Toxicity of Sulfluramid to *Coptotermes formosanus* (Isoptera: Rhinotermitidae)

by

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ABSTRACT

The topical LD_{50} of sulfluramid (N-ethyl perfluorooctane sulfonamide) to the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, was found to be 9.04g/g (8 days after topical application), 8.16g/g (10 days), and 7.78g/g (14 days). When groups of *C. formosanus* were fed filter papers containing sulfluramid, 10ppm elicited complete mortality within 13 days, 100ppm within 5 days, and 1000ppm within 3 days. With 100ppm, visual symptoms of sulfluramid intoxication appeared by the second day of exposure. When termites were exposed to papers treated with up to 20ppm sulfluramid for only 24 hours, no mortality resulted, indicating that repeated feeding is necessary at these low concentrations to achieve a lethal dose. In laboratory bioassays with untreated cardboard from two different sources, one cardboard was more acceptable to *C. formosanus* than the other. When termites were exposed to cardboard containing 100ppm sulfluramid (FirstLine™, FMC Corp.), they readily explored it and died within one week. Feeding was not apparent on the treated cardboard, indicating that a toxic dose was ingested by minimal tasting of the cardboard surface. These laboratory results suggest that (1) sulfluramid concentrations of less than 100ppm may be desirable and effective against *C. formosanus* as a result of the greater susceptibility of this species to sulfluramid in comparison to *Reticulitermes* spp., but must be balanced against the length of the exposure period that would be required to accumulate a lethal dose; and (2) superficially similar bait matrices such as cardboard produced by different manufacturers may differ in their acceptability to *C. formosanus*, and efforts must be made to insure use of a consistently acceptable matrix.

INTRODUCTION

Sulfluramid (N-ethyl perfluorooctane sulfonamide) is a relatively slow-acting toxicant formulated in baits for cockroach, ant and termite control (Vander Meer *et al.* 1985; Ballard 1997, 1999; Scheurer *et al.*

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1999). With respect to termite control, Su et al. (1995) reported a reduction in the size of Formosan subterranean termite, *Coptotermes formosanus* Shiraki, colony populations as a result of the application of wooden blocks impregnated with sulfluramid; although these populations appeared to gradually increase again after the removal of the toxic baits (Su & Scheffrahn 1996). Felix & Henderson (1995) reported the absence of any detectable *C. formosanus* activity following the application of sulfluramid baits around underground cables in manholes; and Henderson & Forschler (1996) concluded that *C. formosanus* colonies occupying river-bound trees were eliminated, based on the absence of any feeding on untreated cardboard, by application of cardboard baits containing either 200ppm or 1000ppm sulfluramid. In an alternative approach using sulfluramid in a topically-applied groomable coating (Myles & Grace 1991), Myles (1994, 1996) also reported a reduction in termite activity as measured by decreasing numbers of termites collected at field sites following sulfluramid applications.

FirstLine™ and FirstLine™ GT (FMC Corp., Philadelphia, PA) termite bait stations, holding a corrugated cardboard bait containing 100ppm sulfluramid (Ballard 1999), are in commercial use throughout the United States (Lewis et al. 1998). In early reports on ongoing field studies, Forschler & Chiao (1998) indicated success against *Reticulitermes* spp. in some field applications when these baits were used in conjunction with spot insecticide treatments; and Gold & Howell (1998) reported suppression of termite foraging activity in the vicinity of the bait placements when the baits were used as stand-alone treatments, but some occurrence of termite foragers and alates in other parts of the treated structures. Thus, in considering the use of sulfluramid baits for *C. formosanus* control in Hawaii, it appeared to us that certain basic information would be useful in developing improved baiting methods for use against this serious pest.

Henderson & Forschler (1996) found that cardboard containing 200ppm and 1000ppm eliminated evidence of *C. formosanus* feeding activity in trees, but these authors were not able to confirm the efficacy of 100ppm sulfluramid in this particular study, and also commented that sulfluramid baits "seemed to have some repellency" in the field, although they were readily explored by termites in the laboratory. Although the current commercial concentration is ten-fold greater than the 10ppm "acceptance threshold" for *C. formosanus* reported by Su & Scheffrahn (1991) in laboratory studies with sulfluramid-impregnated pine wafers, the very different physical structure of solid wood blocks and thin cardboard makes it difficult to extrapolate from this work to try to define the appropriate concentration of sulfluramid in cardboard.

