

Toxicity of Naphthalene and 10 Related Compounds on *Coptotermes formosanus* (Isoptera: Rhinotermitidae)

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Abstract: Naphthalene and ten derivatives were evaluated for initial and residual toxicity, route of penetration and speed of toxic action on *C. formosanus*. In no-choice treated filter paper assays using two colonies, 1'- and 2'-acetonephthone had the greatest contact toxicity followed by 1- and 2-methoxynaphthalene; toxicity of these chemicals was 7- to 38-fold greater than naphthalene. 2, 7- and 2, 6-diisopropyl naphthalene were 4- to 11-fold less toxic than naphthalene. For all chemicals tested, the colony collected from Lake Charles, LA, was more tolerant than that collected from New Orleans, LA. When termites placed on filter papers treated with the estimated 24h LC_{90s}, 2'-acetonephthone followed by 1-methylnaphthalene and 1'-acetonephthone were the fastest acting toxicants killing 50% of workers after ≤ 5h compared to 16h with naphthalene. Workers responded faster than soldiers to 1'-acetonephthone and both responded similarly to 2'-acetonephthone. At the estimated concentrations for 90% contact mortality, termite mortality via inhalation was not significantly different from the controls in 1'-acetonephthone, 2'-acetonephthone and 2-naphthalene methanol treatments. Naphthalene, 2-isopropyl naphthalene, 1- and 2-methylnaphthalene and 1- and 2-methoxynaphthalene were highly volatile causing 61% to 100% termite mortality via their toxic fumes. In no-choice treated sand assays at 100mg kg⁻¹, 1'-, 2'-acetonephthone, 1-, 2-methoxynaphthalene and 2-naphthalene methanol were effective toxicants. 1'- and 2'-acetonephthone maintained their initial toxicity when 1-month residual activity was evaluated. Acetyl substitutions altered the physical and chemical properties of naphthalene moiety to low volatility, more contact toxicity, fast action, and long persistence. This study points to the potential value of 1'- and 2'-acetonephthone in termite control programs.

Key Words: naphthalene derivatives, *C. formosanus*, toxicity, volatility, speed of action, residual activity

Introduction

OF 2,300 species and sub-species of termite so far known in the world, two species of dry wood termite and four species of sand termite have been recorded in Egypt (Kassab et al., 1960 and Helal and Ali 1982). The Formosan subterranean termite was first described in Formosa (Taiwan) in 1909 and was well established in Louisiana, USA in 1966 (Spink 1967). Compared to other subterranean species, *Coptotermes formosanus* Shiraki is more destructive, more difficult to control and is responsible for the greatest costs of termite control (Mauldin et al., 1987, Su and Scheffrahn 2000, Henderson 2001). Big colony size and aggressive foraging behavior of the Formosan subterranean termite complicate its control. A mature colony can contain up to 10 million termites, and its foraging area may cover 3577 m². Moreover, Formosan termites attack both living trees and structural wood, and can form aerial colonies that do not have a ground contact. As a result, once established it has never been eradicated from an area (<http://www.aces.edu/departments/ipm/formoterm.htm>).

Safe alternatives to synthetic pesticides for termite control are needed because some have been reported to cause air pollution (Katsura et al., 1996), contaminate small ponds and poison fish (Carr et al 1997, Kumar and Chapman 2001), and accumulate in body tissues of human (Sim et al., 1998) and other animals

(http://ipm.ncsu.edu/wildlife/cotton_wildlife.html). As part of our continuing search for environmentally safe termite control agents, one of the naphthalene derivatives, 2'-acetonephthone was evaluated on Formosan subterranean termites (Ibrahim et al., 2004 a, b) and determined to be a termite toxicant and repellent; affecting tunneling and feeding behaviors at much lower concentrations compared to some other naturally occurring substances (neem insecticide (Grace and Yates 1992), eugenol (Lin and Yin 1995), nootkatone (Zhu et al., 2001)). The findings with 2'-acetonephthone encouraged us to evaluate more derivatives of naphthalene on the Formosan subterranean termite. Naphthalene derivatives that possess relatively low mammalian toxicity, long stability and low cost were chosen for our current study.

Naphthalene, a bicyclic aromatic hydrocarbon, is known as a toxicant to some insect species (Berger 1998) and is commonly used in houses as a fumigant against cloth moths and carpet beetles (ATSDR 1995). It is used as an external medication to control lice on livestock and poultry (NTP 2000). Naphthalene has low mammalian toxicity, with oral LD_{50s} of 1200 mg kg⁻¹ (guinea pigs), 553 mg kg⁻¹ (mice) and 490 mg kg⁻¹ (rats) (RTECS 1993). In addition, the mutagenic effects of naphthalene *in vitro* and *in vivo* were negative (Tingle et al., 1993, Wilson et al., 1995, US Environmental Protection Agency 1998). However, naphthalene has been reported to cause

hemolytic anemia in the people from Mediterranean countries after long time exposure to very high concentrations (ATSDR 1995). Reduced concentrations of glutathione in rat and mice neonates is also a reported side effect of a long-term naphthalene exposure (NTP 2000, Fanucchi *et al.*, 2000).

