

MEASURING HYDRAMETHYLNON RESISTANCE IN THE GERMAN COCKROACH, *BLATTELLA GERMANICA* (L.)

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Abstract—In a field population (RHA, Roanoke VA, USA) of *Blattella germanica* a decline in the efficacy of hydramethylnon has been documented for eight years. In 1987 >85% reduction of apartment infestations was achieved 4 wk after application, in 1990 reduction declined to 73%, and in 1995 reduction declined to 60%. The susceptibility to five concentrations of hydramethylnon in RHA was compared to a susceptible strain (VPI). The RHA strain demonstrated a low to moderate level of resistance to hydramethylnon.

Delivery of an equal dose of insecticide is important when using a feeding bioassay to determine resistance to insecticides formulated as baits. Resistance ratios at KT_{50} or KT_{90} based on feeding may accurately depict resistance at concentrations of hydramethylnon that provide for a nearly equal amount of bait consumed by the field and susceptible strain. Following starvation there may be a nearly equal amount of bait and active ingredient consumed so that RRs based on LT_{50} s accurately depict the level of resistance. Bioassays based on topical application were free of problems associated with feeding. A discriminating dose of 5 μ g provided consistent RRs based on LT_{50} and KT_{50} estimates

INTRODUCTION

Insecticide resistance in laboratory and field strains of the German cockroach, *Blattella germanica* (L.), has been documented for more than 40 years. The history of resistance in this pest follows closely the emergence of modern household insecticides. As new classes of chemicals became available they were adapted for cockroach control. However, each replacement for an insecticide to which *B. germanica* had developed resistance soon experienced the same problem. This succession began with resistance to chlorinated hydrocarbons in the United States (Heal *et al.*, 1953), and was later reported in other countries (Green *et al.*, 1961; Yasutomi *et al.*, 1966; Privora, 1972). Resistance in field populations has been reported for pyrethrins (Keller *et al.*, 1956), organophosphates, carbamates (Bennett and Spink, 1968, Barson and McCheyne, 1978; Nelson and Wood, 1982; Rust and Reiersen, 1991; Chapman *et al.*, 1993), and pyrethroids (Zhai and Robinson, 1991; Atkinson *et al.*, 1991). Recently, resistance has been reported for pesticides used in baits, including sulfluramid (Schal, 1992) and hydramethylnon (Koehler and Patterson, 1991).

Although the use of toxic baits for domestic and peridomestic cockroach control predates the availability of synthetic insecticides, the effectiveness of early baits was limited. The incorporation of modern insecticides into baits improved control, especially for *B. germanica*. Recent improvements in the toxicants, bait matrix, and application methods have made baits a reliable control strategy (Rust *et al.*, 1983). The effective field performance and tamper-proof delivery system of hydramethylnon-based baits, Combat and Maxforce (The Clorox Co., Pleasanton, CA) led to extensive professional and home use of these products (Milio *et al.*, 1986; Appel, 1990). An inevitable consequence of this use is the development of hydramethylnon resistance in field populations.

Reports of reductions in control of field populations by hydramethylnon were followed by laboratory evaluations of physiological and behavioral resistance to this insecticide (Koehler and Patterson, 1991; Silverman and Bieman, 1993). Confirmation of reduced efficacy, based on percentage reduction of infestations, with resistance ratios based on laboratory evaluations is important to detecting and monitoring resistance in *B. germanica*. However, the methods commonly used for measuring physiological resistance to insecticides applied as liquids may not be suitable for those applied as a bait. Methods used to measure resistance to insecticides formulated as baits may be influenced by such factors as strain differences in feeding habits, food preferences, and behavioral responses to bait ingredients (Reiersen and Rust, 1984; Sparks *et al.*, 1989).

The objectives of the research reported here were to compare the sensitivity of three methods used to detect and quantify hydramethylnon resistance in *B. germanica*: percentage reduction of field populations; ingestion to estimate LT_{50} s and KT_{50} s and determining RRs, and topical application to estimate KT_{50} s and LT_{50} s and determining RRs.