The ten-fold greater topical toxicity of sulfluramid to *C. formosanus* in comparison to *Reticulitermes flavipes* (Kollar) reported by Su & Scheffrahn (1988, 1991), however, raises the question of whether a bait containing a single concentration of sulfluramid is appropriate for both of these termite genera, or whether it would be useful to tailor the toxicant concentration to the particular genus of concern. For example, it has been found with *C. formosanus* that concentrated termite mortality within a particular portion of the nest gallery structure will cause other termites to avoid that area (Su *et al.* 1982). Although the manufacturer has not noted termite mortality within FirstLine™ bait stations placed in the field (Ballard 1999a), lower insecticide concentrations could further decrease the probability of such an occurrence by delaying the onset of symptoms of sulfluramid intoxication. Of course, a mitigating factor in reducing toxicant concentration in a bait is the reduced rate of ingestion of the insecticide by the feeding termites (Henderson & Forschler 1996), and thus the probable extension of the feeding time (or need for multiple feedings) in order to accumulate a lethal dose.

Corrugated cardboard (also known as fiberboard or boxboard) has a long and distinguished history as a material used by researchers to aggregate and collect subterranean termites (c.f., Esenther 1980, French & Robinson 1985, Grace 1989, Howard *et al.* 1982, La Fage *et al.* 1983). However, cardboard is locally produced by different manufacturers from different wood fibers and recycled cellulosic materials and may not be a consistent and homogeneous product. Thus, in addition to assessing the toxicity of sulfluramid against *C. formosanus* in Hawaii and examining dose-time-mortality responses, we were also interested in assessing *C. formosanus* feeding responses to superficially similar cardboard from different sources.

We report here the results of laboratory studies to (1) determine the topical toxicity of sulfluramid to *C. formosanus* in Hawaii, (2) establish the dose-time-mortality responses of *C. formosanus* to a range of concentrations of sulfluramid in paper, (3) determine the efficacy of a single 24-hour feeding exposure to low (8-20ppm) concentrations of sulfluramid, and (4) examine the responses of *C. formosanus* in a laboratory bioassay to untreated cardboard from two different sources and FirstLine™ cardboard containing 100ppm sulfluramid.

MATERIALS AND METHODS

Topical Toxicity. Technical sulfluramid (0.1g) (Griffin Corp., Valdosta, GA) was dissolved in 100ml acetone to make a stock solution. This stock solution was then diluted with acetone to obtain sulfluramid concentrations of 0.1, 0.07, 0.05, 0.35, and 0.025g/l. Termites (*C. formosanus*) were collected from a field colony immediately prior to their use in bioassays using a trapping technique (Tamashiro *et al.* 1973). Five groups of 10 workers (pseudergates, or undifferentiated individuals older than the third instar as determined by size) were weighed to obtain the average worker weight (4.33mg). Insecticide dosages were calculated on the basis of amount of toxicant per unit weight of termite. Using a Model 1002 Micro-jector (Houston Atlas Inc., Houston, TX), 0.52 µl of solution was applied to the dorsal surface of the termite. Termites were then placed on moist Whatman No. 1 filter paper in a petri dish, held in an unlighted incubator at 29°C, and checked daily for mortality. Dead termites were removed from each dish at each mortality check. Three replicates of 10 termites each were treated with each concentration of sulfluramid (and acetone controls). Observations were terminated after 14 days, and the 8-day, 10-day, and 14-day mortality data were subjected to probit analysis (Finney 1962) using a commercial statistical program (SAS Institute 1987).

