LETHALITY AND SOCIAL DISTRIBUTION OF DIETARY ^{14}C MIREX IN RETICULITERMES FLAVIPES (KOLLAR)

By

GLENN R. ESENTHER
and
ELIZABETH A. McMAHAN

March 1979

This article was written and prepared by U.S. Government employees on official time, and it is therefore in the public domain.

FOREST PRODUCTS LABORATORY

MADISON, WISCONSIN · 53705

UNITED STATES DEPARTMENT OF AGRICULTURE

FOREST SERVICE

In Cooperation with the University of Wisconsin

LETHALITY AND SOCIAL DISTRIBUTION OF DIETARY ^{14}C MIREX IN *RETICULITERMES FLAVIPES* (KOLLAR)

by

Glenn R. Esenther and Elizabeth A. McMahan[1/]

ABSTRACT

Radioactive labeled mirex (5,005 dpm/mg; 210 ppm mirex) in red dyed cellulose diet was fed to worker termites to determine its lethality, rate of fecal loss, and rate of transfer between donors and recipients. Minimum lethal dosage of mirex in dead termites was estimated at 0.1 ppm of their original live weight. Fecal loss of ingested mirex began during a 4-hour feeding acquisition interval and the loss was greater than 50% within 24 hours. Efficiency of mirex transfer to recipients was low (at best only 12.7% to large workers). Implication of the results for termite control with insecticidal bait is discussed.

INTRODUCTION

Previous study of ^{14}C-dietary mirex for termites indicated that unnatural solitary feeding and nonhomogeneous distribution of the insecticide in filter paper diet affected the results (McMahan and Esenther 1979).

1/ Respectively, Research Entomologist, Forest Products Laboratory, USDA Forest Service, Madison, Wis. , and Professor, Dept. of Biology, University of North Carolina, Chapel Hill.

In the present study, termites fed as a group, and distribution of insecticide in cellulose powder was homogeneous. This study seeks to determine lethality of ingested mirex, its loss in feces, and its transfer between donors and recipients.

MATERIALS AND METHODS

Reticulitermes flavipes (Kollar)(Isoptera, Rhinotermitidae) were collected at Janesville, Wis., April 28 and July 8, 1976, and maintained in 20-gallon metal trash cans prior to use in trials in May, June, and July.

Specific activity of the ^{14}C mirex was 2.34 x 10^7 disintegrations per minute (dpm) per milligram (McMahan and Esenther 1979). It was presented to the termites in red dyed (0.5% Calco oil red N-1700, American Cyanamid Co.) microcrystalline cellulose powder (Avicel[R], American Viscose Corp.) diet with 50% distilled water dampness and 5,005 dpm/mg mirex radioactivity (210 ppm mirex). Radioactivity in samples was measured as described previously (McMahan and Esenther 1979), and involved perchloric acid digestion of solids.

Radioactivity readings were obtained for feces and either whole termite bodies or body parts. Separate readings of body surface wash and body interior were obtained with surface rinse in 1 ml ice-water chilled acetone or dioxane. The chilled solvent reduced reflex gut evacuation by termites during the washing. Termite body readings were further separated into a digestive tract (gut) reading and a body shell

reading by extracting the gut along with a few distal abdominal segments. Scintillation counting dropped to <50% efficiency if a sample contained too much solid matter; therefore, never more than 10 termites were placed in a scintillation counting vial. For that reason also, mirex was eluted from diet or diet-feces mixture with three acetone (extraction efficiency ca. 58%) or dioxane (extraction efficiency near 100%) washes. Each of these washes involved 2 to 5 ml solvent as required by the quantity of mixture.

Groups of at least 100 larger workers (undifferentiated termites, probably 6th stage or older) were selected for feeding exposure to dietary ^{14}C mirex. Before this exposure, they were placed on moistened sand (15% moisture content) for a 24-hour starvation to clear their digestive tract of colored material. Prior to a donor-recipient experiment, recipients were similarly starved. Duration of ^{14}C-mirex feeding differed for individual experiments. Two sizes of cylindrical plastic containers were used to confine experimental termites during incubation at 25°C: 2.5 x 2.5 cm if group size was <50 and 5.0 x 3.5 cm if group size was >50. Small containers were supplied with 0.5 cc diet and large containers with 1.0 cc.

