Horizontal Transfer of Bait in the German Cockroach: Indoxacarb Causes Secondary and Tertiary Mortality

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ABSTRACT Horizontal transfer of indoxacarb in the German cockroach, *Blattella germanica* (L.), was examined under laboratory conditions. Results show that a single bait-fed adult cockroach (i.e., the donor) transferred indoxacarb to numerous primary recipients (secondary mortality), which then became secondary donors. These recipients subsequently became donors to other cockroaches and caused significant mortality in other members of the aggregation, resulting in tertiary kill. Indoxacarb was effectively transferred among adult cockroaches and resulted in significant secondary mortality. When adult males served as donors and vectored the insecticide to adult males, the donor:recipient ratio affected the mortality of the recipients and the rate of secondary mortality increased with increasing the ratio of donors to recipients. Furthermore, secondary mortality in the untreated cockroaches was significantly affected by the freshness of excretions from the donors, the presence of alternative food, and the duration of contact between the donors and the recipients. Ingested indoxacarb was most effectively translocated when the recipients interacted with freshly symptomatic donors in the absence of alternative food. The transfer of indoxacarb continued beyond secondary mortality and resulted in significant tertiary mortality. Excretions from a single bait-fed adult killed 38/50 (76%) nymphs within 72 h. The dead nymphs then vectored indoxacarb to 20 adult males and killed 16/20 (81%) recipients within 72 h. Behavioral mechanisms involved in the horizontal transfer of indoxacarb may include: contact with excretions, necrophagy, emetophagy, and ingestion of other excretions that originate from the donors.

KEY WORDS bait, *Blattella germanica*, German cockroach, horizontal transfer, indoxacarb

Horizontal transfer of insecticides occurs when active ingredients contained within baits or liquid spray insecticides are passed among individuals within an insect population. The population may be a colony of social insects (e.g., ants [Soeprono and Rust 2004] or termites [Saran and Rust 2007; Hu et al. 2005]), a group of gregarious individuals (e.g., cockroaches [Silverman et al. 1991; Kopanic and Schal 1997, 1999; Gahlhoff et al. 1999; Durier and Rivault 2000, Buczkowski and Schal 2001a,b; Buczkowski et al. 2001] or grasshoppers [Smith and Lockwood 2003]), or an assemblage of normally solitary individuals (e.g., mosquitoes [Chism and Apperson 2003] or fleas [Jacobs et al. 2000]). Active members of a population, most often foraging adults, become exposed to an insecticide, return to the nest or an aggregation, and subsequently pass the insecticide to more sedentary members of the population that often cannot feed independently (e.g., ant larvae, termite soldiers) or do not feed independently (e.g., young cockroach nymphs, ant and termite reproductives). The recipients may obtain the insecticide directly from the donors (e.g., mutual grooming, trophallaxis, necrophagy) or by consuming and/or contacting insecticide-containing excretions deposited by the donors (e.g., coprophagy, emetophagy). Depending on the insect taxon, various mechanisms may be involved. In social insects, horizontal transfer usually involves interactions where both the donors and the recipients are alive and actively interacting. Key among these mechanisms is trophallaxis, which facilitates the spread of bait toxicants (Hu et al. 2005), and mutual grooming which facilitates the spread of liquid spray insecticides (Soeprono and Rust 2004). In contrast, horizontal transfer in cockroaches usually involves dead or dying donors or simply excretions left behind by the donors.

In the German cockroach, *Blattella germanica*, (L.), four mechanisms have been shown to facilitate the horizontal transfer of insecticides. These mechanisms include contact (Durier and Rivault 2000, Buczkowski and Schal 2001a), coprophagy (ingestion of feces; Silverman et al. 1991; Kopanic and Schal 1997, 1999; Buczkowski et al. 2001), necrophagy (ingestion of dead conspecifics; Gahlhoff et al. 1999; Le Patourel 2000), and emetophagy (ingestion of regurgitated bait; Buczkowski and Schal 2001a). Of the four mechanisms, contact facilitates the horizontal transfer of residual spray insecticides, while the remaining three mechanisms facilitate the spread of bait active ingre-

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dients. Coprophagy by first instars seems to be an important mechanism underlying the horizontal transfer of slow-acting insecticides, primarily hydramethylnon (Silverman et al. 1991; Kopanic and Schal 1997, 1999; Buczkowski et al. 2001). Necrophagy has been shown to play an important role in the horizontal transfer of bait active ingredients by Le Patourel (2000) who observed that cockroaches that consumed fipronil-containing baits were subsequently wholly or partially consumed by and induced substantial mortality in third instars. Finally, emetophagy, or the ingestion of insecticide-induced regurgitate, has been shown to be an important mechanism by which fast-acting, emetogenic insecticides (e.g., fipronil) are disseminated within cockroach aggregations (Buczkowski and Schal 2001a).

