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The impact of bran baits treated with the insecticides carbaryl, chlorpyrifos and dimethoate on the survivorship and reproductive success of non-target mouse populations*

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ABSTRACT

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Two species of mice, *Mus musculus* and *Peromyscus maniculatus*, were exposed to insecticide-treated bran bait and the lethal and sublethal effects were monitored. Exposure to ad libitum quantities of chlorpyrifos bait caused significant levels of female adult and juvenile mortality for *M. musculus*. Confinement with chlorpyrifos bait also resulted in a short-term lag in weight gain for adult *M. musculus*, and reduced birthweights and survival of *M. musculus* pups. The time to delivery was significantly longer for *M. musculus* in the carbaryl and dimethoate treatments than in the chlorpyrifos treatment. Exposure to 2.0 g of chlorpyrifos baits caused significant levels of adult and juvenile mortality especially for *P. maniculatus* and the chlorpyrifos bait treatment.

INTRODUCTION

Carbamate and organophosphorus insecticides are routinely used by the agricultural industry to control grasshopper populations. In 1986, an estimated 69 100 l of Sevin XLR (carbaryl), 60 000 l of Lorsban 4E (chlorpyrifos), 42 000 l of Cygon 480 (dimethoate), and 15 500 kg of Hopper Stopper (dimethoate bran bait) were applied in Alberta for grasshopper control (D.L. Johnson and M. Dolinski, unpublished data, 1987). An increasing body of literature examining the negative impact of these insecticides on non-target

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organisms (e.g. Ripper, 1956; Newsom, 1967; Pimentel, 1971; McEwen et al., 1972; Ware, 1980; Brandenburg, 1985; Jepson, 1989) attests to the need for insecticide agents or formulations that would provide suitable levels of pest control, and yet be relatively safe for non-target species.

Bran bait formulations of insecticide have been suggested to be such an agent, and have proven to be efficient control methods. Carbaryl (Foster et al., 1979; Johnson, 1986; Johnson and Henry, 1987; Johnson et al., 1987a,b; Onsager, 1980a,b; Quinn et al., 1989) chlorpyrifos (Johnson, 1986; Johnson et al., 1987a), and dimethoate (Mukerji, 1981; Johnson and Henry, 1987) bran baits provide significant levels of grasshopper control. The baits' lower concentrations of active ingredient and reduced rate of drift result in less residue buildup on crops, and make these baits safer for most non-target organisms.

A few studies have examined the effects of bran bait (targeted for grasshoppers) on non-target arthropods (e.g. Charnetski and Hobbs, 1974; Mukerji, 1981; Quinn et al., 1990; Gregory et al., 1992). In spite of literature demonstrating that populations of small mammals may be adversely affected by the application of aqueous insecticide sprays (e.g. Barrett and Darnell, 1967; Barrett, 1968), similar research has not been conducted on the effects of grasshopper bait on these same populations.

In order to provide baseline data to assess the potential impact of widespread use of bran bait in a grasshopper control program on small mammals, laboratory populations of *Mus musculus* and *Peromyscus maniculatus* were exposed to carbaryl, chlorpyrifos or dimethoate bran bait. Bran bait was offered to breeding pairs of mice along with their regular food to determine the palatability of the bait, as well as its effects on the survival and reproductive success of the mice.

MATERIALS AND METHODS

Experimental animals were *M. musculus* (RML strain) and *P. maniculatus*, reared, at the Agriculture Canada Research Station, Lethbridge, Alta., at a constant air temperature of 20°C, 50% r.h., 12:12 light/dark schedule, with an air flow of 17 air changes h^{-1}. Four-month-old virgin mice were housed in 25 cm×20 cm×12 cm aluminum cages and fed laboratory mouse chow (Wayne Lab-Blox, 24.0% crude protein, 4.0% crude fat, 4.5% crude fiber).

M. musculus

One-hundred and twenty mice (60 breeding pairs) were housed in cages, one pair per cage, arranged in a randomized complete block design. There was a total of 15 blocks, each block consisting of four treatments: carbaryl bait, chlorpyrifos bait, dimethoate bait or no bait (control group). Each cage contained ad libitum quantities of mouse chow and water, as well as one bran

bait feeder consisting of a 2 g vial secured to a petri dish to contain any scattered bran.

Mice of approximately the same size (males, 40.6 g, S.E.M. = 0.13, $n = 60$; females, 34.2 g, S.E.M. = 0.17, $n = 60$), were weighed, put in cages (one male and one female per cage) and allowed to acclimatize for 24 h. After this acclimatization period, mouse chow was removed from each cage and the bran bait feeders were filled with 2.0 g of bait. After 24 h, mouse chow was resupplied to the mice. Every 7 days mice were weighed and moved to fresh cages with clean bedding and fresh bait in feeders but no mouse chow. Mouse chow was resupplied after 24 h. The bran bait feeders were refilled throughout the trials whenever they were more than half empty.

In addition to recording weekly changes in adult weight, daily assessments of behavior and well-being were also made. Upon littering, the time to delivery, number of pups per litter and the birth weight and vitality of the pups were noted. The breeding pairs and pups were subsequently killed with CO_2 gas, following the guidelines of the Canadian Council on Animal Care (C.C.A.C., 1984). The experiment continued for 7 weeks.