MATERIALS AND METHODS

Cockroaches

A susceptible-strain (VPI) German cockroach was used as a standard to determine resistance ratios (RR). The RHA field-strain cockroaches were collected in 1995 from Lincoln Terrace apartments, which are located in Roanoke, VA, USA. At the time of this study RHA had moderate-level resistance to cypermethrin (LD_{50} RR=21) and chlorpyrifos (LT_{50} RR=1.8) (Zhai and Robinson, 1996), and RHA had been exposed to hydramethylnon primarily through consumer-based products (Combat) since 1985. RHA were used in bioassay evaluations within 14 d after removal from the field. The SHA field strain originated from Shanghai, China in 1993, and is maintained as a laboratory colony. This field strain has moderate-level resistance to cypermethrin (LD_{50} RR=14) and chlorpyrifos (LT_{50} RR=1.4) (unpublished data), but has no known exposure to hydramethylnon. All laboratory strain cockroaches were provided rat chow (Mouse/Rat Diet 7001, Teklab, Madison, WI) and maintained at 22–24°C, 55–60% RH, and constant light. Adult male cockroaches were used in all evaluations.

Study Site

Field efficacy of hydramethylnon was evaluated in Lincoln Terrace apartments, which are one- to three-bedroom apartments grouped in 76 buildings with 4 or 6 apartments each. Professional pest control is provided 1–3 times per yr, and consists of liquid insecticide applied with a compressed-air sprayer to kitchens and bathrooms.

Field Efficacy

Field evaluations were performed during 1987, 1990, 1994, and 1995. Apartments were selected on the basis of a total of at least 25 adults and nymphs collected in 24 h on three sticky traps (Mr. Sticky, Long Island, NY) placed in the kitchen and bathroom. The mean number of cockroaches trapped per apartment before treatments was: 87, 1987; 48, 1990; 100, 1994; 57, 1995. A minimum of 10 apartments were used for each evaluation.

Apartments were treated with hydramethylnon bait applied as a dry matrix (1.65% AI) enclosed in plastic stations at the rate of 12 stations (18 g bait) per apartment, or as a gel (2.15% AI) applied in 0.06 g doses to crevices at the rate of approximately 125 doses (7.5 g bait) per apartment. In apartments treated with bait stations, eleven were placed in cabinets and under the refrigerator in the kitchen, and one behind the toilet in the bathroom. In apartments treated with gel baits, doses were applied to crevices at or near the same locations used for the bait stations. The sampling locations for the sticky traps and placement of insecticide stations and gel were nearly identical for all apartments for all years.

Sticky traps were used to establish a pre- and post-treatment assessment of the cockroach infestation in each apartment; percentage reductions for all evaluations were determined 4 wk post-treatment. The method of determining percentage reduction has been described by Zhai and Robinson (1991). Briefly, the number of cockroaches collected in 24 h in sticky traps placed in apartments 4 wk post-treatment was subtracted from the number collected in a 24 h period before treatment; this number was divided by the number trapped before treatment, and then multiplied by 100.

Bait Consumption

Nontoxic bait. The amount of nontoxic bait consumed by VPI, RHA, and SHA was determined using SG gel baits formulated without hydramethylnon (American Cyanamid, Wayne, NJ). Cockroaches (n=30) were placed in 3 covered 0.9 l jars with water and 0.5 g of bait in an uncovered

plastic cap. The shape of the cap (22 mm high, 19 mm dia, and 11 mm deep) permitted cockroaches access to the bait with minimal tarsal contact. The amount of bait consumed daily and cumulatively was determined by weighing the cap containing the bait, then adjusting for the weight gained or lost by the control.

Toxic bait. The amount of toxic bait consumed by the VPI, RHA, and SHA was determined using dilutions of nontoxic and toxic formulations of the SG (2.0% AI), and MF gel baits (2.15% AI), and MF dry bait (1.65% AI) (The Clorox Co., Pleasanton, CA) diluted with rat chow to achieve hydramethylnon concentrations of 0.46% and 0.13%. To evaluate feeding deterrence of hydramethylnon, feeding on the baits with different concentrations of insecticide was compared to feeding on the same bait without insecticide. The methods and materials used for delivering and weighing the bait were the same as for the nontoxic baits.