The majority of studies on the efficacy of baits against cockroaches have focused on primary mortality (Appel 1990, 2003; Ross 1993; Appel and Benson 1995; Kaakeh et al. 1997; Scherer et al. 2005). These studies mainly investigate the efficacy of novel toxicants and often report efficacy data (e.g., LT_{50} values, percentage of mortality, laboratory and field performance, product comparisons, resistance issues). A smaller proportion of studies have examined secondary mortality, whereby the active ingredient is horizontally transferred from donors to recipients (Silverman et al. 1991; Kopanic and Schal 1997, 1999; Gahlhoff et al. 1999; Buczkowski and Schal 2001a,b; Buczkowski et al. 2001). Conspicuously, however, no study has examined the proposition that horizontal transfer may continue beyond secondary mortality and may involve higher levels such as tertiary or quaternary mortality. However, some authors have recently speculated that such phenomenon may exist in colonies of subterranean termites, where trophallaxis and frequent contact among nestmates facilitate the transfer of toxicants (Haagsma and Rust 2005, Hu et al. 2005). Furthermore, Suárez and Thorne (2000) have suggested that termites share food by "trophallactic cascade," whereby foragers pass food to recipients which subsequently become donors to other nestmates. The objective of our study was to examine the horizontal transfer of indoxacarb in aggregations of the German cockroach, *Blattella germanica* (L.). Indoxacarb is a neurotoxic oxadiazine insecticide discovered by DuPont (McCann et al. 2001), and it has been shown to significantly reduce German cockroach populations in laboratory and field studies (Appel 2003, Scherer et al. 2005). However, the horizontal transfer of indoxacarb in the German cockroach has not been examined previously, but effective transfer has been demonstrated in the Formosan termite, *Coptotermes formosanus* Shiraki (Hu et al. 2005). The objective for the first part of our study was to examine secondary mortality with indoxacarb. The effects of donor:recipient ratio, freshness of donor excretions, presence of donors, and presence of alternative food were considered. For the second objective, we focused on determining whether indoxacarb could subsequently be transferred from primary recipients to other members of the aggregation and result in tertiary and quaternary mortality.

Materials and Methods

Insects. Insecticide-susceptible cockroaches that originated from American Cyanamid (Princeton, NJ) were reared in walk-in environmental chambers at 26°C, 60% RH, a photoperiod of 12:12 (L:D) h, and they were provided with water and Harlan Teklad rodent diet (Harlan Teklad, Madison, WI). Nymphs were used early in the developmental stage during the peak feeding period (Young and Schal 1997). Adult males, 10–21 d old, were used to vector the insecticide to other cockroaches.

Secondary Mortality: Transfer of Indoxacarb from Adult Male Donors to Adult Male Recipients. The horizontal transfer of indoxacarb from bait-fed adult males to untreated adult males was investigated. Several donor cockroaches (adult males) were removed from a rearing container, placed in a petri dish provided with a harborage, and starved for 24 h while provided with water. After starvation, the cockroaches were offered Advion bait (Advion Cockroach Gel Bait, DuPont Professional Products, Wilmington, DE; 0.6% indoxacarb) ad libitum. When the cockroaches became symptomatic (≈6 h after ingesting bait), they were transferred to dishes with untreated cockroaches. The transfer of indoxacarb was examined using four ratios of donors to recipients: 1:5, 5:5, 5:10, and 5:20. Mortality in the recipients was recorded at 24, 48, and 72 h. All experiments were designed so that the onset of foraging activity in the recipients (beginning of scotophase or soon thereafter) coincided with the onset of indoxacarb excretions. Control experiments using frozen and thawed males were performed, and all experiments were replicated 10 times.