P. maniculatus

The experimental design for *P. maniculatus* was the same as that for *M. musculus*, with the exception that 64 breeding pairs (128 total) were used (males, 21.7 g. S.E.M. = 2.7, $n = 64$; females, 19.4 g, S.E.M. = 0.23, $n = 64$). In addition, *P. maniculatus* pairs were exposed to the bran baits (2.0 g) for 1 week only, from Day 2 to Day 8 of the experiment.

Bran bait formulation

Commercial formulations of carbaryl (Sevin XLR, Rhone Poulenc Canada, Mississauga, Ont.), and chlorpyrifos (Lorsban 4E, Dow Chemical Canada, Sarnia, Ont.) were used to prepare the bait used in this study. Bran bait formulations included 5% carbaryl, 3% chlorpyrifos and 5.3% dimethoate (active ingredient by weight), concentrations typically used in grasshopper control studies (e.g. Johnson et al., 1987a,b; Boetel et al., 1989a,b; Quinn et al., 1989). The carbaryl bait was formulated as described by Johnson and Henry (1987), and the chlorpyrifos bait was formulated as described by Johnson (1986). Commercially available dimethoate bran bait was used ('Hopper Stopper', Peacock Industries, Saskatoon, Sask.).

Statistical analyses

Analysis of variances were carried out on change in weight of adult mice over time and the time to delivery of mice between treatments using the GLM

procedure of SAS (Statistical Analysis Systems, 1985). Tukey's HSD procedure (Snedecor and Cochran, 1980), was used to perform multiple comparisons between means. Analysis of covariance was performed using the average weight per pup as the dependent variable and the number of pups per litter as the covariates. Non-orthogonal contrasts were used to assess which treatment accounted for the significance within the model. Pup mortality for each bait treatment was adjusted using the modified Abbott's formula (Connin and Kuitert, 1952).

RESULTS

Mortality occurred in the chlorpyrifos bait treatment only (*M. musculus*: females, 40% mortality; males, 0%. *P. maniculatus*: females, 81% mortality; males, 56%). Only one pair of *P. maniculatus* in the chlorpyrifos bait treatment survived to litter, and thus time to delivery, pup weight and pup mor-

TABLE 1

Bran bait provided to *M. musculus*

Treatment	Total supplemented (g)	Average/cage (g)
Chlorpyrifos bait	98	6.5
Dimethoate bait	77	5.1
Carbaryl bait	35	2.4

TABLE 2

Average weights (g) of male and female *M. musculus* over time ($n = 15$ for each treatment)

Treatment	Mean (Week 2)	
	Males	Females
Carbaryl bait	41.0a	34.1a
Control	40.5a	36.7ab
Dimethoate bait	40.0ab	34.9ab
Chlorpyrifos bait	37.8b	32.7b
Treatment	Mean (Week 3)	
	Males	Females
Carbaryl bait	41.9a	39.1a
Control	41.2a	44.6a
Dimethoate bait	40.8ab	41.9a
Chlorpyrifos bait	38.7b	38.0a

Means with the same grouping letter within a week are not statistically different ($P > 0.05$).

TABLE 3

Effect of bran bait on *M. musculus* time to delivery (days)

Treatment	Mean	Grouping
Carbaryl bait	32.4 ± 11	a
Dimethoate bait	29.4 ± 12	b
Control	26.1 ± 10	c
Chlorpyrifos bait	25.5 ± 6	c

Means with the same grouping letter are not statistically different ($P > 0.05$).

TABLE 4

Effect of bran bait on *M. musculus* pup weight

Treatment	Mean	*N*	S.E.M.
Control	1.85	69	0.033
Carbaryl bait	1.88	63	0.031
Dimethoate bait	1.95	63	0.041
Chlorpyrifos bait	1.47	18	0.072

TABLE 5

Adjusted percentage mortality[1] of *M. musculus* pups

Treatment	Initial no.	Final no.	Abbott's % mortality
Control	74	69	–
Carbaryl bait	71	63	4.8
Dimethoate bait	65	63	−4.0[2]
Chlorpyrifos bait	36	18	46.4

[1]Modified Abbott's Percentage Mortality $= 100\ 1 - \left[\frac{T2 \times C1}{T1 \times C2}\right]$

where $T1$ is the total number of pups alive in treated group at birth; $T2$ is the total number of pups alive in treated group after 12 h; $C1$ is the total number of pups alive in control group at birth; $C2$ is the total number of pups alive in control group after 12 h (Abbott, 1925; Connin and Kuitert, 1952).
[2]No significant treatment mortality, $P > 0.05$.

tality could not be calculated for this treatment. *P. maniculatus* adult weight, time to delivery, pup weight and pup survival in the carbaryl and dimethoate bait treatments did not differ significantly from the controls and these results are presented for *M. musculus* only.

The amounts of bran bait to which the *M. musculus* were exposed is shown in Table 1. Mice in the chlorpyrifos bait treatments were most often supple-

mented with additional bran bait, followed by those in the dimethoate and carbaryl treatments.

