper spike with 4 days of field exposure	4	Outdoor exposure	Outdoor exposure	Outdoor exposure	Outdoor exposure	0.75 ± 0.02

[1] Means within each row followed by the same letter are not significantly different ($P > 0.05$) with Student–Newman–Keuls mean separation test (Sokal and Rohlf 1981).

(Table 2). Immediately after spike extraction, followed by drying the filter paper, recovered naled residue was 4.81 ± 0.12 μg (Table 2), equivalent to 80% recovery. Residue recoveries at days 1–4 were 90, 84, 88, and 88%, respectively.

Fourteen-hour overnight field exposure of naled

During the 1st experiment, naled residue recovered from filter paper exposed for 14 h overnight was 2.96 ± 0.06 μg/ml (Table 1), which was equivalent to 49% recovery of the spike concentration under 68% relative humidity. We noticed that the filter papers were relatively dry at the time of collection. Samples showed no significant difference in residue recovery after extracts stored at 4°C. The results of residue recovery were 2.95 ± 0.11, 3.06 ± 0.07, and 3.44 ± 0.08 μg/ml (Table 1), respectively, after 1–3 days of storage at 4°C; this was equivalent to 50, 52, and 57% recovery of the spike, respectively. In the 2nd week's test, average naled residue from filter papers was 1.87 ± 0.07 μg/ml (Table 2), equivalent to 31% recovery of the spike concentration under 76% relative humidity after a 14-h overnight field exposure. At that time we noticed that filter papers were wet at the time of collection. The results did not show significant loss of residue when stored at 4°C (Table 2). Residue recoveries were 30, 31, and 31% after 2, 3, and 4 days, respectively, of storage in hexane solvent.

Comparison of naled degradation in 2 scenarios

Naled was unstable in the field environment when compared with storage in hexane. Analysis of the data showed significant residue reduction due to field exposure. After the 14-h overnight exposure in the field, we recovered 3.44 ± 0.08 μg/ml, equivalent to 57% recovery, during the 1st week of test and 1.84 ± 0.05 μg/ml, equivalent to 31% recovery, during the 2nd week of test (Table 3). Residue recovery was stabilized as soon as samples were placed in hexane (Table 3). Indeed, residue recovery seemed to decline as long as naled was exposed in the field (Table 3).

Table 3. Comparative stabilization of naled degradation under 2 different scenarios: 3 days of indoor storage with hexane after a 1-day outdoor exposure; and all 4 days of exposure conducted outdoors.

Treatments	*n*	Mean and standard error of naled residue degradation after days stored at 4°C[1]					
		Hexane spike	Filter paper spike	Day 1	Day 2	Day 3	Day 4
Week 1: 3 days (day 2, 3, 4) of indoor exposure with hexane	24	6.65 ± 0.26a	5.53 ± 0.19b	3.44 ± 0.08c	3.34 ± 0.08c	3.05 ± 0.06c	2.97 ± 0.02c
Week 2: all exposures outdoors without hexane	24	6.01 ± 0.16a	5.31 ± 0.10b	1.84 ± 0.05c	1.13 ± 0.03d	0.70 ± 0.01e	0.76 ± 0.02e

[1] Means within each row followed by the same letter are not significantly different ($P > 0.05$) with Student–Newman–Keuls mean separation test (Sokal and Rohlf 1981).

Table 4. Naled residue recovered from filter paper and yarn during a field test on June 23, 1999, at Palmetto, FL.

Sampling location	Ground deposit		Drift through air (μg/yarn)
	μg/ filter paper	μg/m²	
Site 1, at edge of forest	30.27	669	28.18
Site 1, at edge of forest	36.97	817	25.83
Site 1, inside forest	19.28	426	12.72
Site 1, inside forest	16.89	373	11.28
Site 1, open field (weather station)	55.94	1,237	73.77
Site 1, open field (weather station)	55.95	1,237	—
Site 2, at edge of forest	33.06	731	14.24
Site 2, at edge of forest	37.57	831	12.44
Site 2, open field	173.36	3,833	27.61
Site 2, open field	154.35	3,413	22.51

Validation of the method by determination of naled from field samples

The range of residue deposition on filter paper was from 373 to 3,833 μg/m² (Table 4). Greater amounts of naled residues were found from filter papers placed in the open area (1,237–3,833 μg/m²) than at the edges of the forest (669–831 μg/m²). The least naled was found on the filter paper placed inside the woods (373–426 μg/m²; Table 4.). Naled concentrations on yarn ranged from 11 to 74μg/ yarn. More naled was found on the yarn placed in an open area (Table 4). The field spike recovery was 92 and 79% for yarn and filter paper, respectively.

DISCUSSION

Naled was found to be unstable in the field environment. About 50–70% of the residue was lost during 14 h in the overnight assays. We do not agree that extracting samples into an organic solvent and evaporating to dryness will obtain optimal recoveries, as stated by Hall et al. (1997). To obtain the best residue recovery, we recommend extracting samples into hexane as soon as samples are collected from the field. The naled extract in hexane can be directly injected into a GC. Naled was stable in hexane solvent for at least 1 wk and perhaps longer (Hall et al. 1997). We have demonstrated that hexane can be used to preserve naled samples and prevent further loss of the residue after sampling. Storage of naled samples in hexane allowed more time for sample shipment and residue analysis. We found no significant difference in naled residue recovery from samples stored at room temperature vs. at 4°C as long as samples were stored in hexane.

Similarly, we found that naled residues degraded rapidly on filter paper under the influence of relative humidity and sunlight (ultraviolet radiation), as reported by Tietze et al. (1996). However, we found that the rate of naled degradation at our test conditions was slower than those authors stated. The significant reduction of naled residue recovery from 57% in the 1st week's assay to 31% in the 2nd week may be due to the changes in relative humidity from 68 to 76%. If this is true, hydrolysis may play an important role in naled degradation. We hypothesize that the percentage of residue recovered in our last sample after day 4 (0.71 μg/ml) and day 5 (0.70 μg/ml) of field exposure was at the same level, which suggests that some of the naled might bind to filter paper and then is released by hexane extraction, although this remains to be determined.

Analysis of the data demonstrated that our rapid extraction method was effective in recovering naled from both filter paper and yarn. Excellent residue recovery was obtained by direct hexane solvent extraction. Our sample collection and preservation protocol was able to recover naled residue from filter paper and yarn at 2 spray sites after aerial naled ULV application. During development of the method, we recovered only 10–20% of naled residue by evaporating 10-ml hexane extracts to dryness with a gentle helium breeze under a hood and resuspension with 1 ml of hexane. Therefore, we do not recommend extracting of naled residue by evaporation to dryness as stated by Hall et al. (1997). We found that the naled residue is stable in hexane solvent under both room temperature and at 4°C for at least 1 wk.

In conclusion, we have developed and validated a simple but effective method to reliably recover naled residues without the confounding effects attributed to rapid degradation. This method should allow more reliable determination of residue deposits of this adulticide after aerial ULV application to maximize effectiveness against mosquitoes while minimizing impacts to nontarget species.

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