To determine the amount of dry or gel bait consumed, the percentage gain or loss of weight by representative baits maintained as control was incorporated into all measurements. The weight of the bait at the beginning of the test was recorded, then daily weights were obtained, and the final weight was adjusted by the percentage gain or loss in the control. The mean insecticide dose ingested per cockroach with the bait was calculated by re-adjusting the cumulative bait consumption to the equivalent weight at the beginning when the insecticide concentration was known.

Bioassay

Knockdown (KT) and death (LD, LT) were considered distinct responses to the insecticide. The criteria for knockdown were the inability of a cockroach to right itself when on its back, and able to walk when turned on its legs. The cockroach was considered dead when it was unable to walk in response to probing when turned on its legs.

Feeding. The bioassay based on feeding was conducted with SG gel bait and MF gel bait diluted with the identical nontoxic bait formulation to achieve hydramethylnon concentrations of 1.0, 0.5, 0.25, 0.12, and 0.06%, and with a dry bait diluted with rat chow to achieve concentrations of 0.46% and 0.13%. KT_{50} s (days) were estimated by confining 30 VPI and RHA to three 0.9 l glass jars with approximately 0.5 g of bait, and water. The toxic effect was recorded every 6 h for 3 d following the first day of exposure, then recorded daily until 90% were killed in the highest two consecutive concentrations, or until there was no change in the cumulative mortality for three consecutive days in the lower concentrations. Prior to the feeding bioassay using dry bait the cockroaches were removed from food but not water for 48 h. Following starvation the toxic bait was provided for 24 h, then replaced with rat chow.

Topical. The topical application bioassay to estimate LT_{50} and KT_{50} was conducted with technical hydramethylnon (99.0% [AI], Chem Service Inc., West Chester) dissolved in acetone to achieve four doses (2, 3, 4, 5 μ g), and applied to the ventral mesothorax of 30 VPI, RHA, and SHA using a calibrated, automatic microapplicator (Burkard Manufact. Co., Herts, UK). Following topical application of 1, 2, 3, and 5 μ g of hydramethylnon RHA and VPI ($n=10$) were provided rat chow as food. The total amount (mg) of food consumed after 7 d and the amount (μ g) per cockroach were determined.

End point toxicity. The end point of toxicity is when more than 90% of the cockroaches were knocked down, or when no additional cockroaches appeared poisoned for three days, and the cumulative mortality was unchanged.

Data Analysis

Data were subjected to probit analysis (SAS Institute, 1982, $n=30$ insects per test). Resistance ratios (RR) were calculated by the formula: LT or KT RHA or SHA / LT or KT VPI.

RESULTS

Field Efficacy

The percentage reduction of infestations of RHA in Lincoln Terrace apartments achieved by hydramethylnon baits declined over the nine year period (1987–1995) of evaluation (Table 1).

Application of the dry bait achieved 95% (range 90–100%) reduction in 1987, 86% (range 78–100%) in 1990, and 76% (range 28–98%) reduction in 1994. Application of the gel resulted in 60% (range 18–100%) reduction in 1995.

Bait Consumption

Non toxic bait. There were distinct differences in the total amount of nontoxic SG bait consumed by the three strains during the 9-day period (Table 2). SHA consistently consumed more bait on daily basis than either VPI or RHA. Total bait consumption by VPI (220.2 mg) was more than 10% less than RHA (262.2 mg) and SHA (292.3 mg).

Toxic bait. The amount of toxic SG gel bait consumed at the end of a 7-day period increased as the hydramethylnon concentration decreased from 1.0% to 0.12% (Table 3). The largest amounts of bait consumed per cockroach were at the 0.25 and 0.12% concentrations. VPI consumed approximately 10% more of the toxic bait than RHA.

Table 1. Percentage reduction of German cockroach infestations in apartments 4 wk after treatment with hydramethylnon dry or gel baits.

Year	Precount ¹	Percentage Reduction	Range
1987	86	95	90–100
1990	48	86	78–100
1994	100	76	28–98
1995	57	60	18–10

¹ Mean number of cockroaches present in the apartments before treatment.