To our knowledge there are no publications on the effect of naphthalene and the derivatives we tested on termites, except that naphthalene was surprisingly found in termite carton nests at 50.56-214.6 $\mu\text{g kg}^{-1}$ and is believed to constitute a unique chemical defense strategy against natural enemies of the Formosan subterranean termite (Chen *et al.*, 1998 a). In addition, Formosan subterranean termites have been found to follow trails of naphthalene (Chen *et al.*, 1998 b) and its derivative, 2-naphthalene methanol (Stowell 1997) and may be useful as termite bait additives. A derivative of naphthalene, *N,N*-naphthalolylhydroxylamine, was evaluated for its efficiency as a fungicide and a termiticide (Green *et al.*, 1997). Also copper naphthenate has been proven to be effective in preventing the consumption of wood by the aggressive Formosan termite in field and laboratory tests (<http://www.merichem.com/Copper/CuNapRpt11.htm>).

Naphthalene derivatives selected for our study are naturally occurring chemicals found in petroleum oil (ATSDR 1995, Irwin *et al.*, 1997). 2-Methylnaphthalene was identified as a volatile constituent of dried legumes at concentrations that ranged 2.8 to 49.2ppb (Lovegreen 1979). 1'- and 2'-acetonephthone were identified in corn bud essential oil (Thompson *et al.*, 1974) and both are listed by Fisher Scientific CANADA ([http://www.fishersci.ca/msds2.nsf/EView/1/11647/\\$file/msds-11647.html](http://www.fishersci.ca/msds2.nsf/EView/1/11647/$file/msds-11647.html)) and chemical Land (<http://www.chemicaland21.com/arokorhi/specialtychem/perchem/2/ACETONAPHTHONE.htm>) as major constituents in the fragrances of perfumes and household products. 2, 6 Diisopropylnaphthalene is used to inhibit sprouting in potatoes held in the storage (EPA 1999) and also used to prepare Naproxen [2-(6-methoxy-2-naphthyl) propionic acid], which is used as a non-steroidal anti-inflammatory drug (James *et al.*, 1994). Di-isopropylnaphthalene (D-IPN) is used as a solvent for ink and has been identified in samples of food packaging materials made from recycled board and in some samples of food (MAFF 1999).

We compared the performance of these derivatives on the Formosan subterranean termite since they have low mammalian toxicity (Irwin *et al.*, 1997) compared to commonly used termiticides (Bloomquist 1996); their oral rat LD_{50} s ranged 599 to $>5000\text{mg kg}^{-1}$ (<http://www.sigmaaldrich.com/>, <http://ptcl.chem.ox.ac.uk/MSDS/ME/2/methoxynaphthalene.html>). Also they contain no chemical groups, which would be structurally altering for potential mutagenicity (naphthalene, 1-methylnaphthalene and 2-methylnaphthalene (Irwin *et al.*, 1997); 2, 6-Diisopropylnaphthalene (EPA 1999); 2-isopropylnaphthalene and 2'-acetonephthone (Honda *et al.*, 1991); di-isopropylnaphthalene (Huntingdon Life Science 1999). In addition, they are relatively stable and inexpensive. The current study includes toxicity, speed of toxic action, route of penetration and longevity of these chemicals in relation to substitutions on the fused-ring system of naphthalene.

Materials and Methods

Termites and Chemicals. Termites from two colonies were used in this study. Termites from colony A were collected from an island along the Calcasieu River in Lake Charles, LA on January 6, 2003. Termites from colony B were collected from Brechtel Park, New Orleans, LA on January 15, 2003. Naphthalene (98% purity); 1'-acetonephthone (98%); 2'-acetonephthone (99%); 1-methoxynaphthalene (98%); 2-methoxynaphthalene (99%), 2-naphthalene methanol (98%); 1-methylnaphthalene (95%); and 2-methylnaphthalene (97%) were purchased from Aldrich Chem. Co. Inc., Milwaukee, WI. 2-Isopropylnaphthalene (95%); 2, 6-diisopropylnaphthalene (99%); and 2, 7-diisopropylnaphthalene (95%) were purchased from TCI American, Portland, OR. Absolute ethanol (Ethyl alcohol USP, absolute-200 proof, Aaper Alcohol and Chemical Co. DSP-KY-417, Shelbyville, KY) was used as a solvent for all chemicals except naphthalene and 2, 6-diisopropylnaphthalene, which were dissolved in n-hexane (J T Baker Chemical Co, Phillipsburg, NJ).

