[Jpn. J. Sanit. Zool. Vol. 46 No. 4 p. 397-403 1995]

Laboratory test on secondary killing effect of hydramethylnon by coprophagous uptake of *Blattella germanica*

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(Received: 13 July 1995; Accepted: 25 September 1995)

Key words: hydramethylnon, secondary killing effect, coprophagy, German cockroach.

Abstract: As it was reported that hydramethylnon was excreted in the feces of bait-fed German cockroaches before being killed and coprophagous uptake by other cockroaches resulted in high mortality, we observed how long such a secondary killing effect lasts and how effectively kill them in the laboratory condition. A group of 30 1st instar nymphs was exposed to the feces excreted by 25 male bait-fed cockroaches for 10 days and the mortality rate was counted, and new group was re-introduced with 10 days intervals until the mortality dropped to the control level. The mortality rate was 81.1% in average of 1st–5th tests, 61.5% in 6th–14th tests and 15.2% in 15th–18th tests, showing 57.6% (943 dead/1,636 exposed) of the cumulative mortality rate during 180 days of the study period. When 25 4th–5th instar nymphs were exposed to hydramethylnon instead of 25 males, the mortality rate was 79.5%, 37.8%, 17.9% in average of 1st–6th tests, 7th–13th tests and 14th–17th tests, respectively, showing 48.9% (799 dead/1,635 exposed) of the cumulative rate during 180 days of the study period.

Introduction

Since 1980s, the poison bait method has been widely applied for domestic cockroach control in Korea, and many different types of formulations and chemicals are available in market nowadays. Hydramethylnon is one of the most widely accepted chemicals in Korea, and it has completely different mode of action in toxicity from all other chemicals such as chlorinated hydrocarbon, organophosphate, carbamate and pyrethroid compounds.

Hydramethylnon is a new member of the amidinohydrazone class of insecticides (Lovell, 1979) and is selectively toxic to chewing and/or sponging insects by inhibiting mitochondrial electron transport (Hollingshaus and Little, 1984; Hollingshaus, 1987). Silverman *et al.* (1991) reported that considerable amount of hydramethylnon was excreted in the feces of all stages of bait-fed German cockroaches before being killed because of delayed activity of the chemical, and coprophagous uptake of hydramethylnon by other cockroaches resulted in high mortality.

We observed how long such a secondary killing effect by coprophagous uptake lasts through other individuals and how effectively kill them in the laboratory condition.

Materials and Methods

Test room. Tests were carried out in an insectary under the standard conditions of 25°C and 55% relative humidity, with a light : dark cycle of 14 : 10 hr.

Arena. A plastic box with a tight lid, sized 33 cm wide × 53 cm long × 17 cm high was used for the arena. All upper sides of the arena were greased about 5 cm deep with a thin even coat of the vaseline and mineral oil mixture, making sure to miss any spots to prevent cockroaches escaping. A wide opening (15×30 cm) covered with fine muslin net were made on the lid for ventilation. A small entrance on a edge of the paper cup was cut and put it upside-down at a corner of the arena for providing harvorage of the test cockroaches. A small tissue culture dish (3.5 cm in diameter × 1 cm in high) was filled with tap water with a piece of sponge floated, and placed in the middle of the arena. The water was re-filled every day. Laboratory animal food was also provided in a small tissue culture dish from start to end of the test. Only when the cockroaches were exposed to the poison bait, the food was taken away.

Cockroaches tested. NIH strain of *Blattella germanica* was used for the test. They have been reared in large plastic boxes where many cardboard-made tubes are piled for their harborage. When the test started, a harborage tube was transferred into a small transparent plastic box with a tight lid, and cockroaches harboured in the tube were anesthetized by introducing CO_2 gas into the box. The target stage of cockroaches were then picked and introduced in the arena.