Table 2. Amount (mg) of nontoxic SG gel bait consumed by laboratory (VPI) and field strains (RHA, SHA) of German cockroach males (n=30) during a consecutive 10 days.

Day	VPI	RHA	SHA
1–3	98.7	128.9	104.6
4	20.6	19.6	28.1
5	11.4	13.8	29.6
6	21.3	21.5	38.9
7	26.5	33.1	30.2
8	23.9	23.1	29.4
9	18.2	22.2	31.5
Total	220.6	262.2	292.3

Table 3. Total amount (mg) of SG gel bait containing four concentrations of hydramethylnon consumed by VPI and RHA cockroaches (n=30) in seven days.

% Conc.	Total amount (mg) consumed		\bar{x} mg/cockroach	
	VPI	RHA	VPI	RHA
1.0	38.2*	10.7*	1.27	0.54
0.5	45.5	24.0	1.51	1.20
0.25	62.6	52.9	2.08	1.76
0.12	92.4	77.3	3.07	2.57

*20 cockroaches tested for each strain

Bioassay

Feeding. At the 0.06% and 0.12% concentration SG gel bait the KT_{50} s for RHA were 7.74 and 4.94, and for VPI they were 5.48 and 3.52, respectively; the RR at KT_{50} was 1.4 for both concentrations (Table 4). Using the 0.13% MF dry bait the LT_{50} s for RHA and VPI were 4.10 and 3.06, respectively; and the RR at LT_{50} was 1.3 (Table 5). The total consumption of 0.13% dry bait for VPI and RHA was 3.5 and 4.0 mg, and the hydramethylnon dose ingested per cockroach was 4.5 and 5.2 μ g, respectively. The LT_{50} estimates using 0.46% dry bait were not significantly different based on overlap of the 95% CI. The total consumption of 0.46% bait for VPI and RHA was 4.62 and 4.82 mg, and the dose ingested per cockroach was 21.3 and 22.2 μ g, respectively. The KT_{50} - and KT_{90} estimates using the MF toxic gel bait were not significantly different at all concentrations based on overlap of the 95% CI (Table 6). The amount of bait VPI and RHA consumed per cockroach was variable at these concentrations and may have resulted in unequal doses of insecticide, and ingestion may have occurred at different times.

Topical. LT_{50} s and KT_{50} s derived from the 5 μ g per cockroach dose provided a reliable estimate of the resistance ratio (Tables 7, 8). At the lower rates (1–4 μ g) the LT_{50} estimates were less reliable because the time to achieve LT_{50} for RHA was more than 14 d. The LT_{50} and LT_{90} estimates for VPI were 5.31 and 7.39, and for RHA they were 8.24 and 13.5; the RRs at LT_{50} and LT_{90} were 1.5 and 1.8, respectively. The KT_{50} and KT_{90} estimates for VPI were 4.66 and 6.33, and for RHA they

Table 4. KT_{50} s and KT_{90} s (days) for VPI and RHA fed five concentrations of hydramethylnon SG gel bait in nonchoice tests.

% Conc	KT_{50} (95% CI) ¹	RR	KT_{90} (95% CI) ¹	Slope (SEM)	RR
VPI					
1.0	2.24 (2.33–2.62) ^a	–	2.83 (2.51–3.25) ^b	18.7 (2.5)	–
0.5	2.66 (2.51–2.83)	–	3.19 (2.92–3.67)	16.4 (2.1)	–
0.25	3.13 (2.92–3.36)	–	4.31 (3.81–5.49)	9.2 (1.1)	–
0.12	3.52 (3.23–3.85)	–	5.59 (4.64–8.00) ^c	6.4 (0.9)	–
0.06	5.48 (4.94–6.08)	–	8.19 (6.62–12.0) ^d	7.3 (1.0)	–
RHA					
1.0	2.44 (2.14–2.78) ^a	1.0	3.35 (2.51–4.68) ^b	9.3 (1.8)	1.0
0.5	3.23 (2.92–3.59)	1.2	6.03 (4.60–10.4)	4.7 (0.7)	1.9
0.25	4.23 (3.64–4.92)	1.3	8.80 (5.84–19.5)	4.0 (0.6)	2.0
0.12	4.94 (4.15–5.89)	1.4	10.4 (6.60–27.4) ^c	3.9 (0.7)	1.9
0.06	7.74 (7.23–8.29)	1.4	11.2 (9.92–14.7) ^d	7.8 (0.8)	1.3

¹Lethal knockdown (days) estimates followed by the same letter are not significantly different based on overlap of 95% CI.