Acute Toxicity. For each chemical treatment, filter papers (Whatman # 2, 55mm diameter, Whatman International Ltd, Maidstone, England) were fitted in plastic Petri dishes (6cm diameter by 1.5cm high) and coated with the tested concentrations dissolved in 250 μl solvent. Concentrations used for establishing the toxicity lines were varied according to the chemicals tested. For 1'- and 2'-acetonephthone, the concentrations were ranging from 1 to 20 μgcm^{-2} ; for 1- and 2-methoxynaphthalene, the concentrations were ranging from 3 to 60 μgcm^{-2} . For 2-naphthalene methanol, 1- and 2-methylnaphthalene and 2-isopropylnaphthalene, concentrations assayed with colony B were ranging from 10 to 300 μgcm^{-2} ; for the rest of chemicals, concentrations were from 100 to 2000 μgcm^{-2} . Filter papers treated with solvent only served as the control. Five replicates were performed for each treatment. Containers were left for 4h uncovered at ambient conditions and then 10 workers were inoculated and placed in each container after the filter paper was moistened with 250 μl double distilled water (DDH_2O). Petri dishes were covered and incubated (26.4°C, 59% RH, darkness) for 24h before worker mortality was recorded. For each chemical and colony the 24h mortality data were corrected using Abbott's transformation (Abbott, 1925), then probit analysis results were established (Finney, 1971).

Speed of Toxic Action. For each chemical evaluated, six plastic containers (5.5cm diameter by 3.7cm high) each were provided with a Whatman # 2-filter paper. In three containers, filter papers were coated with the appropriate amount of the chemical in 250 μl solvent to yield a concentration equivalent to the 24h LC_{90} as $\mu\text{g cm}^{-2}$. Filter papers in the other three containers were coated with 250 μl solvent and served as a control. After drying at ambient conditions (4h), filter papers in the six containers were moistened with 250 μl DDH_2O and provided with 100 workers from colony A. The containers were covered with their lids and an opaque black sheet and kept at laboratory conditions for short-term observations. Ten

readings at one-hour intervals followed by 7 readings every two hours were made to record mortality.

Based on the data obtained with the previous experiment, another experiment was conducted using the most effective chemicals, 1'-acetonaphthone, 2'-acetonaphthone, 1- and 2-methoxynaphthalene on workers and soldiers from colony B. The previous technique was used except: 1) a diagnostic concentration ($20\mu\text{g cm}^{-2}$) was used because the number of soldiers was not enough to establish the probit analysis results; 2) eight replicates of 10 soldiers or 10 workers each was used for each chemical assay and control; and 3) observations were recorded for 10h at one hour intervals and an additional reading was recorded after 24h. For each chemical, mortality at each time interval was corrected with control mortality using Abbott's formula (Abbott, 1925) and probit analysis results were calculated (Finney, 1971).

Route of Exposure. To establish whether toxicants are transmitted by physical contact or via inhalation, a technique developed by Delgarde and Rouland-Lefevre 2002 was used with some modifications. Worker mortality in the untreated enclosures away from any physical contact with the chemicals tested was used as an indicator of the inhalation route of penetration. We chose the concentrations that induced the same toxicity response via physical contact (24h LC90s) to avoid effects that may be related to the variation in the toxicity of the chemicals. For each chemical and colony tested, Whatman # 2-filter papers were fitted on the bottom of 48 plastic containers (5.5cm diameter by 3.7cm high). Twenty-four containers were marked "treated" and filter paper in each container was coated with $250\mu\text{l}$ of the chemical solution adjusted to have the 24h LC₉₀ as $\mu\text{g cm}^{-2}$. Filter papers in another 24 containers were left untreated. The 48 containers of the control were handled the same except that filter paper in each of the 24 containers that marked "treated" received $250\mu\text{l}$ solvent only. Treated containers with either chemical solution or solvent were kept 4h uncovered at ambient conditions for solvent evaporation. Filter papers in all containers were wetted with $250\mu\text{l}$ DDH₂O followed by providing 20 workers. For either control or chemical treatment, the 48 containers were divided to 6 sub-groups as replicates of which each was consisting of 4 containers with treated filter papers and 4 with untreated filter papers, were housed together uncovered in a large plastic container (20cm diameter by 7.8cm high). The large plastic container was covered with its lid and incubated (26.4°C, 59% RH, darkness) for 24h. This technique allowed for the dispersion of the chemical vapors from the treated into the untreated containers preventing direct contact between termites in the untreated enclosures and the chemical. Mortality in treated and untreated enclosures was recorded and corrected with the corresponding mortality in the controls (Abbott, 1925). For each chemical, among the two colonies (A & B) and the two routes of exposure (physical contact & inhalation), mortality percentages were analyzed using SAS GLM procedure followed by Tukey's Studentized Range (HSD) Test (SAS Institute, 1999).

A second experiment was conducted to confirm the previous finding with 2-naphthalene methanol, 1'- and 2'-acetonaphthone. The same technique described above was used except using termites from different colony (colony

B) and mortality in treated and untreated enclosures was recorded for 6 days at 1-day intervals.