Dose-Time-Mortality Feeding Response

Serial dilutions of a stock solution of technical sulfluramid in acetone (0.003g/ml) were applied by pipette in 2ml aliquots to individual Whatman No. 2 filter papers (9cm dia., ca. 0.6g) to obtain concentrations of sulfluramid on the papers of 0 (acetone controls), 1, 10, 100, or 1000ppm on a weight/weight basis (wt. a.i./wt. paper). Each air-dried paper was placed in a glass petri dish, with humidity provided by wetting a second filter paper secured to the inside of the cover with 1.5ml water. Termites (*C. formosanus*) were collected from an active field colony as described above, 20 workers were placed in each dish, and the dishes were held in an unlighted incubator at 29°C. There were three replicates of each insecticide concentration. Termites were monitored daily for 15 days for mortality, and the visible activity of survivors was rated on a scale of high, moderate, and low activity.

Single 24-hour Feeding Exposure

A stock solution of technical sulfluramid in acetone (1g/l) was serially diluted with acetone to obtain solution concentrations of 10, 4, and 2µg/ml. Whatman No. 2 filter papers (9cm dia.) were oven dried at 90°C for 18 hours, cooled in a desiccator and weighed. Based upon these individual paper weights, an appropriate amount (4ml acetone for the controls, and ranging from 2.21-3.72ml for each treatment) of one of the dilute solutions was applied to each paper by pipette to achieve sulfluramid concentrations on the papers of 0, 8, 10, 12, 15, or 20ppm on a weight/weight basis (wt. a.i./wt. paper). Each air-dried paper was placed in a glass petri dish, with a second damp (1.25ml water) filter paper secured to the inside of the cover to maintain humidity in the dish. Termites (*C. formosanus*) were collected from a field colony as previously described, and 100 workers were placed in each dish, and held in an unlighted incubator at 29°C for 24 hours, with six replicates for each insecticide concentration. After 24 hours, surviving termites in each replicate were transferred to a clean petri dish containing damp filter paper, and returned to the incubator. Termite activity was monitored daily, and destructive mortality counts were made of three replicates on day 8 and the remaining three replicates on day 15 (14 days after the 24-hour sulfluramid exposure).

Response to Treated and Untreated Cardboard

FirstLine™ bait stations containing corrugated cardboard with 100ppm sulfluramid were supplied by J. B. Ballard, FMC Corp., Philadelphia, PA. In order to assess the acceptability of untreated corrugated cardboard, samples were obtained from the eastern United States (Source A, obtained by J. B. Ballard) and from a local supplier in Hawaii (Source B, GBC Boxes & Packaging, Honolulu, HI). The cardboard samples were cut into 2.5 x 2.5cm squares, and six stacked squares were held together with an insect pin and placed on a square of aluminum foil on the surface of damp sand (150g silica sand, moistened with 30ml distilled water) at one side of a glass screw-top jar (8cm dia. x 10cm high). A Douglas-fir wood wafer (2.5 x 2.5 x 0.75cm) was oven dried, weighed, and placed on the surface of the sand on the opposite side of the jar to create a two-choice situation. Formosan subterranean termites were collected as previously described, and 200 termites (180 workers and 20 soldiers) were placed in each jar between the cardboard and the wood wafer. There were five replicates of each of the three treatments (FMC FirstLine™ cardboard with 100ppm sulfluramid, source A untreated cardboard, source B untreated cardboard). The test units were placed within an unlighted incubator at 28 C, and examined weekly to assess termite activity. After eight weeks, surviving termites were counted to determine mortality, the amount of cardboard consumed was estimated by visual examination, and the wood wafers were oven dried and weighed to measure mass loss from termite feeding.

RESULTS AND DISCUSSION

Depending upon the length of time after exposure, the topical LD_{50} values for sulfluramid on *C. formosanus* in Hawaii were 9.04µg/g (8 days), 8.16µg/g (10days), and 7.78µg/g (14 days). These are in the same range as the 14-day LD_{50} values previously reported for *C.*

formosanus in Florida of 9.94µg/g (Su & Scheffrahn 1988) and 6.95µg/g (Su & Scheffrahn 1991). Based on the results obtained by Su & Scheffrahn (1988, 1991) using similar methodology with *R. flavipes*, this would support the conclusion of those authors that topically applied sulfluramid is ca. ten-fold more toxic to *C. formosanus* than to *R. flavipes*.