Secondary Mortality: Role of Fresh Excretions, Donor Presence, and Alternative Food. The role of freshness of donor excretions, the presence of symptomatic donors, and the presence of alternative food on the level of secondary mortality was examined. The experiment consisted of six treatments. In treatment 1, untreated cockroaches were exposed to freshly symptomatic donors in the absence of alternative food. This treatment examined the maximum level of secondary mortality that can be achieved when the only source of food available to untreated cockroaches were the donors and/or excretions from the donors. The donors were fed the bait for 2 h and transferred into the experimental dish at the onset of the symptoms (≈6 h after ingesting the bait). In treatment 2, untreated cockroaches were exposed to freshly symptomatic donors in the presence of alternative food. Therefore, untreated cockroaches had a choice to feed on standard laboratory diet or excretions produced by the donors. In treatment 3, untreated cockroaches were exposed to donors that had been dead for 24 h in the absence of alternative food. Such donors only provided dry residues that adhered to their bodies. In treatment 4, untreated cockroaches were exposed only to excretions left behind by the donors and had

no access to alternative food. Symptomatic donors were first allowed to deposit excretions in the dish for 24 h, and they were removed before adding untreated cockroaches. Treatment five was a control experiment in which the recipients were exposed to untreated donors (frozen and thawed) in the absence of alternative food. Treatment 6 was a control experiment in which the recipients were exposed to untreated donors (frozen and thawed) in the presence of alternative food. For each treatment, two ratios of donors to recipients were tested: one adult male to five adult males and five adult males to 10 adult males. All tests were performed in 9-cm i.d. dishes by using recipients previously acclimated to the dish for 24 h and provided with water and harborage (10-cm^2 piece of folded cardboard). Whenever alternative food was provided, it consisted of standard laboratory diet (rat chow). All experiments were designed so that the onset of foraging activity in the recipient cockroaches coincided with the onset of indoxacarb excretions (≈6 h after consuming the bait). Mortality in the recipients was recorded at 24, 48, and 72 h, and each treatment was replicated 10 times.

Tertiary and Quaternary Mortality. Tertiary mortality was examined as the horizontal transfer of indoxacarb from first instars that died by feeding on excretions from bait-fed adult males to adult male recipients. The two-part study first examined the horizontal transfer of indoxacarb from adult males to first instars (secondary mortality). Subsequently, the transfer of indoxacarb from first instars to adult male recipients (tertiary mortality) was examined.

The first objective was to determine the number of nymphs that can be affected by excretions produced by a single adult male donor (secondary mortality). First instars were used as recipients because they readily ingest adult excretions (e.g., feces: Kopanic and Schal 1999, regurgitated bait: Buczkowski and Schal 2001a), and they are ideally the main target of pest control efforts as they comprise a large fraction of the population (DeMark et al. 1993). Fifty first instars were placed in a 9-cm i.d. dish, provided with water, harborage (10-cm^2 piece of folded cardboard), and acclimated to the dish for 24 h without food. A single symptomatic male was added to the dish at the end of the acclimation period. Mortality in the nymphs was examined at 24, 48, and 72 h, and 10 replicates were performed.

Results showed that excretions from a single adult male are capable of killing 38 ± 4 first instars. Thus, the tertiary transfer of indoxacarb from 38 first instars to either 20 or 30 untreated adult males was examined. Dishes were set up as described above (single donor male plus 50 first instars) to obtain the secondary donors. The first instars interacted with the symptomatic male for 12 h, and then they were then transferred to a clean dish that contained untreated males starved for 24 h. The dish was provisioned with water, harborage, but no food. Mortality in the recipients was recorded at 24, 48, and 72 h. Control experiments using frozen and thawed nymphs were performed, and all experiments were replicated 14 times.

Quaternary mortality was examined by placing 10 symptomatic adult males (donors) in a 9-cm i.d. dish that contained 25 first instars that had been starved for 24 h. The donor males were males that had died as a result of tertiary mortality, i.e., having contact with 38 first instars that died from having contact with a single bait-fed adult male. The males were allowed to interact with the dying first instars (tertiary mortality) for 12 h. The next day, 10 symptomatic males were selected and placed in a dish with 25 first instars that had been starved for 24 h while provided with water. In addition, the dish contained a cardboard shelter and was provisioned with water, but no food. Mortality in the male recipients was examined at 24, 48, and 72 h, and eight replicates were performed.

Statistical Analyses. All data analyses were performed using SAS 8.1 statistical software (SAS Institute 2002). Nonparametic one-way analysis of variance (ANOVA) tests (Wilcoxon signed rank test) were performed to examine the influence of treatment (indoxacarb) on recipient survival in interactions with donor cockroaches (secondary, tertiary, and quaternary mortality). This was accomplished by using the PROC NPAR1WAY procedure. Comparisons among treatments or among exposure times (24, 48, and 72 h) consisted of analysis of variance (ANOVA) (PROC ANOVA) on mean cumulative percentage of mortality by treatment category. Each ANOVA was followed by the least significant difference (LSD) *t*-test to test for significant differences between means. The level of significance was set at $\alpha = 0.05$.