Initial and Residual Activity. Two hundred gram sand (fine blasting sand # 4, Cement Products Inc., Baton Rouge, LA) was held in a plastic container (20cm diameter by 7.8cm high) and mixed with 25ml from a stock solution [$800\text{mg (AI) litre}^{-1}$ ethanol] for each chemical, to yield a final concentration of 100mg kg^{-1} sand. The same amount of sand receiving the same volume of ethanol served as a control. Containers having chemical- and ethanol-treated sand were kept uncovered overnight at ambient conditions for ethanol evaporation. To evaluate the initial toxicity, 100g of treated sand was mixed with 10ml DDH₂O. Blaine Test Disc (S & S # 597, 12.7mm diameter, Keene, NH) was centered in a plastic Petri dish (6cm diameter by 1.5cm high). Eleven grams of wetted sand was placed in each Petri dish. Sand in each Petri dish was leveled and packed, and then 20 workers from colony A were placed on the surface of the sand. Each treatment was replicated 10 times. Petri dishes were covered with their lids and incubated (26.4°C, 59% RH, darkness) for 11 days (checked daily to observe mortality and suitable moisture). On day 11, the bottoms of the containers were scanned to fix the image of tunnels and the filter paper disc and printed actual size. The number of living workers in each replicate was then counted. Squared areas of consumed filter paper and the tunnels constructed were measured from the printed images. For studying the residual activity of the tested chemicals, the other half of each batch of sand was kept at 26.4°C, 59% RH and darkness for 1-month in a glass jar. Stored sand was evaluated on workers from the same colony using exactly the same technique as described above. Among treatments, mean percentages of mortality, mean tunnel areas and mean food consumption were subjected SAS GLM procedure followed by Tukey's (HSD) Test (SAS Institute 1999).

Results

Acute Toxicity: of the 11 chemicals tested, 1'- and 2'-acetonaphthone had the greatest acute toxicity after 24 h exposure to treated filter paper (Table 1). 1'- and 2'-acetonaphthone exhibited similar toxicity and were significantly more toxic than 1- and 2-methoxynaphthalene. 1- and 2-methoxynaphthalene were more toxic than the rest of the chemicals including naphthalene. Toxicity of 1', 2'-acetonaphthone, 1- and 2-methoxynaphthalene (based on the LC₅₀s, Table 1) was 7 to 38-fold (for colony A) and 14 to 22-fold (for colony B) greater than that of naphthalene. Acute toxicity of 2-naphthalene methanol (colony B), 1-methylnaphthalene (colony B) and 2-methylnaphthalene (colonies A & B) was not significantly different from naphthalene (based on the overlap of 95% CL of the LC₅₀s, Table 1). 1-Methylnaphthalene (colony A), 2-isopropylnaphthalene (colony A), 2-naphthalene methanol (colony A), 2, 6- and 2, 7-diisopropylnaphthalene (colonies A & B) were significantly less toxic than naphthalene. For all of the tested chemicals, colony A (Lake Charles) was significantly more tolerant (based on the non overlap of 95% confidence limits of the LC₅₀s) than colony B (New Orleans). Of the 11 chemicals tested, 2, 6-diisopropylnaphthalene was the least toxic chemical;

moreover, workers from colony A did not respond to any of the tested concentrations of 2, 6-diisopropylnaphthalene up to 2000 $\mu\text{g cm}^{-2}$.

Speed of Toxic Action: 2'-Acetonaphthone followed by 1-methylnaphthalene and 1'-acetonaphthone were the fastest acting toxicants (based on the $\text{LT}_{50\text{s}}$, Table 2). 1- and 2-methoxynaphthalene were relatively slow acting compared to 1-methylnaphthalene and 1'- and 2'-acetonaphthones; but they acted similarly and significantly faster than naphthalene, 2-naphthalene methanol, 2, 6-diisopropylnaphthalene and 2, 7-diisopropylnaphthalene.

2 Isopropylnaphthalene and 2-methylnaphthalene had statistically the same speed of action of 1-methoxynaphthalene. 2-Naphthalene methanol and 2, 6-diisopropylnaphthalene took longer time to induce similar toxicity response.

The potency and fast action of 1', 2'-acetonaphthone, 1- and 2-methoxynaphthalene encouraged us to re-evaluate

them at 20 $\mu\text{g cm}^{-2}$ on workers and soldiers from colony B (Table 3). 1'- and 2'-acetonaphthone were similarly active on workers and both resulted in 100% worker mortality after 5h (LT_{50} was 2.58h and 2.37h; respectively). However, when they were evaluated on soldiers, 1'-acetonaphthone was significantly slower acting than 2'-acetonaphthone; inducing 100% mortality after 10h and 7h, respectively (LT_{50} was 5.62h and 3.30h, respectively). Workers responded significantly faster than soldiers to 1'-acetonaphthone; however, both responded similarly to the toxic action of 2'-acetonaphthone. In general, 1'- and 2'-acetonaphthones were ca 3-fold faster acting on workers than 1- and 2-methoxynaphthalene. Although 1- and 2-methoxynaphthalene induced 77% and 100% worker mortality, respectively after 10h; however, their speed of toxic action was not significantly different (Table 3). Soldiers did not show signs of toxicity after 10h exposure to either of these two tested chemicals; however after 24h exposure, all termite workers and soldiers were dead.