The forms, relative sizes, and vigor of termites in a colony varies during the year (Esenther 1977). Therefore, additional information on termite weight changes may be of value. Our larger-sized workers (LW) had an average weight near 3.0 mg in May and near 4.0 mg in July. Caste composition of source colonies in July indicated that the termites were

at or near peak vigor. Designations and average relative sizes for other forms were:

smaller workers (SW) . . . . . ca. 1/2 LW size

small nymphs (SN) . . . . . slightly smaller than LW

neotenic reproductives (NR) . . . . . ca. twice LW size

soldiers (S) . . . . . slightly larger than LW

Specific details for individual experiments follow.

Feeding–Lethality Relationship

An initial group of 95 LW was confined on dyed ^{14}C diet. At 10–minute intervals for 10 to 90 minutes, 5 LW were selected which appeared to have ingested the most red diet. Each termite in these 9 groups, plus a group of 5 nonexposed LW, was placed on plain, moist, cellulose diet in a separate container (total, 50 containers). Thereafter, at 7 daily inspections any moribund or dead termite was prepared for acetone body wash and body determinations of radioactivity. All termites remaining at the 7th (final) inspection were prepared for dpm counting. Acetone elutions of fecal ^{14}C from the cellulose diet in each container also were prepared for dpm counts.

Fecal Loss of ^{14}C

Fecal loss of ingested ^{14}C seemed to begin about 4 hours into a feeding acquisition interval (McMahan and Esenther 1979); therefore, an experiment was initiated to investigate further the rate at which ingested ^{14}C was lost in feces. Five groups of 50 LW were confined on the standard

mirex diet. From each group, 3 sets of 5 workers with the most red in their gut were selected after 4 hours and again after 8 hours of feeding exposure (total, 6 sets from each 50 LW group). The 1st set was prepared for acetone body wash, gut, and body shell radioactivity counts immediately. The 2nd and 3rd sets were placed on cellulose diet for either one or two days. At the end of their post-acquisition feeding, they too were prepared for the three types of dpm counts. Acetone washes of feces-diet mixtures from sets 2 and 3 also were prepared for radioactivity counts.

Soldier Pick-Up of Dietary ^{14}C

R. flavipes soldiers (S) were used to measure body surface pick-up of dietary ^{14}C mirex because they cannot self feed or groom. Five S were confined on standard ^{14}C diet for 4 hours. Radioactivity in their acetone body wash, gut, and body shell was determined.

Donor-Recipient Transfer of ^{14}C

Donors were fed red-dyed cellulose powder for three days prior to a 24-hour starvation period and a 5-hour mirex feeding period. Intense redness of their body tissues permitted easy distinction between them and recipients.

Five replicates of three series (A, B, and C) of donor-recipient groups were initiated. For combinations of number and type in these series, see Table III. After ^{14}C-mirex exposure of donors, each donor-recipient group mingled on plain moist cellulose diet for 24 hours

before dpm counts of dioxane body wash and body were made. The ^{14}C activity in dioxane wash of the feces-diet mixture also was determined.

RESULTS

Feeding-Lethality Relationship

Selection of the reddest termites from a pool of workers after the 10 minute intervals of ^{14}C-mirex acquisition identified reasonably well those termites having the most ^{14}C mirex. Only one obviously erroneous dpm reading was recorded (Table I, footnote 2). Low elution efficiency of acetone made estimates of fecal radioactivity suspect, enlarging the probable degree of error in estimates of total dpm pick-up during feeding acquisition. Nevertheless, average total dpm pick-up per termite progressed from 0 to 50.8 dpm in the 0 to 30 minute feeding sets. The average pick-up was between 35.0 and 95.8 dpm in the 40 to 90 minute sets. These had erratic progression of pick-up with increasing time because selection of redder termites became increasingly difficult after nearly all termites in the selection pool had redness in their gut. Total dpm pick-up by individual termites in the 40 to 90 minute sets ranged from 15 dpm to 217 dpm (avg. 52.1). Only data for the 0 to 50 minute sets were summarized in Table I because of the difficulty in selecting the reddest termites after longer feeding intervals.

Insecticidal moribundity began with the shortest (10 minute) ^{14}C-mirex feeding interval. With all the other feeding intervals, moribundity or death was nearly complete. During the 7-day post-acquisition period,

the average number of days for mortality, including moribundity, declined from >7 to 4.2 days in the 0 to 30 minute sets. Thereafter, sets with long (40 to 90 minute) acquisition intervals stabilized at mortality in 4 to 5 days.