Results

Secondary Mortality: Transfer of Indoxacarb from Adult Male Donors to Adult Male Recipients. Indoxacarb was effectively transferred among adult males and resulted in significant secondary mortality (Table 1). As expected, the rate of secondary mortality increased with increasing the ratio of donors to recipients. Secondary mortality was the fastest when five donors interacted with five recipients (1:1 ratio) and 94 ± 4% of the recipients died within 24 h and 100% died by 48 h. The rate of secondary mortality decreased as the ratio of donors to recipients decreased; however, complete mortality in the recipients was still achieved at 72 h when five donors interacted with either 10 or 20 recipients (Table 1). The level of secondary mortality was the lowest when one donor interacted with five adults and resulted in 77 ± 5% mortality in the recipient males at 72 h. However, this result is still highly significant as only 20 ± 6% of recipients died in the control experiment (Wilcoxon signed rank test: $Z = 3.13$, $P = 0.008$, $n = 8$).

Secondary Mortality: Role of Fresh Excretions, Donor Presence, and Alternative Food. The results of experiments that tested the effect of freshness of donor excretions, the presence of symptomatic donors, and the presence of alternative food on secondary mortality are presented in Table 2. The highest level of secondary mortality was observed when the recipients were exposed to freshly symptomatic donors in

Table 1. Cumulative percentage of mortality (± SEM) in German cockroach adult males exposed to bait-fed adult males (secondary mortality)

Ratio donors: recipients	Level of horizontal transfer	Treatment	Mean cumulative % mortality (± SEM)		
			24 h	48 h	72 h
1 adult:5 adults	Secondary	Indoxacarb	40 ± 8b, a	57 ± 11b, ab	77 ± 5b, b
		Control	6 ± 4a, a	17 ± 5a, ab	20 ± 6a, b
5 adults:5 adults	Secondary	Indoxacarb	94 ± 4b, a	100 ± 0b, a	100 ± 0b, a
		Control	3 ± 3a, a	9 ± 6a, a	11 ± 6a, a
5 adults:10 adults	Secondary	Indoxacarb	83 ± 9b, a	87 ± 6b, a	100 ± 0b, a
		Control	0 ± 0a, a	10 ± 4a, ab	16 ± 4a, b
5 adults:20 adults	Secondary	Indoxacarb	54 ± 4b, a	90 ± 2b, b	100 ± 0b, b
		Control	3 ± 2a, a	8 ± 2a, b	10 ± 2a, b

Means within a given ratio followed by the same letter are not significantly different ($P \leq 0.05$). The first letter indicates within-column comparisons by Wilcoxon signed rank test, and the second letter indicates within-row comparisons by Tukey's HSD test.

the absence of alternative food. This treatment resulted in the death of 77 ± 5% of recipients when one donor interacted with five recipients and 100 ± 0% mortality when five donors interacted with 10 recipients. In treatment 2, the recipients were exposed to freshly symptomatic donors in the presence of alternative food. Secondary mortality was significantly lower when alternative food was present and resulted in the death of 30 ± 5% of recipients when one donor interacted with five recipients (treatment 1 versus treatment 2; Wilcoxon signed rank test: $Z = 3.17$, $P = 0.007$, $n = 8$,) and 59 ± 10% mortality when five donors interacted with 10 recipients (Wilcoxon signed rank test: $Z = 3.00$, $P = 0.01$). However, secondary mortality in the presence of alternative food was still significantly higher than mortality in the control experiment (treatment 2 versus treatment 6; Table 2, 5:10 ratio). In treatment 3, the recipients were exposed to donors that had been dead for 24 h and had no access to alternative food. This treatment resulted in 66 ± 10% mortality in the recipients at 72 h (1:5 ratio), not significantly different from treatment 1 (Wilcoxon signed rank test: $Z = 1.06$, $P = 0.307$).

When the recipients were exposed only to excretions left behind by the donors and had no access to alternative food (treatment 4) the mortality in the recipients decreased significantly relative to treatment 1 where the recipients had access to symptomatic donors that exuded fresh excretions. In the 1:5 ratio, 43 ± 9% of recipients died when exposed to residues only and 77 ± 5% of recipients died when exposed to symptomatic donors (Wilcoxon signed rank test: $Z = 1.86$, $P = 0.04$, $n = 8$). In the 5:10 ratio, 45 ± 5% of recipients died when exposed to residues only and 100 ± 0% of recipients died when exposed to symptomatic donors (Wilcoxon signed rank test: $Z = 3.38$, $P = 0.004$, $n = 8$). However, secondary mortality in treatment 4 was still significantly higher than mortality in the control experiment (treatment 4 versus treatment 5; Table 2).