Chemicals. Two formulations were tested. One is Combat Super Bait, active ingredient of which is 2% hydramethylnon, and the other is Roach Bait, active ingredient of which is 0.6% chlorpyrifos. When tested, the plastic made disc station was removed and the bait was wrapped by a piece of sealing film for preventing chemical contamination of the legs and other parts of the exposed cockroaches, on which a tiny opening (1 mm in diameter) was made for feeding.

Preliminary experiment. Twenty-five males were introduced in each of six arenas. After 2 days of starvation period, hydramethylnon bait in arena A and B and chlorpyrifos bait in arena C and D were put, and arena E and F remained for control. When all cockroaches were killed, the dead cockroaches and poison baits were removed. In arena A, C and E 25 males and 25 1st instar nymphs, and in arena B, D and F 25 1st instar nymphs were introduced. Water and laboratory animal food were supplied in all arenas. The live and dead cockroaches were counted every day by stage for 10 days. The tests were triplicated and the average of mortality rate was given.

Experiment 1. Twenty-five males were introduced in each arena. Three arenas were used at the same time. After 2 days of starvation period, hydramethylnon bait was introduced in an arena, and chlorpyrifos bait in another arena. The third arena was kept for control. The mortality was counted after 3 days of the exposure, and all the live and dead males and the poison bait were removed. The feces excreted by the bait-fed cockroaches in the arena were kept remained. Thirty 1st instar nymphs were then introduced in each arena. Laboratory animal food was provided throughout the test period. Every day the dead ones were counted and removed. On 10th day, the remained cockroaches were removed, and 30 1st instar nymphs were newly introduced and observed their mortality for 10 days. The successive tests were continued, with 10 days intervals until no more mortality was observed.

Experiment 2. All the test procedures were exactly identical to the Experiment 1, except that 25 4th–5th instar nymphs were introduced instead of adult males, and exposed to the poison bait.

When the control mortality rate was higher than 5%, the test mortality rate was corrected by the Abbott formula.

$$\text{The corrected test mortality rate} = (\text{test mortality rate} - \text{control mortality rate}) / (100 - \text{control mortality rate}) \times 100.$$

Both experiments were triplicated and average mortality rate was given.

Results

Preliminary experiment

In order to evaluate the effect of males' presence on coprophagous uptake of 1st instar nymphs, the comparative experiment was carried out at the beginning of this study and the result is presented in Table 1. The mortality rate of the 1st instar nymphs was not different between two groups, showing 92.5% when males was present and 93.5% when the 1st instar nymphs alone in case of hydramethylnon bait, and 52.4 and 53.3%, respectively in case of chlorpyrifos bait.

Table 1 Comparison of coprophagous uptake of hydramethylnon and chlorpyrifos on the 1st instar nymphs of *B. germanica* with and without males (average of triplicate tests).

Chemical	% mortality* ($\alpha \pm$S.D.)		
	When males present		1st nymphs alone
	Males	1st nymphs	
Hydramethylnon	77.8±3.9	92.5±4.2	93.5± 2.9
Chlorpyrifos	22.2±6.9	52.4±9.9	53.3±13.6
Control	5.5±1.9	6.6±3.4	5.0± 1.9

* % mortality was accessed after 10 days of the exposure to feces excreted by the poisoned males.

Table 2 Laboratory test on secondary killing effect of poison baits by coprophagy against *Blatella germanica* males (triplicate tests).

Successive test No.**	Hydramethylnon 2%		Chlorpyrifos 0.6%		Control	
	No. tested	Mort. %	No. tested	Mort. %	No. tested	Mort. %
Males exposed to bait for 3 days	75	98.7	75	100	50	2.0
1	93	93.5	90	45.6	90	4.4
2	91	80.2	89	11.2	86	2.3
3	89	74.2*	90	6.7*	92	6.5*
4	91	79.1	91	2.2	90	4.4
5	98	78.6	93	5.4	90	3.3
6	90	65.4	89	0	59	3.4
7	90	60.0	90	0	60	1.7
8	90	63.3	90	3.4	60	0
9	91	61.5	90	0	60	3.3
10	94	63.8	—	—	59	1.7
11	89	61.8	—	—	60	0
12	91	58.2	—	—	60	0
13	91	59.5*	—	—	60	5.0*
14	90	60.2*	—	—	60	5.0*
15	90	27.8	—	—	62	4.8
16	90	13.3	—	—	56	3.8
17	89	10.6*	—	—	60	8.3*
18	89	9.0	—	—	60	0
19	90	4.4	—	—	60	1.7
20	90	3.3	—	—	59	0
21	91	3.3	—	—	59	3.4
22	60	0	—	—	60	1.7