Amount of radioactivity in the bodies of termites permits an estimate of the minimum lethal dosage for mirex. Table I shows that a total of 21 termites became moribund or died. Nine of these termites averaged only 7.1 dpm (range 2 to 10) in the body (including the gut) at the time of death. Relative to 0.210 nanograms (ng) mirex per 5,005 dpm, that average is equivalent to 0.3 ng mirex. Because average live worker weight was 3 mg, the 0.3 ng converts to lethality with retention of 0.1 ppm mirex per live weight of a termite. One of the survivors had 12 dpm radioactivity in its body, but it is not known how much of this activity was absorbed in body tissue or remained in the gut.

Fecal Loss of ^{14}C

Data on distribution of ^{14}C after 4- or 8 hour acquisition feeding and 0, 1, or 2 day post-acquisition intervals were summarized (Table II). Immediately after acquisition, the termites had about 20% less total dpm with the 8-hour feeding than with the 4-hour feeding. This difference indicates that the rate of ^{14}C gained through ingestion during the last four hour part of the 8-hour feeding period was surpassed by the rate of ^{14}C lost through defecation. Reflex gut evacuation from handling termites with forceps also may have reduced ^{14}C in the 8-hour acquisition set because the ^{14}C mirex would have more time to accumulate near the anus.

Total amount of radioactivity was progressively smaller in termites with increasing post-acquisition interval. Inefficient acetone elution of fecal ^{14}C may account for such loss. Total percentage of radioactivity either in feces or unaccounted for in sets with post-acquisition intervals was 73.5% (1-day set) and 75.7% (2-day set) after 4-hour acquisitions, and 56.9% (1-day set) and 80.0% (2-day set) after 8-hour acquisitions. Very little of the relative total dpm in these sets remained in the termite body shell (13.5% or less).

Soldier Pick-Up of Dietary ^{14}C

After confinement on mirex diet for 4 hours, the 5 soldiers had accumulated 1,540 total dpm (avg. 308). This was considerably more than when only radioactive feces were available to the termites (McMahan and Esenther 1979). Nearly all of the ^{14}C (92.4%) was in the acetone body wash. Small amounts were recovered in the body shell (6.0%) and gut (1.6%), but this may have been residue left by the inefficient acetone wash. If workers also had been present, a considerable amount of the body surface radioactivity would have been removed by the grooming habit of the termites. The results, therefore, indicate that body surface contamination may contribute notably to termite radioactivity.

Donor-Recipient Transfer of ^{14}C

Data on distribution of ^{14}C after uniting donors and recipients for 24 hours are summarized in Table III. After acquisition feeding, the ^{14}C carried over by individual groups of 5 donors had a range of 774 to 5,366 dpm, and with groups of 50 donors the range was 24,219 to 47,846 dpm.

This considerable variability occurred in most of the sets. Nevertheless, the low of 774 dpm shows that appreciable amounts of radioactivity always were available for transfer to recipients. The replicates of donors in series A, B, and C came from the same five replicates of donor selection groups. The total dpm range for the donors from these groups was 40,782 to 64,857, which indicates that variability of radioactivity in donor sets involved differences in acquisition rates by both individual termites and groups.

The low percentage of total radioactivity in feces (avg. 0.5 to 5.0% for the 3 series) contrasts greatly with the more than 50% total dpm in feces in our previous experiments. Perhaps in the previous experiments the combination of dye and radioactive material in the diet caused excessive defecation. In the present experiment, dye and radioactivity were acquired from separate diets; this procedure did not result in major loss of fecal radioactivity from donors. Natural fluctuation in the condition of termites (Esenther 1977) also may contribute to such differences.

Transfer of ^{14}C from donors to recipients had low efficiency; most of the ^{14}C remained with the donors (66.3 to 90.1% of total dpm), primarily in the body. Transfer of radioactivity to the bodies of recipients was greatest when the donor-recipient ratio was 1:5 (series B; transfer of 24.2% total dpm) and decreased with lower ratios (1:1 series A, 7.6% of total dpm and 2:1 series C, 7.7% of total dpm). The greatest transfer in the 3 series was to the LW type recipients and the least to the S type.

Except in series A, the transfer of ^{14}C into SW, SN, or NR forms was less than half of that into the LW form. The slight transfer of ^{14}C mirex to NR forms was unexpected because the nutritional requirements for egg production should have made neotenic females the most voracious recipients. In mixed caste groups, therefore, dietary exchanges may occur predominantly between the larger sized R. flavipes workers.