Table 5. LT_{90} s and LT_{90} s (days) for VPI and RHA cockroaches starved for 48 h then fed MF dry bait diluted with rat chow to achieve two concentrations of hydramethylnon in non-choice tests for 24 h.

% Conc.	LT_{50} (95% CI) ¹	RR	LT_{90} (95% CI) ¹	Slope (SEM)	RR
VPI					
0.46	2.58 (2.02–3.30) ^a	–	3.60 (2.86–4.70) ^b	8.8 (2.2)	–
0.13	3.06 (2.73–3.43)	–	4.68 (3.97–6.29) ^c	6.9 (1.1)	–
RHA					
0.46	2.95 (2.95–3.36) ^a	1.1	4.32 (3.56–5.86) ^b	7.7 (1.4)	1.4
0.13	4.10 (3.75–4.49)	1.3	6.01 (4.90–8.69) ^c	7.7 (1.1)	1.3

¹Lethal time (days) estimates followed by the same letter are not significantly different based on overlap of 95% CI.

Table 6. KT_{50} s and KT_{90} s (days) for German cockroaches MF toxic gel bait diluted nontoxic gel to achieve four concentrations of hydramethylnon in non-choice tests.¹

% Conc.	KT_{50} (95% CI) ²	RR	KT_{90} (95% CI) ²	Slope (SEM)	RR
VPI					
0.47	2.87 (2.57–3.20) ^a	–	4.49 (3.77–6.17) ^e	6.60 (0.96)	–
0.23	3.24 (2.95–3.56) ^b	–	4.42 (3.77–5.67) ^f	9.51 (1.50)	–
0.12	3.69 (3.44–3.96) ^c	–	4.65 (4.10–5.65) ^g	12.8 (1.73)	–
0.06	4.32 (3.99–4.68) ^d	–	5.59 (4.81–7.07) ^h	11.4 (1.64)	–
RHA					
0.47	2.71 (2.43–3.02) ^a	0.94	3.80 (3.24–4.86) ^e	8.76 (1.28)	0.85
0.23	3.23 (2.90–3.59) ^b	1.00	4.66 (3.72–6.65) ^f	8.02 (1.30)	1.05
0.12	3.60 (3.33–3.90) ^c	0.98	4.69 (4.07–5.86) ^g	11.2 (1.57)	1.01
0.06	4.42 (4.03–4.84) ^d	1.02	5.99 (4.97–8.01) ^h	9.73 (1.47)	1.07

¹Insecticide dose (μ g) per cockroach, VPI: 0.47%=34.7, 0.23%=10.7, 0.12%=7.4, 0.06%=3.0; RHA: 0.47%=31.6, 0.23%=11.4, 0.12%=11.8, 0.06%=5.1

²Lethal knockdown (days) estimates followed by the same letter are not significantly different based on overlap of 95% CI.

Table 7. LT_{50} s and LT_{90} s (days) for VPI and RHA cockroach strains treated topically with hydramethylnon at the rate of 5 μ g per cockroach.

Strain	LT_{50} (95% CI)	RR	LT_{90} (95% CI) ¹	Slope (SEM)	RR
VPI	5.31 (4.87–5.78)	–	7.39 (6.46–9.39) ^a	8.94 (1.11)	–
RHA	8.24 (7.52–9.04)	1.5	13.5 (11.9–20.1) ^b	5.92 (0.77)	1.8
SHA	6.42 (5.80–7.11) ^a	1.2	10.6 (8.62–16.2) ^{ab}	5.80 (0.78)	1.4

¹Lethal time (days) estimates followed by the same letter are not significantly different based on overlap of 95% CI.