* Mortality rate was corrected by Abbott formula in case that the control mortality gave higher than 5%. ** In each test, 30 1st instar nymphs were introduced and mortality was observed for 10 days.

Experiment 1

Mortality rate of *B. germanica* males exposed to hydramethylnon and chlorpyrifos for 3 days was 98.7 and 100%, respectively. After removal of all the males dead and alive, the mortality rate of the 1st instar nymphs exposed to the feces through 22 successive tests is shown in Table 2 and Fig. 1. High mortality rate of the early instars was given from the 1st test group through the successive test groups until 18th test, showing mortality rate of 81.1% (93.5–78.6%) in average of the 1st–5th tests, 61.5% (65.4–60.2%) in average of the 6th–14th tests, and

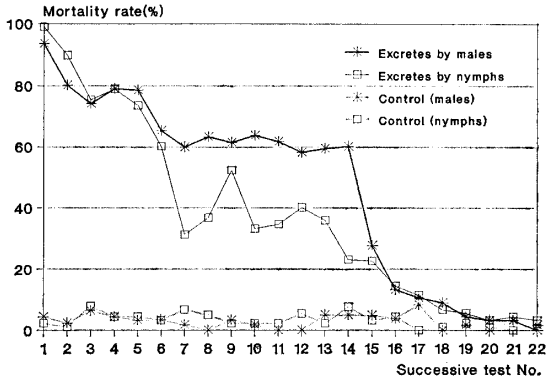

Fig. 1 Laboratory test on secondary killing effect of hydramethylnon by uptakes of feces excreted by males and old nymphs of *B. germanica*.
Each test was triplicated.

Table 3 Laboratory test on secondary killing effect of poison baits by coprophagy against 4th–5th instar nymphs of *Blatella germanica* (triplicate tests).

Successive test No.**	Hydramethylnon 2%		Chlorpyrifos 0.6%		Control	
	No. tested	Mort. %	No. tested	Mort. %	No. tested	Mort. %
4th–5th nymphs exposed to bait for 3 days	75	96.0	99	97.0	75	2.7
1	92	98.9	120	38.5	90	2.2
2	89	89.8	104	24.4	90	1.1
3	93	75.5*	120	9.4*	90	7.8*
4	90	78.9	120	4.4	90	4.4
5	91	73.6	120	3.7	90	4.4
6	93	60.2	120	3.4	89	3.3
7	92	31.3*	119	5.0*	90	6.7*
8	90	36.8*	118	4.0*	91	5.0*
9	90	52.2	120	4.2	89	2.2
10	90	33.3	90	1.7	90	2.2
11	92	34.8	90	5.0	90	2.2
12	99	40.2*	91	0	92	5.4*
13	89	36.0	90	0	90	2.2
14	90	23.1*	—	—	92	7.6*
15	88	22.7	—	—	90	3.3
16	90	14.4	—	—	90	4.4
17	88	11.4	—	—	89	0
18	89	6.7	—	—	89	1.1
19	90	5.6	—	—	90	2.2
20	90	3.3	—	—	90	1.1
21	90	4.4	—	—	89	0
22	60	3.3	—	—	90	2.2

* Mortality rate was corrected by Abbott formula in case that the control mortality gave higher than 5%. ** In each test, 30 1st instar nymphs were introduced and mortality was observed for 10 days.