Filter papers containing 1000, 100, or 10ppm sulfluramid elicited complete mortality of *C. formosanus* within 3, 5, and 13 days, respectively (Table 1). These are similar to the results reported by Henderson & Forschler (1996) from exposure to treated cardboard, in which 100ppm also resulted in complete mortality within 5 days, and 10ppm within 8 days. As did Henderson & Forschler (1996), we also noted that the termites exhibited symptoms of intoxication several days before dying (Table 1). *C. formosanus* exhibited a decrease in activity by the second day of exposure to 100ppm sulfluramid, severe lethargy by the third day, followed by high mortality on day four, and total mortality by the fifth day (Table 1). Thus, use of a lower toxicant concentration might be useful to minimize the probability of mortality occurring in the vicinity of the bait with this termite species, or the rapid appearance of severely intoxicated individuals within the colony who would be likely to be segregated, decreasing the probability of trophalactic transfer of the toxicant. Although the manufacturer has not observed termite mortality within the commercial bait stations (Ballard 1999a), possibilities that might further increase the distribution of toxicant through the colony are obviously worth considering.

Since 10ppm sulfluramid proved effective in eliciting complete termite mortality within a reasonable period of time (13 days) under conditions of constant exposure to the treated paper, our next bioassay was intended to determine whether prolonged and repeated feeding was

Table 1. Activity and cumulative mean percentage mortality of *Coptotermes formosanus* workers (3 groups of 20 workers per treatment) exposed to filter papers treated with sulfluramid for 15 days.

Conc.	Cumulative Mean Percentage Mortality Over 15 Days of Sulfluramid Exposure														
(ppm)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	0[a]	2[a]	2[a]	2[a]	2[a]	2[a]	10[a]	12[a]	13[a]	17[a]	17[a]	17[a]	18[a]	18[a]	18[a]
1	0[a]	2[a]	2[a]	2[a]	2[a]	2[a]	3[a]	5[a]	5[a]	5[a]	5[a]	5[a]	5[a]	5[a]	8[a]
10	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[b]	2[c]	33[c]	88[c]	97[c]	100		
100	0	0[b]	0[c]	93[c]	100										
1000	2[b]	93[c]	100												

[a]Surviving termites show high activity.
[b]Surviving termites show moderate activity.
[c]Surviving termites show low activity.

necessary to accumulate a lethal dose of sulfluramid at concentrations in this low range, or whether a single 24-hour feeding period would result in delayed mortality. Obviously, a "time bomb" effect would be more desirable than a scenario of repeated feeding followed by rapid mortality once a lethal dose was accumulated. However, our results (Table 2) indicate that a single 24-hour exposure to paper containing sulfluramid concentrations up to 20ppm is not sufficient to cause mortality. Thus, the dose-time-mortality pattern apparent in Table 1 indicates that within the range of 10 to 100ppm, feeding continues until a lethal dose is achieved, followed by death within ca. 3 days. Determining the ideal bait concentration thus requires balancing the time until the onset of lethargic symptoms and death against the length of the bait exposure and number of repeated visits to the bait that may be required to accumulate a lethal dose.

When Formosan subterranean termites were exposed to cardboard containing 100ppm sulfluramid from FirstLine™ stations, they readily explored the cardboard, despite the presence of Douglas-fir wood as an alternative food source, and all died within one week (Table 3). Interestingly, there was no visible damage to the treated cardboard (although a very small amount of wood was removed), indicating that minute "tasting" of the cardboard surface was sufficient to accumulate a lethal sulfluramid dose.

When termites were exposed to the two different untreated cardboards, one of these was fed upon to a much greater extent than the other, indicating that there was some feeding deterrence (as distinguished from immediate repellence) associated with the Source A cardboard (Table 3). These results indicate that quality control steps are important to insure that a bait matrix is consistently acceptable to the

Table 2. Mean percentage mortality of *Coptotermes formosanus* workers following a single 24-hour exposure to filter papers treated with low concentrations of sulfluramid.