No significant difference was detected between the two control experiments (treatments 5 and 6), indicating that lack of food alone is not a factor that significantly contributes to cockroach mortality, at least during the 72-h starvation period (Table 2).

Tertiary and Quaternary Mortality. In the test involving the transfer of indoxacarb from a single adult male to 50 first instars, 18/50 (35%) nymphs died within 8 h after contacting the symptomatic male. In contrast, no nymphs died in the control experiment involving a frozen and thawed donor cockroach (Wilcoxon signed rank test, $Z = 4.00$, $P = 0.0008$; $n = 10$). Furthermore, mortality in first instars increased significantly over time, and 38/50 (76%) of nymphs were dead 72 h after the addition of the symptomatic male (Fig. 1; Table 3). The transfer of indoxacarb continued

Table 2. Cumulative percentage of mortality (± SEM) in adult male recipients exposed to bait-fed donors or donor excretions (secondary mortality)

Ratio donors: recipients	Treatment	Mean cumulative % mortality (± SEM)		
		24 h	48 h	72 h
1 adult:5 adults	1 = fresh bait-fed donor, food absent	40 ± 8a, b	57 ± 11a, ab	77 ± 5a, a
	2 = fresh bait-fed donor, food present	13 ± 7b, b	28 ± 7b, a	30 ± 5b, a
	3 = aged bait-fed donor, food absent	37 ± 5a, c	51 ± 6a, b	66 ± 10a, a
	4 = residues only, food absent	3 ± 3b, c	30 ± 11b, b	43 ± 9b, a
	5 = control donor, food absent	6 ± 4b, b	17 ± 5b, a	20 ± 6b, a
	6 = control donor, food present	3 ± 3b, c	11 ± 6b, b	17 ± 8b, a
5 adults:10 adults	1 = fresh bait-fed donor, food absent	83 ± 9a, a	87 ± 6a, b	100 ± 0a, a
	2 = fresh bait-fed donor, food present	20 ± 5b, b	50 ± 9b, a	59 ± 10b, a
	3 = aged bait-fed donor, food absent	87 ± 6a, a	90 ± 5a, a	94 ± 3a, a
	4 = residues only, food absent	5 ± 2c, b	35 ± 3b, a	45 ± 5b, a
	5 = control donor, food absent	0 ± 0c, b	10 ± 4c, a	16 ± 4c, a
	6 = control donor, food present	4 ± 2c, a	6 ± 3c, a	7 ± 4c, a

Means within a given ratio followed by the same letter are not significantly different by Wilcoxon signed rank test ($P \leq 0.05$). The first letter indicates within-column comparisons, and the second letter indicates within-row comparisons.

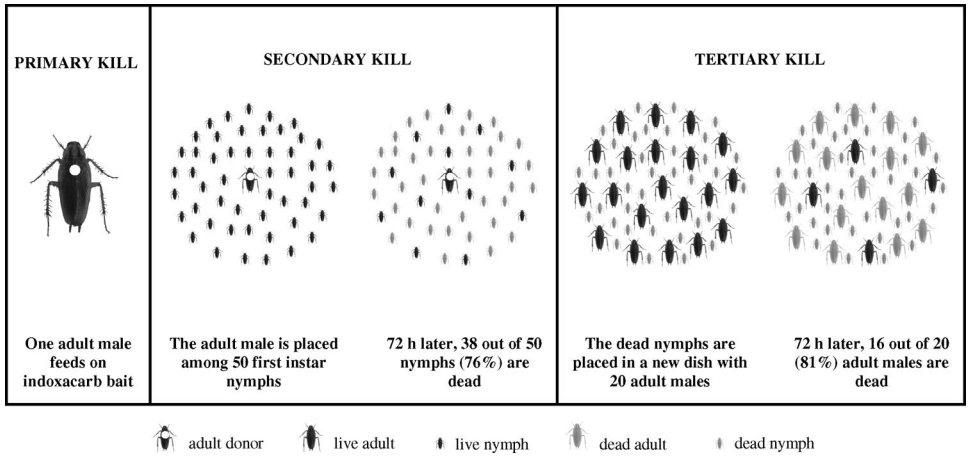

Fig. 1. Diagram illustrating cascading horizontal transfer of indoxacarb in the German cockroach. A single primary donor (adult male) transfers indoxacarb to first instar primary recipients (secondary mortality). The primary recipients then become secondary donors and transfer indoxacarb to adult males (tertiary mortality).

beyond secondary mortality and resulted in significant tertiary mortality (Fig. 1; Table 3). When 20 adult males were exposed to 38 first instars that died by having contact with the primary donors (bait-fed adults), 81 ± 3% of adult males died in 72 h (Fig. 1). In contrast, only 5 ± 1% of males died in the controls (Wilcoxon signed rank test: $Z = 4.50$, $n = 14$, $P = 0.0001$). In all tests involving tertiary and quaternary mortality relatively few recipients died in the first 8 h, and mortality in the recipients increased significantly with time (Table 3).