15.2% (27.8–9.0%) in average of the 15th–18th tests. From the 19th test on 190 days after the first test, the mortality rate dropped to the control level. In case of chlorpyrifos, the 1st test group of the 1st instar nymphs exposed to the feces showed 45.6% of mortality rate, the 2nd test group showed 11.2% of mortality, and thereafter the mortality rate dropped to the control level.

Experiment 2

Mortality rate of the old nymphs fed hydramethylnon bait and chlorpyrifos bait for 3 days was 96.0 and 97.0% respectively. The result is shown in Table 3 and Fig. 1. High mortality rate of the 1st instar nymphs exposed to the feces showed from the 1st test to 17th test, giving 79.5% (98.9–60.2%) mortality in average of the 1st–6th test groups, 37.8% (52.2–31.3%) mortality in the 7th–13th test groups and 17.9% (23.1–11.4%) in the 14th–17th test groups in average. The mortality dropped to the control level from the 18th test after 180 days since the test started. In case of chlorpyrifos, the mortality rate was 38.5% in the 1st test group, 24.4% in the 2nd test group, and thereafter dropped to the control mortality level.

The cumulative record of the successive tests is given in Table 4. The cumulative number of the 1st instar nymphs exposed to the feces excreted by 75 hydramethylnon poisoned males (25 males × 3 tests) was 1,636 through the 1st–18th successive tests during 180 days of the observation period, of which 943 young nymphs were killed giving 57.6% mortality rate. Total 1,635 1st instar nymphs were exposed to the feces excleted by 75 old nymphs poisoned by hydramethylnon and 799 young nymphs were killed through 18 successive tests during 180 days giving 48.9% mortality rate. From the results of both experiments, it can be calculated

Table 4 Cumulative mortality of successive tests on secondary killing effect of hydramethylnon by coprophagy against *B. germanica* (tests triplicated).

Successive test No.**	Cumulative mortality* by coprophagous uptake excreted by					
	75 males			75 4th–5th instar nymphs		
	No. tested	No. dead	Mort. %	No. tested	No. dead	Mort. %
1	93	87	93.5	92	91	98.9
2	184	160	87.0	181	170	93.9
3	273	226	82.8	274	250	91.2
4	364	299	82.1	364	321	88.2
5	462	376	81.4	455	388	85.3
6	552	435	78.8	548	444	81.0
7	642	489	76.2	640	477	74.5
8	732	546	74.6	730	513	70.3
9	823	602	73.1	820	560	68.3
10	917	662	72.2	910	590	64.8
11	1,006	717	71.3	1,002	622	62.1
12	1,097	770	70.2	1,101	665	60.4
13	1,188	826	69.5	1,190	697	58.6
14	1,278	882	69.0	1,280	723	56.5
15	1,368	907	66.3	1,368	753	55.0
16	1,458	919	63.0	1,458	777	53.3
17	1,547	935	60.4	1,546	793	51.3
18	1,636	943	57.6	1,635	799	48.9

* Mortality of 1st instar nymphs. ** In each test, 30 1st instar nymphs were introduced and mortality was observed for 10 days.

that 13 young nymphs and 11 young nymphs were killed by 1 male and 1 old nymph, respectively by coprophagous uptake during the period of 180 days.