Table 1. Contact toxicity of naphthalene and 10 derivatives on Formosan subterranean termite workers

Chemical	Colony, n ^a	Slope \pm SE	χ^2 , df, p	LC ₅₀ ^c (95% CL)
Naphthalene	A, 1050	4.05 \pm 0.39	21.20, 18, 0.2694	264.6 (232.4-296.4) c
	B, 900	2.95 \pm 0.22	18.81, 15, 0.2225	100.9 (83.8-116.9) d
1'-Acetonaphthone	A, 800	4.08 \pm 0.18	19.53, 13, 0.1076	6.9 (6.3-7.6) g
	B, 500	7.15 \pm 1.15	9.44, 7, 0.2226	5.0 (2.8-5.8) h
2'-Acetonaphthone	A, 800	3.48 \pm 0.15	14.52, 13, 0.3383	7.9 (7.1-8.8) g
	B, 600	6.69 \pm 0.57	2.24, 9, 0.9871	4.6 (4.2-4.9) h
1-Methoxynaphthalene	A, 750	6.51 \pm 0.54	20.11, 12, 0.065	36.1 (32.8-39.4) f
	B, 600	3.84 \pm 0.27	14.79, 9, 0.0969	7.4 (6.5-9.2) g
2-Methoxynaphthalene	A, 800	3.26 \pm 0.21	11.23, 13, 0.5916	29.6(25.8-33.9) f
	B, 650	5.15 \pm 0.38	15.94, 10, 0.1014	7.2 (5.9-8.3) g
2-Naphthalene methanol	A, 900	1.10 \pm 0.12	8.11, 15, 0.9193	991.1(772.4-1382.3)
	B, 850	2.20 \pm 0.14	18.31, 14, 0.193	110.3 (91.4-129.1) d
1-Methylnaphthalene	A, 750	3.17 \pm 0.34	13.01, 12, 0.3683	451.3 (350.5-541.6) b
	B, 500	1.89 \pm 0.14	5.74, 7, 0.5704	74.4 (42.0-107.6) de
2-Methylnaphthalene	A, 650	4.94 \pm 0.40	15.37, 10, 0.1191	277.3 (235.3-312.3) c
	B, 450	2.42 \pm 0.20	11.87, 6, 0.0649	79.6(33.4-121.1) def
2-Isopropylnaphthalene	A, 700	2.0 \pm 0.30	7.93, 11, 0.7196	1108.8 (894.6-1583.8) a
	B, 500	2.42 \pm 0.18	8.58, 7, 0.2842	47.4 (36.9-57.1) ef
2, 6-Diisopropylnaphthalene	A	ND ^b	ND	ND
	B, 800	5.71 \pm 0.64	12.34, 13, 0.5	1153.4 (1024.6-1255.3)a
2, 7-Diisopropylnaphthalene	A, 750	2.64 \pm 0.16	18.15, 12, 0.1112	1139.7 (1022.7-1286.2)a
	B, 650	3.49 \pm 0.22	23.36, 10, 0.0095	429.9 (358.5-501.9) b

^aNumber of workers tested. ^bNot determined.

^cThe LC₅₀s are the lethal concentrations ($\mu\text{g cm}^{-2}$) for 50% of termite workers.

LC₅₀s followed by the same letters are not significantly different (based on the overlap of 95% confidence limits).

Table 2. Logit time-probit analysis results of naphthalene and 10 derivatives on Formosan subterranean workers^a

Chemical	Slope ^b \pm SE	χ^2 , df, p	LT ₅₀ (95% CL) ^d
Naphthalene	6.69 \pm 0.33	15.34, 12, 0.2234	16.06 (14.96 – 17.32)b
1'-Acetonaphthone	5.04 \pm 0.25	17.39, 11, 0.0969	4.67 (4.27 – 5.07)f
2'-Acetonaphthone	5.67 \pm 0.38	5.09, 5, 0.405	3.06 (2.82 – 3.28)g
1-Methoxynaphthalene	4.45 \pm 0.18	7.51, 15, 0.9419	8.65 (5.61 – 12.35)cde
2-Methoxynaphthalene	6.69 \pm 0.30	10.75, 15, 0.7701	9.45 (9.15 – 9.76)d
2-Naphthalene methanol	6.97 \pm 2.04 ^c	2.07, 15, 1	38.95 (31.06 – 87.84)a
1-Methylnaphthalene	2.95 \pm 0.21	17.59, 6, 0.0073	3.46 (2.43 – 4.56)fg
2-Methylnaphthalene	2.23 \pm 0.11	17.84, 15, 0.2712	7.12 (6.53 – 7.73)e
2-Isopropylnaphthalene	4.07 \pm 0.23	16.57, 10, 0.0844	11.09 (10.34.-11.85)c
2, 6-Diisopropylnaphthalene	5.24 \pm 0.76 ^c	6.94, 15, 0.9593	33.99 (29.71 – 42.95)a
2, 7-Diisopropylnaphthalene	3.54 \pm 0.19	9.90, 15, 0.826	16.65 (15.78 – 17.67)b

^aTermite workers from colony A were used.

^bNumber of insects on which each probit analysis based was 600.

^cThe estimated LC₉₀s with colony B were used to evaluate the speed of toxic action on colony A.

^dLT₅₀s expressed as time in hours required to kill 50 of termite workers.

LT₅₀s followed by the same letters are not significantly different (based on the overlap of 95% confidence limits).