No significant quaternary mortality was observed and only 12 ± 4% of recipient nymphs died from contacting adults that died from contacting nymphs that died from contacting adults that died from eating the bait and 9 ± 3% of nymphs died in the control test (Wilcoxon signed rank test: $Z = 0.54$, $P = 0.600$, $n = 8$).

Discussion

Secondary Mortality: Transfer of Indoxacarb from Adult Male Donors to Adult Male Recipients. Indoxacarb was transferred from adult donors to adult recipients and caused significant secondary mortality. As predicted, the rate of secondary mortality increased with increasing the ratio of donors to recipients. Hu et al. (2005) documented significant effect of donor: recipient ratio on the horizontal transfer of topically applied indoxacarb in the Formosan termite. Furthermore, the authors investigated the effect of dose applied to donor versus donor:recipient ratio and showed that the effect of dose on secondary mortality was stronger than the effect of the ratio.

Behavioral observations (although not quantified in this study) revealed that exudates from indoxacarb-fed cockroaches were highly attractive to recipient cockroaches, especially first instars. Observations of nymphs feeding on excretions from adult donors revealed that the mechanisms underlying horizontal transfer of indoxacarb may include contact with excretions, necrophagy (ingestion of dead conspecifics), emetophagy (ingestion of regurgitated bait, as per Buczkowski and Schal 2001a), and ingestion of excretions that originate from the digestive or reproductive organs in the donors. Contact may have been important as recipient cockroaches inadvertently contacted liquid exudates while feeding on excretions emanating

Table 3. Cumulative percentage of mortality (± SEM) in German cockroach adult males exposed to first instars (tertiary and quaternary mortality)

Ratio donors:recipients	Level of horizontal transfer	Treatment	Mean cumulative % mortality (± SEM)			
			8 h	24 h	48 h	72 h
1 adult:50 nymphs	Secondary	Indoxacarb	35 ± 4b, a	62 ± 8b, b	69 ± 8b, b	76 ± 7b, c
		Control	0 ± 0a, a	4 ± 1a, ab	6 ± 1a, b	8 ± 1a, c
38 nymphs:20 adults	Tertiary	Indoxacarb	3 ± 1a, a	51 ± 6b, b	68 ± 5b, c	81 ± 3b, d
		Control	0 ± 0a, a	1 ± 1a, a	4 ± 2a, b	5 ± 1a, b
38 nymphs:30 adults	Tertiary	Indoxacarb	1 ± 1a, a	26 ± 2b, b	43 ± 2b, c	57 ± 2b, d
		Control	0 ± 0a, a	1 ± 1a, a	1 ± 1a, a	2 ± 1a, a
10 adults:25 nymphs	Quaternary	Indoxacarb	0 ± 0a, a	8 ± 3a, b	11 ± 4a, b	12 ± 4a, b
		Control	0 ± 0a, a	4 ± 3a, ab	8 ± 3a, b	9 ± 3a, b

Means within a given ratio followed by the same letter are not significantly different ($P \leq 0.05$). The first letter indicates within-column comparisons by Wilcoxon signed rank test, and the second letter indicates within-row comparisons by Tukey's HSD test.