The data permit comment on the possible lethality of mirex in recipients. The average ingestion of mirex by the least efficient recipient (S type) was 3.0 ng (series A), 0.7 ng (series B), or 4.0 ng (series C) mirex per termite. All of these averages are near the previously estimated minimum lethal dosage for large workers, but if fecal loss of the ingested mirex was similar to that by the workers the soldiers would be unlikely to die from the mirex. By such reasoning, few recipients acquired a lethal dose. This analysis agrees with our previous notation (McMahan and Esenther 1979) that little mortality occurred in a donor-recipient experiment.

DISCUSSION

Goals of this study included estimation of a minimum lethal dosage for mirex in termites, rates of ingestion, and transfer of dietary ^{14}C-mirex between donors and recipients. The pick-up of ^{14}C mirex by termites was somewhat erratic during 10- to 90-minute feeding intervals (lethality experiments), but it was reasonably uniform and high, on the

average, with a 4- to 5-hour acquisition interval (fecal loss and donor-recipient experiments). Acquisition feeding for 8 hours proved less satisfactory because the rate of ingestion was offset by the rate of defecation (fecal loss experiment). Use of a more efficient solvent than acetone to elute ^{14}C from feces should improve the precision in similar experiments.

Average minimally lethal retention in the body of worker termites was 0.1 ppm mirex at the time of death. This supports Beard's (1974) conclusion that R. flavipes is highly sensitive to mirex. Sensitivity of termites in natural colonies has not been established.

Estimation of insecticide dosage for decayed wood termite bait in field trials requires a large safety factor to compensate for an unknown, but large, tolerance to mirex in natural populations of Reticulitermes (Esenther 1979). Direct ingestion of the 210 ppm mirex in our diet was fatal to nearly all the termites with greater than a 10-minute acquisition time. Esenther (1979) and McMahan and Esenther (1979), however, noticed an increase in tolerance to mirex in donor-recipient groups. Although Esenther and Beal (1978) demonstrated that 10 mg mirex (4,000 ppm) in bait blocks was effective in suppressing foraging activity of Reticulitermes, Beard (1974) concluded that 1 mg in the bait also would be effective. Unfortunately any further work with mirex in bait will have only academic value because the Environmental Protection Agency is unlikely to accept any use of this insecticide.

Continuing work with anti-termitic agents in decayed wood bait will have to consider the impact of insecticidal bait on the termites. Low dietary transfer of insecticide between donors and recipients indicates that lethality of bait treatment results primarily from direct ingestion by some of the foragers. Hence foragers must make repeated contact with bait before enough of them are affected to have a serious impact on a colony. Indeed, field trial results of Esenther and Beal (1974; 1978) show that bait treatment required about a year before termite activity was reduced to a low level and maintained there. Their results also show that each contact or recontact with bait blocks by termites involved typically the removal of only part of the block. Perhaps an anti-termitic agent other than mirex will not suppress the initial contact with a bait block quite so quickly; if so, a greater number of foragers will ingest the agent. Another agent also might be transferred more readily to recipients; however, the dilution factor in such transfers indicates that only a microbial disease-type agent might have a significant impact on the recipients.

STATEMENT

Mention of a chemical does not constitute a recommendation; only those chemicals registered by the U.S. Environmental Protection Agency may be recommended, and then only for uses as prescribed in the registration--and in the manner and at the concentration prescribed. The list of registered chemicals varies from time to time; prospective users, therefore, should get current information on registration status from Pesticides Regulation Division, Environmental Protection Agency, Washington, D.C. 20460.

Mention of tradenames is for readers' convenience only and should not be construed as a recommendation by the U.S. Department of Agriculture.

REFERENCES

Beard, R. L. 1974. Termite biology and bait block method of control. Conn. Agric. Exp. Stn. Bull. 748. 19 pp.

Esenther, G. R. 1977. Fluctuation of survival, growth and differentiation in biweekly samples of Reticulitermes flavipes workers. Mater. and Org. 12:49-58.

Esenther, G. R. 1979. Estimation of insecticidal dosage for wood termite baits from three bioassays. [In preparation.]

Esenther, G. R. and R. H. Beal. 1974. Attractant-mirex bait suppresses activity of Reticulitermes spp. J. Econ. Entomol. 67:85-88.