Conc. (ppm)	Mean Percentage Mortality ± SD[a,b] Day 1	Day 8	Day 15
0	0.5 ± 0.8	7.3 ± 0.6	31.0 ± 12.5
8	1.2 ± 1.3	7.7 ± 1.5	15.3 ± 3.1
10	1.0 ± 1.3	5.3 ± 2.3	15.3 ± 2.5
12	0.5 ± 0.8	9.3 ± 7.0	11.7 ± 3.5
15	1.3 ± 1.2	9.0 ± 4.6	15.3 ± 5.1
20	1.7 ± 2.1	10.3 ± 6.7	23.0 ± 9.5

[a]Day 1 = six replicates of 100 workers per treatment. Mortality was subsequently evaluated in three of these replicates on Day 8 and in the remaining three replicates on Day 15.
[b]SD = standard deviation.

Table 3. Mean percentage of cardboard bait consumed, amount of wood consumed, and percentage mortality of *Coptotermes formosanus* (5 groups of 180 workers and 20 soldiers per treatment) in an 8-week, two-choice bioassay in which each cardboard bait was paired with a Douglas-fir wood wafer.

Cardboard	Cardboard Consumed (% ± SD)[b]	Wood Consumed (grams ± SD)[b]	Termite Mortality (% ± SD)[b]
100ppm sulfluramid	0.00 ± 0.00	0.05 ± 0.03	100.00 ± 0.00[a]
Source A Untreated	12.50 ± 5.00	0.77 ± 0.10	10.63 ± 1.84
Source B Untreated	39.00 ± 8.94	0.73 ± 0.23	8.70 ± 2.80

[a]All termites exposed to cardboard containing 100ppm sulfluramid died during the first week.
[b]SD = standard deviation.

relevant termite species when working with a material such as cardboard with a relatively high level of variation.

In sum, the results of our laboratory assays confirm the high toxicity of sulfluramid to *C. formosanus*, and suggest that (1) sulfluramid concentrations of less than 100ppm may be desirable and effective against *C. formosanus* as a result of the greater susceptibility of this species to sulfluramid in comparison to *Reticulitermes* spp., but must be balanced against the length of the exposure period that would be required to accumulate a lethal dose; and (2) superficially similar bait matrices such as cardboard produced by different manufacturers may differ in their acceptability to *C. formosanus*, and efforts must be made to insure use of a consistently acceptable matrix. Continuing research on the behavioral responses of different termite species and regional populations is critical in improving baiting methodology to have maximum impact on the variety of economically important species found in the United States and around the world.

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REFERENCES

Ballard, J.B. 1997. Termite baiting 101. Pest Control 65(2):30, 34, 36, 40.

Ballard, J.B. 1999. Systematic Termite Control: a termite (Isoptera: Rhinotermitidae) IPM program for the pest control professional. Proceedings of the 3rd International Conference on Urban Pests (W.H. Robinson, F. Rettich & G.W. Rambo, Eds.). Czech University of Agriculture, Prague, Czech Republic, 19-22 July 1999. pp. 367-371.

Ballard, J.B. 1999a. Personal communication.

Esenther, G.R. 1980. Estimating the size of subterranean termite colonies by a release-recapture technique. International Research Group on Wood Preservation Document No. IGR/WP/1333, 6 pp.

Felix, J. & G. Henderson. 1995. Debugging New Orleans' phone lines. Pest Control Technology 23:76, 78, 151.

Finney, D.J. 1962. Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve, Second Edition. Cambridge: Cambridge University Press. 318 pp.

Forschler, B.T. & E. Chiao. 1998. Field and laboratory tests of sulfluramid treated cardboard and FirstLine™ termite baits. Proceedings of the National Conference on Urban Entomology. San Diego, California, 26-28 April 1998. P. 113.

French, J.R.J. & P.J. Robinson. 1985. A technique used on mounds of *Coptotermes lacteus* to screen potential bait substrates. Journal of the Australian Entomological Society 24:111-112.

Gold, R.E. & H.N. Howell, Jr. 1998. Field tests of sulfluramid baits against subterranean termites. Proceedings of the National Conference on Urban Entomology. San Diego, California, 26-28 April 1998. P. 114.