from the donors. Necrophagy was apparent as some cadavers had their heads detached and abdomens hollowed out. Even more apparent was emetophagy, or feeding on indoxacarb-induced regurgitate. Buczkowski and Schal (2001a) originally described fipronil-induced emetophagy in the German cockroach as suggested that a similar process may happen with other fast-acting, neurotoxic insecticides. Indeed, cockroaches dying of indoxacarb poisoning exuded liquid excretions that were attractive and lethal to conspecifics. The nymphs aggregated around the symptomatic donors and horizontal transfer of indoxacarb seemed to occur by contact with and/or ingestion of the regurgitated bait. Buczkowski and Schal (2001a) demonstrated that fipronil excretion coincided with the onset of the paralytic symptoms, most of the excreted fipronil (79%) was eliminated during the first 12 h after ingestion of bait, >74% of fipronil was excreted from the oral region in the form of regurgitate, and first instars preferentially visited the oral region of dying cockroaches and ingested the liquid exudates. Indoxacarb also seemed to be emetogenic, and it is likely that the origin and the time-course of indoxacarb excretion are similar to that described for fipronil. A third mechanism that may facilitate the horizontal transfer of indoxacarb is contact or ingestion of excretions that originate from the digestive and/or reproductive systems. Cockroaches dying of indoxacarb poisoning seemed to excrete some liquids from their digestive tract. More importantly; however, symptomatic male cockroaches everted their genitalia and voided the contents of their accessory sex glands resulting in the excretion of uric acid which occurred as a white secretion. This secretion looked milky when fresh and subsequently hardened to a flaky, chalk-white powder. Male German cockroaches normally sequester urates in accessory sex glands and release them in conjunction with the spermatophore during mating, a form of paternal investment of nitrogen into the progeny (Mullins and Keil 1980). The secretion of uric acid seems to be unique to indoxacarb poisoning, because we have not observed this phenomenon with any other insecticide, including fipronil. First instars were attracted to the rectal region of dying cockroaches, and they seemed to feed on excretions and/or secretions from the posterior end. However, it remains unknown whether the secretions that originate from the reproductive organs actually contain any indoxacarb or whether the nymphs actually ingested any of the uric acid that was released. Further studies using a radioactive tracer will be needed to examine the time-course of indoxacarb excretion, the origin of indoxacarb-containing residues, and the total amount of indoxacarb that is exuded by the donors and thus available to the recipients. Furthermore, the amount of indoxacarb that each recipient acquires over time and the overall distribution of indoxacarb in cockroach aggregations will need to be examined.

Secondary Mortality: Role of Fresh Excretions, Donor Presence, and Alternative Food. We designed various experiments to test the relative importance of the initial contact between the treated and untreated cockroaches, the role of fresh excretions, the role of excreted residues, and the role of alternative food presence on the transfer of indoxacarb. In treatment 1, the recipient cockroaches were in proximity to symptomatic donors that served as the source of indoxacarb-containing excretions and did not have access to any alternative food. This treatment resulted in the highest level of secondary mortality (100% at 72 h in the 5:10 ratio), indicating that excretions from symptomatic donors are highly attractive and lethal to recipient cockroaches, especially when food is absent. In treatment 2, the recipient cockroaches also interacted with symptomatic donors, but had access to alternative food (rat chow). Relative to treatment 1, the presence of alternative food significantly reduced secondary mortality; however, in the 5:10 ratio, the level of secondary mortality was still significantly higher than mortality in the control assays. This clearly demonstrates that cockroaches are not forced to feed on donor excretions as a result of starvation, but rather feed on the excretions voluntarily, even when given access to regular laboratory diet. This indicates that excretions from symptomatic donors are highly attractive to other cockroaches. Previously, Buczkowski and Schal (2001a) used time-lapse video and demonstrated that exudates from fipronil-fed cockroaches were highly attractive to first instars, which preferentially visited the oral region of dying cockroaches, the major source of liquid excretions. Furthermore, Buczkowski and Schal (2001a) observed that when given a choice between a symptomatic cockroach, rat chow, and water, 38% of first instar contacts were with the donor, 44% with rat chow, and 18% with water, demonstrating that cockroaches willingly ingest donor exudates even when alternative food is available and in proximity.

The role of exudates that emanate from dying cockroaches was evaluated by exposing recipient cockroaches to fresh and older residues from indoxacarb bait-fed cockroaches, with and without contact with symptomatic donors. We hypothesized that the level of secondary mortality would be higher when recipients interact with freshly symptomatic donors rather than donors that have been dead for 24 h (treatment 1 versus 3). This is because fresh excretions are readily available for ingestion by the recipients, unlike older excretions that dry out and stick to the substrate. However, the difference in secondary mortality caused by fresh versus older donors ($77 \pm 5\%$ versus $66 \pm 10\%$ at 72 h) was not statistically significant. This suggests that 1) older residues of indoxacarb continue to be lethal to conspecifics beyond the initial 24 h, or 2) ingestion and/or contact with fresh excretions is less important for secondary mortality and necrophagy plays a major role. Preliminary observations indicated that emetophagy plays a major role in the horizontal transfer of indoxacarb when the donor cockroaches are still alive and actively producing oral and rectal excretions. Later, as the donor cockroaches and the excretions they produce dry out, the role of emetophagy diminishes and necrophagy and perhaps

coprophagy become more important. Buczkowski and Schal (2001b) examined the role of initial donor presence (and the residues they excreted) on secondary mortality in the German cockroach and determined that mortality of untreated cockroaches was significantly lower when they were initially prevented from contacting fipronil-fed donors and residues they excreted (cumulative 7-d mortality: fresh donors present, 85 ± 5% versus aged donors present, 18 ± 3%). The authors concluded that the effectiveness of excreted fipronil residues quickly diminished over time as the deposits dried and adhered to the dead cockroaches and the substrate. Excreted indoxacarb residues alone, in the absence of the donors (treatment 1 versus treatment 4) caused significantly lower mortality in the untreated cockroaches when they were prevented from interacting with indoxacarb-fed donors and could only contact residues deposited by the donors. This confirms that the majority of indoxacarb is transferred via excretions that are directly associated with symptomatic donors.