Table 8. KT_{50} s and KT_{90} s (days) for VPI and RHA cockroach strains treated topically with hydramethylnon at the rate of 5 μ g per cockroach.

Strain	LT_{50} (95% CI) ¹	RR	LT_{90} (95% CI) ¹	Slope (SEM)	RR
VPI	4.66 (4.21–5.15) ^a	–	6.33 (5.45–8.04) ^b	9.60 (1.28)	–
RHA	7.07 (6.41–7.81)	1.5	12.2 (9.98–18.4) ^c	5.42 (0.66)	1.9
SHA	5.31 (4.67–6.04) ^a	1.1	10.2 (7.72–17.3) ^{bc}	4.55 (0.63)	1.6

¹Lethal time (days) estimates followed by the same letter are not significantly different based on overlap of 95% CI.

were 7.07 and 12.2; the RRs at KT_{50} and KT_{90} were 1.5 and 1.9, respectively. The LT and KT estimates for SHA were not significantly different from VPI based on the overlap of the 95% CI.

There were significant differences in the amount of rat chow consumed in 7 d by VPI and RHA receiving the 1 μ g and 2 μ g hydramethylnon as topical applications. VPI treated with 1, 2, and 3 μ g consumed a total of 68.9 mg (6.8 μ g per cockroach), 32.7 mg (5.0 μ g per cockroach), and 32.7 mg (3.8 μ g per cockroach), respectively. RHA treated with 1, 2, and 5 μ g consumed a total of 14.4 mg (1.9 μ g per cockroach), 4.8 mg (0.6 μ g per cockroach), and 3.9 mg (0.4 μ g per cockroach), respectively.

DISCUSSION

The decline in percentage reduction of RHA apartment infestations achieved by hydramethylnon is attributed to a gradual increase in resistance to this insecticide in the Lincoln Terrace German cockroach population. Resistance is confirmed by the results of the feeding and topical bioassays which indicate a RR at LT_{50} of 1.4–1.5. This level of resistance may be the result of the availability and frequent use of (Combat) bait stations by the tenants. Although hydramethylnon baits became available to consumers in 1985, there was only limited use of this product in Lincoln Terrace prior to 1987 (personal observation) when 95% reduction was achieved. Continued and perhaps increased use of this effective control method between 1987 and 1995 may have selected for resistance in RHA, which is evident in the 86% (1990) to 60% (1995) decline in efficacy. Koehler and Patterson (1991) reported 50–60% reduction of apartment infestations after about three years of hydramethylnon use; and they reported low-level resistance (LT_{50} RR=1.4) in the field strain.

Failure of insecticides to manage or control field populations is often the first indication of resistance in *B. germanica* (Heal *et al.*, 1953; Gradidge, 1960; Zhai and Robinson, 1990; Koehler and Patterson, 1991). Although control failure is an important component of studies of putative insecticide resistance (Ball, 1981), resistance ratios obtained from bioassays of field-collected individuals are necessary to determine the magnitude of the problem (Green *et al.*, 1961; Zhai and Robinson, 1991). Methods used to measure physiological resistance to hydramethylnon include feeding and topical bioassay; however, the relatively slow action of this insecticide (Hollingshaus, 1987), and the behavior aspects of feeding and bait consumption may influence the KT_{50} and LT_{50} estimates and the resistance ratios. Confirmation of resistance with feeding bioassays may require preliminary evaluations of bait consumption by both the susceptible and field strain.

VPI consumed significantly less of the nontoxic gel bait than the two field strains. These overall differences in feeding (amount consumed) between laboratory and field strains may be the result of differences associated with field and laboratory populations. The mass rearing conditions which include abundant food adjacent to the harborage, and the long (190 generations) colonization history of VPI may have selected for reduced feeding. Other examples of differences between VPI and RHA include reduced movement in the presence or absence of insecticide residues (Akers and Robinson, 1989; Zhai and Robinson, 1991), and in the frequency of antennal and leg grooming (Zhai and Robinson 1996; Robinson, 1996). There may be similar differences in other susceptible and field strains.