Male and female *M. musculus* experienced delayed weight gains in the chlorpyrifos bait treatment. Average weights for male *M. musculus* were significantly lower in the chlorpyrifos bait treatment than in the control or carbaryl bait treatments for Week 2 and Week 3 (Table 2). *M. musculus* female average weights were significantly lower for mice exposed to chlorpyrifos bait than for control mice for Week 2 (Table 2). Mouse weights were not significantly different between treatments for the other weeks of the study (data not shown).

A significant interaction between *M. musculus* time to delivery and treatment was recorded. The time to delivery was significantly longer for *M. musculus* in the carbaryl and dimethoate bait treatments than for mice in the control or chlorpyrifos bait treatments (Table 3). The time to delivery was not significantly different between control mice and chlorpyrifos bait mice.

M. musculus pup weight was significantly lower in the chlorpyrifos bait treatment than in the other treatments (Table 4). There was a significant interaction between *M. musculus* pup weight and treatment ($P<0.05$). *M. musculus* pups in the chlorpyrifos bait treatment also incurred significantly higher mortality than pups in the other treatments (Table 5).

DISCUSSION

Significant levels of mortality were observed for both species of mice upon exposure to chlorpyrifos bran bait. Although it was not possible in these experiments to measure the amount of bran bait ingested by the mice (owing to its incorporation into their bedding), preliminary feeding trials demonstrated this amount to be minimal. Consequently, the observed levels of mortality may be more a result of the dermal toxicity than the oral toxicity of the bait. Both species of mice avoided contact with carbaryl bait, leaving their bait feeders virtually untouched. Mice in the chlorpyrifos and dimethoate bait treatments, however, routinely scattered the bait throughout their cages, as shown by the greater weight of bait provided them. Mice in these treatments were therefore exposed to more bran bait.

Carbaryl bait was less toxic to the mice than chlorpyrifos bait, possibly owing to the apparent reluctance of the mice to come into contact with carbaryl bait. Also, carbaryl has a lower mammalian toxicity (oral LD_{50} to rats=250 mg kg^{-1}, carbaryl; 145 mg kg^{-1}, chlorpyrifos, Merck Index, 1983). In addition, while both carbamate (carbaryl) and organophosphorus (chlorpyrifos) insecticides act by inhibiting cholinesterase activity at synaptic junctions, the effects of carbamate-induced inhibition are short-lived and reversible (Kurtz et al., 1989), unlike organophosphorus inhibition.

Female *M. musculus* and *P. maniculatus* were more susceptible to the effects of chlorpyrifos bait than males. This difference may reflect the smaller size and greater surface area to body mass ratio of the females, and thus, relatively greater area over which dermal toxicity can occur. Alternatively, the apparent gender differences may be physiological in nature and stem from hormonal or biochemical differences which favor the accumulation of insecticide. Klevay (1971), for example, demonstrated that male rats were up to 12 times more efficient than females in eliminating endrin through their bile systems. The apparent intolerance of female mice to this organophorus insecticide indicates the potential for significant disruptions to small mammal fecundity with the use of chlorpyrifos bait in a grasshopper control program.

The time to delivery for *M. musculus* was significantly longer for mice exposed to carbaryl and dimethoate bran bait, suggesting some influence of the baits on the estrous cycle of these mice. The significance of this effect on naturally breeding colonies of mice was illustrated by Barrett (1968), who found that a single field application of carbaryl delayed cotton rat (*Sigmodon hispidus*) reproduction by as much as 4 weeks, leading to a lag in population density. Budreau and Singh (1973) noted that laboratory mice exposed to dimethoate experienced a longer reproductive interval, a condition these authors attributed to the insecticide's effect on production of follicle stimulating hormone. The delay in time to delivery measured in this study, however, would probably have no significant effect on field populations of mice.

On average, *M. musculus* pups in the chlorpyrifos bait treatment had smaller litters, fewer of the adults littered, and the pups had significantly smaller birthweights and were less healthy than pups in the carbaryl or dimethoate bait treatments (as shown by their higher mortality shortly after birth). In addition, evidence of teratogenesis in several litters and the indirect evidence of resorption (lack of littering in several females which had previously been obviously pregnant) suggest that the effect of chlorpyrifos bait on offspring was more severe than the data indicate. These results are comparable to those of Deacon et al. (1980) who observed significant levels of fetotoxicity when chlorpyrifos was orally administered to pregnant mice.

The incorporation of chlorpyrifos into a bran bait rather than an aqueous spray formulation may not offer any clear environmental advantages to small mammal populations. Carbaryl and dimethoate baits, however, did not appear to affect either species of mouse, with the exception of a short-term delay in the length of time to littering. The use of carbaryl and dimethoate bran baits to control grasshoppers would therefore not significantly impact mouse, and possibly other small mammal, populations. The rate of bran bait used to control grasshoppers is low enough that field populations of mice would encounter very little bait. However, as mice display no avoidance behavior to chlorpyrifos bait, active foraging in the field could potentially put them into contact with toxic levels of this bait. Field trails are necessary to confirm this.

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