Grace, J.K. 1989. A modified trap technique for monitoring *Reticulitermes* subterranean termite populations (Isoptera: Rhinotermitidae). Pan-Pacific Entomologist 65:381-384.

Henderson, G. & B.T. Forschler. 1996. Termite bait screening using naturally-infested trees. Proceedings of the 2nd International Conference on Insect Pests in the Urban Environment (K.B. Wildey, Ed.). Heriot-Watt University, Edinburgh, Scotland, 7-10 July 1996. pp. 449-458.

Howard, R., S.C. Jones, J.K. Mauldin & R.H. Beal. 1982. Abundance, distribution, and colony size estimates for *Reticulitermes* spp. (Isoptera: Rhinotermitidae) in southern Mississippi. Environmental Entomology 11:1290-1293.

La Fage, J.P., N.-Y. Su, M.J. Jones & G.R. Esenther. 1983. A rapid method for collecting large numbers of subterranean termites from wood. Sociobiology 7:305-309.

Lewis, R., J.B. Ballard & G. Cramer. 1998. Systematic Termite Control . . . results in the real world-the Orkin® experience in Florida. Proceedings of the National Conference on Urban Entomology. San Diego, California, 26-28 April 1998. pp. 109-112.

Myles, T.G. 1994. A new approach to subterranean termite control. Proceedings of the Annual Meeting of the Canadian Wood Preservation Association 15:61-79.

Myles, T.G. 1996. Development and evaluation of a transmissible coating for control of subterranean termites. Sociobiology 28:373-457.

Myles, T.G. & Grace, J.K. 1991. Behavioral ecology of the eastern subterranean termite in Ontario as a basis for control. Proceedings of the Ontario Ministry of the Environment Technology Transfer Conference. Vol. 2:547-544.

SAS Institute. 1987. SAS/STAT Guide for Personal Computers, Version 6 Edition. Cary, North Carolina: SAS Institute Inc.

Scheurer, S., M. Heising & M. Thiele. 1999. Fluoro-octane-sulfonamide (Alstar®) for controlling *Tapinoma melanocephalum* (Fabr.) (Hymenoptera: Formicidae). Proceedings of the 3rd International Conference on Urban Pests (W.H. Robinson, F. Rettich & G.W. Rambo, Eds.). Czech University of Agriculture, Prague, Czech Republic, 19-22 July 1999. pp. 221-224.

Su, N.-Y. & R.H. Scheffrahn. 1988. Toxicity and lethal time of N-éthyl perfluorooctane sulfonamide against two subterranean termite species (Isoptera: Rhinotermitidae). Florida Entomologist 71:73-78.

Su, N-Y. & R.H. Scheffrahn. 1991. Laboratory evaluation of two slow-acting toxicants against Formosan and eastern subterranean termites (Isoptera: Rhinotermitidae). Journal of Economic Entomology 84:170-175.

Su, N-Y. & R.H. Scheffrahn. 1996. Fate of subterranean termite colonies (Isoptera) after bait applications-an update and review. Sociobiology 27:253-275.

Su, N.-Y., R.H. Scheffrahn & P.M. Ban. 1995. Effects of sulfluramid-treated bait blocks on field colonies of the Formosan subterranean termite (Isoptera: Rhinotermitidae). Journal of Economic Entomology 88:1343-1348.

Su, N.-Y., M. Tamashiro, J.R. Yates & M.I. Haverty. 1982. Effect of behavior on the evaluation of insecticides for the prevention of or remedial control of the Formosan subterranean termite. Journal of Economic Entomology 75:188-193.

Tamashiro, M., J.K. Fujii & P.Y. Lai. 1973. A simple method to observe, trap and prepare large numbers of subterranean termites for laboratory and field experiments. Environmental Entomology 2:721-722.

Vander Meer, R.K., C.S. Lofgren & D.F. Williams. 1985. Fluoroaliphatic sulfones: a new class of delayed-action insecticides for control of *Solenopsis invicta* (Hymenoptera: Formicidae). Journal of Economic Entomology 78:1190-1197.