The influence of hydramethylnon on deterring consumption of the SG gel bait is indicated by the reduced amount of bait consumed by VPI and RHA during the 7-day exposure to four concentrations. Unlike consumption of nontoxic bait in which RHA regularly consumed more than VPI, when exposed to toxic bait RHA consumed less than VPI at all concentrations tested. A similar reduced-feeding response to hydramethylnon by RHA was shown after exposure to topical applications. For example, during the 7 days following the topical application of 2 μ g hydramethylnon VPI consumed a total of 32.7 mg (5.0 μ g per cockroach) of untreated rat chow, while RHA consumed only 4.8 mg (0.6 μ g per cockroach).

Apparently, RHA is capable of detecting and reducing food consumption when exposed to concentrations of hydramethylnon >0.12%. This stimulus-dependent behavior response in RHA may be an important component, along with physiological mechanisms, of hydramethylnon resistance in RHA. Reduced feeding on hydramethylnon bait would limit the amount of toxicant delivered to the insect and delay the effect of the toxic dose. At low (<0.12%) concentrations the cockroach consumes only a small quantity and a sublethal dose of the toxic bait before detecting the insecticide. After detecting the presence of the insecticide it may seek an alternative source of food in the household environment and avoid further contact with the toxic bait. However, limited exposure to hydramethylnon results in reduced feeding of nontoxic (alternative) food. Both oral and topical exposure to hydramethylnon significantly reduced the feeding of RHA compared to VPI on either toxic or untreated (hydramethylnon) nontoxic food.

The behavioral component of reduced feeding in hydramethylnon resistance in the German cockroach may limit the use of feeding bioassays to determine LT - or KT_{50} RRs. The dose of insecticide delivered to susceptible and field strain cockroaches during a feeding bioassay may vary according to concentration and the amount of bait consumed. Unequal doses delivered to the field and susceptible strain may influence the LT - and KT_{50} estimates. However, starving the

cockroaches for 48 h before providing the toxic bait decreased strain and behavioral differences in feeding. Starvation also assured better timing of delivery of the toxicant to both strains, since both RHA and VPI begin feeding almost immediately after exposure to the bait. For example, when exposed to the 0.13% MF dry bait following starvation the 24 h consumption per cockroach for VPI and RHA was 3.5 mg (4.5 μ g AI) and 4.0 mg (5.2 μ g AI), respectively; when exposed to 0.46% MF dry bait the consumption for VPI and RHA was 4.62 mg (21.3 μ g AI) and 4.82 mg (22.2 μ g AI), respectively. These data show that starving increased the potential of all cockroaches consuming nearly the same amount of bait and acquiring a nearly equal dose of insecticide.

The use of several concentrations of hydramethylnon in the feeding and topical bioassays was critical to obtaining accurate LT- and KT_{50} estimates. At low (<1%) concentrations there was a delayed response to the toxicant and the time to achieve LT- and KT_{50} s was not confined to a few hours or days, as is the case with high concentrations. Distinguishing between the conditions of knockdown and death at 6–8 h intervals during the first 24–48 h of feeding bioassays provides reliable estimates of KT_{50} and LT_{50} and LT_{90} s for hydramethylnon. The mode of action of this insecticide is slow but cumulative (Hollingshaus, 1987), with the result that cockroaches may be knocked down or die hourly during the 2 d following the first exposure to bait.

The 0.12% gel bait and the 0.13% dry bait provided consistent 1.3 RRs at KT_{50} and LT_{50} estimates. At these concentrations the hydramethylnon dose delivered to each strain was nearly equal. The RRs determined for RHA were similar (LT_{50} [days] RR=1.4) to those reported for a field population of German cockroaches with a history of exposure and control failure similar to RHA (Koehler and Patterson, 1991).