Esenther, G. R. and R. H. Beal. 1978. Insecticidal baits at field plot perimeters suppress Reticulitermes. J. Econ. Entomol.

McMahan, E. A. and G. R. Esenther. 1979. Tracing radioactive mirex in Reticulitermes flavipes (Isoptera: Rhinotermitidae). Sociobiology (in press).

Table I.--Distribution and lethality of dietary ^{14}C mirex at 7 days after 0 to 50 minute feeding intervals

Acquisition interval	Radioactivity[1/] (dpm) in				Number dead	Average number days to death
	Termite		Feces	Total		
	Body wash	Body				
Min	($\bar{X}$ ± SD)	($\bar{X}$ ± SD)	($\bar{X}$ ± SD)	($\bar{X}$ ± SD)		
0	0.0 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.0 ± 0.00	0	>7.0
[2/]10	3.0 ± 2.94	3.75 ± 4.35	1.25 ± 1.50	8.0 ± 8.21	2	>6.3
20	10.0 ± 6.44	13.0 ± 6.44	2.2 ± 1.31	25.2 ± 12.11	5	5.2
30	13.4 ± 15.11	14.6 ± 8.32	22.8 ± 15.00	50.8 ± 14.96	5	4.2
40	12.4 ± 8.33	18.6 ± 12.13	6.8 ± 5.76	37.8 ± 12.23	[3/]4	>4.8
50	9.8 ± 4.82	13.2 ± 6.02	12.0 ± 16.32	35.0 ± 16.77	5	4.4

1/ Relative to feeding acquisition of 5 large workers as replicates.
2/ Excludes one replicate with 147 dpm in body wash but 0 dpm in both body and feces.
3/ Survivor had 12 dpm in body.

Table II.--Distribution of radioactivity at 0, 1, and 2 days after 4- and 8-hour acquisition intervals by 5 replicates of 5 worker termites in groups[1/]

Acquisition	Post acquisition	Distribution of relative % dpm in				Total dpm acquired	
		Body wash	Gut	Body shell	Feces	$\bar{X}$ ± SD	Relative percent[2/]
H	D						
4	0	20.1	48.8	31.1	--	610.2 ± 127.93	100.0
	1	5.1	16.8	4.6	56.5	359.8 ± 185.81	83.0
	2	8.1	11.6	5.2	44.9	310.9 ± 86.23	69.8
8	0	23.7	27.6	26.7	--	474.4 ± 162.30	78.1
	1	15.1	14.5	13.5	27.2	353.1 ± 240.08	70.3
	2	4.6	7.3	8.1	32.7	227.9 ± 101.51	52.7

1/ Corrected for 58% acetone elution efficiency of fecal radioactivity.

2/ Based on radioactivity immediately after 4-hour acquisition feeding as 100%.

Table III. --Distribution of ^{14}C radioactivity (dpm) at 24 hours after donor and recipient termites united

Experimental series	Percentage of total dpm in Donor: Number and form	Donor: Bodies	Donor: Body wash	Recipient: Number and form	Recipient: Bodies	Recipient: Body wash	Feces	Total dpm[1/] ($\bar{X}$ ± SD)
A	5 LW	82.6	1.3	5 LW	12.7	0.5	2.8	1,723.7 ± 870.97
	5 LW	87.5	1.8	5 SW	8.2	0.7	1.8	3,248.8 ± 242.07
	5 LW	88.1	3.3	5 SN	5.6	0.5	2.4	2,873.4 ± 221.71
	5 LW	76.9	4.0	5 NR	9.7	1.2	8.2	3,514.5 ± 1,240.05
	5 LW	89.5	5.6	5 S	1.7	0.9	2.2	3,723.4 ± 1,040.47
	Average	84.92	3.20		7.58	0.76	3.38	3,016.74 ± 789.538
B	5 LW	61.5	4.8	5 LW	11.5	1.4	5.0	2,103.4 ± 480.73
				5 SW	3.2	0.6		
				5 SN	4.3	0.6		
				5 NR	4.4	1.2		
				5 S	0.8	0.6		
Total	5	61.5	4.8	25	24.2	4.4	5.0	
C	50 LW	80.3	9.8	5 LW	4.4	0.5	0.5	31,014.0 ± 12,363.36
				5 SW	1.2	0.3		
				5 SN	1.0	0.2		
				5 NR	0.8	0.3		
				5 S	0.3	0.2		
Total	50	80.3	9.8	25	7.7	1.5	0.5	

1/ Five replicates.