Tertiary and Quaternary Mortality. This study demonstrates a chain reaction in which a primary donor transfers insecticide to primary recipients, which then become secondary donors. A single donor delivered a lethal dose of indoxacarb to a considerable number of young nymphs (secondary mortality). These recipients subsequently became donors to other cockroaches and were capable of causing significant mortality in other members of the aggregation (tertiary kill). Although this novel phenomenon has only been demonstrated with indoxacarb, it is possible that other insecticides may also exhibit tertiary mortality.

A single symptomatic adult excreted enough indoxacarb to cause mortality in 38 ± 4 first instars. The nymphs acquired enough insecticide from the primary donor to further kill 16 ± 1 adult males. This is a remarkable result given that presumably the amount of insecticide available for transfer at each step decreases substantially. First, the primary donor most likely excreted only a relatively small amount of the ingested insecticide and the remainder became sequestered within the dead donor. Buczkowski and Schal (2001b) examined the time course of fipronil excretion in the German cockroach and determined that an adult male cockroach excreted, on average, 4.5% of the fipronil it ingested, mainly due to emesis. Second, the primary recipients most likely only acquired a small proportion of the insecticide excreted by the primary donor, whereas the rest adhered to the body of the donor and the substrate. Indeed, previous studies show that the transfer of emetogenic insecticides (i.e., fipronil) quickly diminishes as liquid excretions dry out and become unavailable to the recipients (Buczkowski and Schal 2001a,b). Third, the primary recipients (and later secondary donors) themselves most likely sequestered the majority of indoxacarb they acquired, and excreted only a small proportion. It remains unknown whether some nymphs acquired the majority of indoxacarb from the primary donor and then vectored the insecticide to the recipients or whether all nymphs contributed more or less equally to tertiary mortality. Further studies using radiolabeled indoxacarb will be necessary to examine the movement of indoxacarb. The origin and time course of indoxacarb excretion, the total amount of indoxacarb excreted at each level, and the distribution of indoxacarb among the individuals involved will be especially critical in determining the flow of indoxacarb through a cockroach population. Such studies also could help determine why little quaternary mortality was observed.

Observations indicated that the behavioral mechanisms involved in tertiary mortality may include those involved in secondary mortality. These include contact with excretions, necrophagy, emetophagy, and contact and ingestion of other excretions that originate from the donors. Necrophagy seems to play a role as starved adults consumed some first instars, often completely. Relative to secondary mortality, emetophagy seemed to play a lesser role in facilitating tertiary mortality, for two reasons. First, the nymphs (secondary donors) never ingested any bait directly and it remains unknown whether they actually produced any oral exudates that could facilitate emetophagy. The nymphs may have ingested bait regurgitated by the primary donors, but it remains unknown whether the ingested regurgitate was subsequently regurgitated. Although the attraction of nymphs to symptomatic, bait-fed adults was clearly apparent in the first phase of the study, feeding by adults on oral or rectal excretions from the nymphs was not observed. Second, the nymphs are much smaller than the adults, so any oral excretions produced by the nymphs might be miniscule. The nymphs exhibited typical symptoms of indoxacarb poisoning (e.g., distended bodies, paralysis); however, no liquid excretions were observed emanating from the nymphs. Again, studies using a radiolabeled tracer will be necessary to quantify the amount of indoxacarb acquired and voided by the nymphs.

What remains to be determined is the importance of horizontal transfer of indoxacarb and other insecticides for the management of field populations of cockroaches. Comparative studies that examine horizontal transfer in various settings (e.g., residential units versus swine facilities) and use various cockroach strains might be especially informative. Given the exceptionally high propensity of indoxacarb to be transferred among cockroaches, it is likely that some transfer might occur in natural populations. Future studies should also focus on determining whether indoxacarb is chemically altered in excretions. Indoxacarb is a proinsecticide and it is activated in vivo by the insect's enzymes (McCann et al. 2001). This changes the molecular structure and the toxic properties of indoxacarb. It remains to be determined whether oral and rectal exudates from the donors contain the inactive parent compound, metabolically activated compound, or a mixture of both.

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