Table 3. Logit time-probit analysis results when Formosan subterranean termite workers and soldiers (colony B) were exposed to filter paper treated with the tested chemicals at 20µg cm⁻²

Chemical	Termite group ^a	Slope ± SE	χ ² , df, P	LT ₅₀ (95% CL) ^b
1'-Acetonaphthone	Worker	6.74 ± 0.55	6.57, 3, 0.0869	2.58 (2.21 to 3.69)b
	Soldier	4.44 ± 0.29	11.10, 8, 0.1961	5.62 (5.25 to 5.99)a
2'-Acetonaphthone	Worker	5.41 ± 0.42	5.60, 3, 0.1328	2.37 (1.45 to 2.99)b
	Soldier	5.96 ± 0.43	2.83, 5, 0.7262	3.30 (2.71 to 3.85)b
1-Methoxynaphthalene	Worker	4.68 ± 0.44	12.05, 8, 0.1490	6.57 (5.99 to 7.43)a
	Soldier	ND ^b	ND	> 10h
2-Methoxynaphthalene	Worker	8.28 ± 0.52	7.14, 8, 0.5216	6.44 (5.50 to 7.64)a
	Soldier	ND ^b	ND	ND

^aNo = 80.

^bLT₅₀s expressed as time in hours required to kill 50 of termite workers.

^cSoldier mortality was not significant during the first 10h observations, however 100% mortality was observed after 24h exposure.

For each column, LT₅₀s followed by the same letters are not significantly different (based on the overlap of 95% confidence limits).

Route of Exposure: with the exception of 2, 6-diisopropylnaphthalene, the estimated concentrations for killing 90% of termite workers via physical contact resulted in 24h contact mortality ranging from 59 to 99% (colony A) and 79 to 100% (colony B) that were not significantly different among chemical treatments (Table 4). However, inhalation mortality was significantly varied between treatments. 1'- and 2'-acetonaphthone (for the two colonies) followed by 2-naphthalene methanol and 2, 7-diisopropylnaphthalene (for one colony) induced inhalation mortality that was not significantly different from the control. In the assays of the two colonies, naphthalene, 1- and 2-methylnaphthalene, 1- and 2-methoxynaphthalene and 2-isopropylnaphthalene were highly volatile. In each of the 6 treatments, mortality via physical contact and via inhalation was not significantly different; moreover, both were significantly different from the controls (Table 4). Inhalation mortality with workers from colony B in 2, 7-diisopropylnaphthalene treatment was negligible; however, 58% inhalation mortality was achieved with workers from colony A (Table 4). In 2, 6-diisopropylnaphthalene treatment, no remarkable mortality was achieved in either treated or untreated enclosures.

For each row, means with the same small letters are not significantly different (P > 0.05). * df = 1, 10 in 2-naphthalene methanol and 2, 6-diisopropylnaphthalene treatments

Repeating the experiment with the low-volatile chemicals (1'- and 2'-acetonaphthones and 2-naphthalene methanol) for a 6 day observation period (data not shown in a table) revealed that mortality of workers (from colony B) via physical contact with 1'-acetonaphthone was 75% and 92.5% in the first two successive days compared to 0 and 2.5 % worker mortality in the control. Cumulative inhalation mortality in the untreated enclosure of the 1'-acetonaphthone treatment did not exceed 12.5% on day 6 compared to 17.5% in the control. 2'-Acetonaphthone induced 100% mortality after 24h when termite workers were in physical contact with the 24h LC₉₀-treated filter paper. However, inhalation mortality in the untreated enclosure was only 32.5% compared with 17.5% mortality in the control. In the enclosures, which had, filter paper treated with 2-naphthalene methanol, mortality via physical contact was 12.5, 75, 86.25 and 100% in the first four

successive days. The corresponding mortality via inhalation was 11.25%,

Initial and Residual Activity: mortality percentages, tunnel areas and filter paper consumption of Formosan subterranean workers measured on day 11 in response to 100 mg kg⁻¹ treated sand in initial and residual assays.

Initial Activity. 1'-Acetonaphthone, 2'-acetonaphthone, 1-methoxynaphthalene,

2-methoxynaphthalene and 2-naphthalene methanol were the only effective toxicants, resulting in 98.5% to 100% worker mortality compared to 11.5% in the control on day 11 (Table 5). Complete mortality was recorded at day 2 for 1'-acetonaphthone, 2'-acetonaphthone and at day 3 in 2-methoxynaphthalene. Similar toxic effect required 5 days in 1-methoxynaphthalene and >11 days in 2-naphthalene methanol. Food consumption and tunnel construction were negligible in these treatments after 11 days exposure. We also observed that the abdomens of all dead workers changed to dark blue in 1-methoxynaphthalene treatment. Mortality of termite workers after 11 days exposure to naphthalene and the other tested derivatives was not significantly different from the control (Table 5). Tunnel areas were significantly reduced in all chemical treatments except for 1-methylnaphthalene. Also feeding activity was significantly reduced in all treatments except 1-methylnaphthalene, 2-methylnaphthalene and 2, 6-diisopropylnaphthalene. In 1-methylnaphthalene treatments termites tunneled as long as control, however their feeding activity was significantly greater than the control (Table 5).