Discussion

Although many arthropods have a habit of coprophagous uptake for obtaining nutrients, coprophagy by cockroaches has little attention. Schowalter and Crossley (1982) speculated that coprophagy had some effect on the non-feces consumption rate by the cockroach detritivone *Gromphadorhina patentosa*. Silverman *et al.* (1991) reported that hydramethylnon was found in the feces excreted by the hydramethylnon bait-fed cockroaches, and measured the amount of hydramethylnon recovered in the feces, by the stage, being 1.3 μg in 1st–2nd instars, 13.8 μg in 3rd–4th instars, 41.1 μg in 5th instars, 53.8 μg in nongravid females, 12.2 μg in gravid females and 35.8 μg in males. The average amount of excreted hydramethylnon was equivalent to 32.8% of the ingested amount. These differences by stage can explain the reason why our Experiment 1 and Experiment 2 showed different cumulative mortality rate (57.6–48.9%). Experiment 1 and Experiment 2 were tested with the feces excreted by males and old nymphs (4th–5th instars), respectively. Silverman *et al.* (1991) also noticed considerable mortality when individual German cockroaches were placed in containers harboring feces excreted by cockroaches that fed on hydramethylnon bait, and reported that the mortality rate of young nymphs was much higher in the group with males together than the group of the youngs alone, showing 20.4±4.6% and 1.8±0.95%, respectively. However, our study result (Table 1) showed, that the mortality rate of the 1st instar nymphs was not different between two groups, showing 92.5% when males present and 93.5% when the 1st instar nymphs alone. And the mortality rate in our study was much higher than the result of Silverman *et al.* (1991), *i.e.* 93.5% *vs.* 1.8%. Shimamura *et al.* (1994) also carried out similar test with our preliminary experiment; 1st–2nd instar nymphs together with males and females were exposed to the feces of the hydramethylnon-fed cockroaches, showing 52.3% mortality rate in 1st–2nd instar nymphs, 65.5% in males and 50.0% in females on 18 days after exposure. We can not explain what factor(s) caused such different results. The further studies are required.

In both experiment of chlorpyrifos bait, mortality was shown only in the 1st–2nd test groups, which is not clear whether this mortality resulted from the secondary killing effect through coprophagous uptake or bait contamination by the exposed cockroaches. Even though prudential care was paid not to contaminate the arenas by the chemicals (hydramethylnon and chlorpyrifos), small particles of the chemicals might adhere to mouth part and/or tarsi of the cockroaches and the arenas would be contaminated. However, judging from the result that because the toxic action of chlorpyrifos is very fast and kills cockroaches in a few hours, bait-fed cockroaches would excrete very few amount of the feces or none. It can be answered only by analyzing the excretes of the chlorpyrifos exposed cockroaches.

It should be mentioned that the mortality rate of 1st instar nymphs of the 1st–18th test groups would be resulted from coprophagous uptake of the feces excreted by not only the males/old nymphs which fed hydramethylnon but also the 1st instar nymphs which fed hydramethylnon-contained feces. In other words, there was not only secondary killing effect but thirdly and forthly killing effect.

Acknowledgements

The authors wish to thank the Medical Entomology Division, National Institute of Health for sharing the NIH strain of *B. germanica*. This study was funded by the Clorox Company, Oakland, California, U.S.A.

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摘　要

チャバネゴキブリの糞摂取による hydramethylnon の二次毒性実験

hydramethylnon bait を摂取したチャバネゴキブリの糞に hydramethylnon が含まれていて，仲間のゴキブリが，この糞を食べて死ぬことが報告されている（Silverman *et al.*, 1991）．本実験では，この二次毒性の持続期間と，致死率を観察した．チャバネゴキブリの雄成虫25匹に，hydramethylnon bait を3日間与えた後，糞だけを test box の中に残して，ゴキブリと薬剤を取り除き，30匹の一齢期幼虫を dog food と一緒に入れて，10日間致死率を観察した．さらに，新しく30匹の一齢期幼虫を入れて，10日間の致死率を観察することを18回繰り返した．その結果，1～5 次実験の平均致死率 81.1%，6～14 次実験の致死率 61.5%を示し，180日間の18次実験までの1,636匹中，943 匹（57.6%）が致死した．雄成虫の代りに4～5齢期幼虫を入れて hydramethylnon を食べさせ，排泄した糞による致死率は，1～5 次実験で 83.3%，6～15 次実験で 41.2% を示し，180 日間の 18 次実験まで，1,635 匹中の 799 匹（48.9%）の一齢期幼虫が致死した．