Bioassays based on topical application were free of problems associated with feeding, such as the delivery of an equal dose to all individuals, and the differences in excretion. The topical LT_{50} and KT_{50} RRs of 1.5 (see Tables 7 and 8) are consistent with the RRs derived from gel and dry baits (see Tables 4 and 5). The 5 μ g dose provided knockdown and mortality responses within 10 d, while the responses to 4, 3, and 2 μ g doses extended more than 21 d and may be impractical for bioassay. The 5 μ g dose may be considered a useful discriminating dose for estimating LT_{50} s and KT_{50} s and determining RRs for German cockroach strains suspected of hydramethylnon resistance.

CONCLUSIONS

Measuring insecticide resistance in field populations of German cockroaches includes using surface-contact methods (Keller *et al.*, 1956; Zhai and Robinson, 1992) to estimate KT_{50} s, and topical application methods (Scott, 1986) to estimate LC_{50} s. However, surface-contact methods may not accurately assess insecticide susceptibility in *B. germanica* (Milio *et al.*, 1984; Wadleigh *et al.*, 1987; Zhai and Robinson, 1992). The inability of surface-contact methods, such as the use of treated jars, to adequately depict susceptibility is due primarily to behavioral differences between laboratory (susceptible) and field strains of German cockroaches. Strain differences in movement, and subsequent unequal accumulation of insecticide on the tarsal pads, results in the surface-contact assays providing an over- or under-estimation of resistance (Zhai and Robinson, 1992a; 1992b). For most contact insecticides topical application methods provide the most accurate RRs.

Problems with the delivery of an equal dose may also occur when using a feeding bioassay to determine resistance to insecticides formulated as baits. Data presented here, and by others, indicate food consumption differences in German cockroach strains, and differences in feeding deterrence imparted by concentrations of insecticides in baits (Cochran, 1994). Our data indicate that RRs at KT_{50} or KT_{90} based on feeding may accurately depict resistance at concentrations of hydramethylnon that provide for a nearly equal amount of bait consumed by both strains. Consumption of equal quantities of toxic bait delivers an equal dose of insecticide within the same time period, and when this occurs the feeding bioassay may simulate a topical method. At low concentrations (<1%) and following starvation there may be a nearly equal amount of bait and active ingredient consumed by all strains, and the LT_{50} and KT_{50} estimates will be spread over several days. The use of high (>1%) concentrations of hydramethylnon in bioassays based on feeding may provide conflicting information on resistance because the response occurs within a short period of time, which makes KT_{50} and LT_{50} estimates difficult, and the dose consumed by the

strains may be unequal. For example, Schal (1992) reported hydramethylnon resistance (LT_{50} [days] $RR=1.1-1.4$) in laboratory-based field strains of German cockroach that had little or no history of exposure to this insecticide. The LT_{50} estimates were based on a feeding bioassay using only one concentration (1.65% AI) and without monitoring of consumption. In our study, the LT_{50} and KT_{50} estimates for SHA, which had no history of exposure to hydramethylnon, were not significantly different from VPI based on the overlap of the 95% CI. Koehler and Patterson (1991) also used several bait concentrations and reported no resistance in a laboratory-based field strain with no history of exposure to hydramethylnon. Feeding bioassays based on single concentrations of the toxicant may not provide sufficient data to determine resistance ratios, and resistance should be confirmed by field control data (Ball, 1981).

The behavioural component of insecticide resistance in German cockroaches has received little study but may be an important factor in field populations. Hostetler and Brenner (1994) hypothesized that pesticide selection led to high levels of physiological resistance in a field strain most likely because pesticide detection and avoidance requires the absorption of at least a small amount of pesticide. Resistance may not increase in a population if the cockroaches possess a stimulus-dependent behaviour that allows detection of the pesticide with little or no bodily contact. Our data indicate that the RHA strain is capable of detecting low concentrations of hydramethylnon in baits and subsequently reducing feeding in response to detection. This behavioral component of hydramethylnon resistance may limit the development of high levels of resistance to this insecticide if there is a discriminating concentration that limits selection. The capacity to detect and avoid hydramethylnon may set the level of physiological resistance in RHA (Hostetler and Brenner, 1994).

ACKNOWLEDGMENTS

We thank Robert A. Barlow (Urban Pest Control Research Center) for assistance in obtaining cockroach strains, insecticide evaluations, and data analysis.

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