Residual Activity. 1'- and 2'-acetonaphthone maintained their initial efficiency, killing 99.5% and 100% of the termites, respectively. Moreover, all termites died within three days in 2'-acetonaphthone and consequently complete inhibition of food consumption and tunneling activity was observed (Table 5). 1-Methoxynaphthalene, 2-methoxynaphthalene and 2-naphthalene methanol lost most of their initial toxic effects when their residual activity was assayed; however, food consumption and tunnels constructed in the three treatments in addition to 2-isopropylnaphthalene; 2, 6- and 2, 7-

diisopropylnaphthalene treatments was significantly reduced compared to the control. Compared to the control, tunnels constructed in the treatments of 2-isopropylnaphthalene and 2, 7-diisopropylnaphthalene were significantly shorter, however, no significant effect on food consumption was achieved (Table 5).

Discussion

Naphthalene and its derivatives are dicyclic aromatic hydrocarbons. We found that substitutions on the naphthalene moiety significantly altered the toxicity, speed of action, route of penetration, volatility and consequently the residual activity. Of the 10 naphthalene derivatives tested, it was evident that an acetyl group attached to naphthalene in either the 1- or 2-position significantly improved the toxicity and the speed of toxic action. 1'- and 2'-acetonaphthone had the greatest contact toxicity that was 20- to 38-fold greater than naphthalene. At the same time, this modification altered the route of penetration from inhalation to contact entry and consequently increased the persistence compared to the rest of tested naphthalene derivatives. In a previous study with 2'-acetonaphthone (Ibrahim *et al.*, 2004 a), termites placed on 40 $\mu\text{g cm}^{-2}$ treated filter paper died within 6-8 h; however survival of termites exposed in two-choice assays to treated filter paper with the same concentration were not effected for up to 15 days. Greater and faster contact toxicity together with a relatively higher persistence of 1'- and 2'-acetonaphthone were accompanied by lower inhalation toxicity. It has been previously reported that toxicity and lipophilicity of naphthalene derivatives increased and volatility decreased as the alkylation increased (Irwin *et al.*, 1997, Knightes *et al.*, 2000). 1'- and 2'-acetonaphthone are not alkyl derivatives of naphthalene, however they are more water soluble, less lipophilic, less volatile and more toxic than naphthalene. The Log (p) is lower (2.36) for the two acetyl derivatives compared to naphthalene (3.05). In contrast, Log (H) is higher (4.407 – 4.662) for the two acetyl derivatives compared to naphthalene (1.667-2.019). This insures that the physical properties that allow the chemical to reach its site of action in enough concentration, together with, the affinity of the molecule to its site of action (target site sensitivity) are complementary factors for its toxicity. The methoxy group attached to naphthalene in either the 1- or 2-position significantly improved the initial toxicity and the speed of action; however, this modification maintained the high volatility, which allowed the chemical when applied to sand to lose most of toxicity within 1-month. Toxicity was significantly diminished when two isopropyl groups were attached to naphthalene in 2, 6 or 2, 7 positions. Those two chemicals exhibited the highest Log (p) values (5.44 \pm 0.49) and the lowest Log (H) values (1.089-1.166) compared to 3.05 \pm 0.49 and (1.667-2.019) for naphthalene.

To our knowledge this is the first study regarding the toxicity of naphthalene derivatives on any insect species. However, derivatives of the allylamine antimycotic terbinafine with varied substitution at the naphthalene ring system have been evaluated for their antifungal activity (Nussbaumer *et al.*, 1993). They found that substitutions that increase lipophilicity were much more important for toxicity than the electronic density distribution and /or steric requirements. In our study we found that acetyl substitution increased the toxicity and speed of action when compared to naphthalene itself probably through reduced volatility but not through increase lipophilicity. Naphthalene is less toxic, more lipophilic, less water soluble, and more volatile than the two acetyl substitutions.

For any insecticide, alterations in the electrophilic properties (Abdel-Aal *et al.*, 1977, Coats 1983, Ford *et al.*, 1989, Konno and Shishido 1994), the hydrophobicity (Singh 2001), the flexibility and steric changes (Hudson *et al.*, 1992) can affect the affinity of an insecticide to its site of action. In addition, substitutions on the original molecule may affect the cuticular penetration and metabolic degradation (Abernathy *et al.*, 1971, Metcalf *et al.*, 1974). Changes in the molecule structure may also result in kinetic changes in the function of receptors (Narahashi 2000). 1'- and 2'-acetonaphthones when compared to other tested naphthalene derivatives may increase their selectivity toward insects. Non-volatile chemicals are more valuable in pest control for indoor safety and outdoor persistence. The low volatility of 1'- and 2'-acetonaphthone allows them to persist longer under field conditions than volatile chemicals. Both maintained their initial activity in treated sand when 1-month longevity was considered. High toxicity to insects is not always associated with high mammalian toxicity (Browning *et al.*, 1948), even if the mode of action is the same for both. However, differences can be due to the route of entry into the tissue. Low volatile pesticides are more selective than strongly volatile chemicals because mammals are mostly exposed to chemicals through inhalation. This study points to the potential value of 1'- and 2'-acetonaphthone in termite control programs.

Table 4. Percentages of corrected mortality ^a of Formosan subterranean termites exposed to the estimated LC_{90s} of naphthalene and its homologues through physical contact or/and inhalation

Colony A Colony B	Colony A		Colony B		<i>F</i> ; MSD; df = 3, 20*; <i>P</i>
Treatment	Physical contact and inhalation	Inhalation	Physical contact and inhalation	Inhalation	
Control	0 (B)	0 (D)	0 (B)	0 (B)	---
Naphthalene	81.83 ± 15.08a (A)	80.41 ± 15.20a (AB)	100a (A)	100a (A)	1.08; 41.513; 0.3789
1'-Acetonaphthone	58.96 ± 10.63b (A)	13.78 ± 5.99c (CD)	93.46 ± 3.95a (A)	0c (B)	40.65; 26.529; < 0.0001
2'-Acetonaphthone	71.18 ± 13.63a (A)	32.83±11.07b (BCD)	100a (A)	9.68 ± 2.78b (B)	21.18; 34.481; < 0.0001
1-Methoxynaphthalene	59.38 ± 17.51a (A)	60.93 ±17.93a (ABC)	82.93 ± 11.07a (A)	67.77 ± 17.92a (A)	0.45; 63.474; 0.7203
2-Methoxynaphthalene	88.71± 8.44a (A)	84.17 ± 11.65a (AB)	100a (A)	86.76 ± 8.87a (A)	0.71; 32.775; 0.5571
2-Naphthalene methanol	ND	ND	78.76 ± 14.21a (A)	25.84 ± 5.03b (B)	12.85;32.896; < 0.0050
1-Methylnaphthalene	99.38 ± 0.64a (A)	100a (A)	100a (A)	100a (A)	1.0; 1.237; 0.4133
2-Methylnaphthalene	76.15 ± 16.17a (A)	74.99 ± 16.97a (AB)	100a (A)	100a (A)	1.51; 45.463; 0.2426
2-Isopropylnaphthalene (beta-)	88.47 ± 6.51a (A)	94.79 ± 4.82a (A)	83.03 ± 13.33a (A)	70.97± 15.75a (A)	0.87; 42.987; 0.4742
2, 6-Diisopropylnaphthalene	ND	ND	29.41 ± 13.03a (B)	23.46 ± 13.30a (B)	0.09; 21.961; 0.7654
2, 7-Diisopropylnaphthalene	65.32 ± 17.41a (A)	58.39 ± 16.59a (ABC)	87.43 ± 10.48a (A)	3.39 ± 2.25b (B)	8.37; 37.652; 0.0008
<i>F</i> ; MSD; df; <i>P</i>	5.19; 56.806 9, 50; < 0.0001	8.45; 54.927; 9, 50; < 0.0001	16.08; 38.14; 11, 60; < 0.0001	26.11; 39.973 11, 60; < 0.0001	---

^aData expressed as mean ± SE. For each column, means with the same capital letters are not significantly different (*P* > 0.05).

Table 5. Mortality percentages, tunnel areas and filter paper consumption^a of Formosan subterranean workers measured on day 11 in response to 100 mg kg⁻¹ treated sand in initial and residual assays

Initial activity 1-Month longevity						
Treatment	% Mortality	Tunnel area (cm ²)	Consumption (mm ²)	% Mortality	Tunnel area (cm ²)	Consumption (mm ²)
Control	11.50 ± 1.07b	8.50 ± 2.67a	8.09 ± 2.33b	12.5 ± 1.34c	8.21 ± 0.94ab	31.0 ± 8.63a
Naphthalene	20.0 ± 4.54b	3.02 ± 1.43bc	0.89 ± 0.46c	23.0 ± 5.07bc	5.17 ± 0.72bcd	9.5 ± 5.70abc
1'-Acetonaphthone	100a	0c	0c	99.5 ± 0.50a	0.06 ± 0.03f	0c
2'-Acetonaphthone	100a	0c	0c	100a	0f	0c
1-Methoxynaphthalene	100a	0.40 ± 0.13c	0c	33.0 ± 5.07bc	0.15 ± 0.04f	1.0 ± 0.67c
2-Methoxynaphthalene	100a	0.74 ± 0.47c	0c	44.5 ± 6.08b	0f	3.0 ± 0.82bc
2-Naphthalene methanol	98.5 ± 0.76a	0.46 ± 0.25c	0.03 ± 0.03c	33.0 ± 5.23bc	0.58 ± 0.0.37ef	1.0 ± 0.67c
1-Methylnaphthalene	14.5 ± 2.04b	6.94 ± 1.71ab	37.94 ± 11.08a	13.0 ± 1.11c	9.05 ± 1.77°	30.0 ± 10.47ab
2-Methylnaphthalene	11.5 ± 1.07b	1.52 ± 1.09c	7.99 ± 2.71b	13.5 ± 3.34c	6.49 ± 0.83abc	11.5 ± 8.37abc
2-Isopropylnaphthalene (beta-)	18.0 ± 2.38b	2.46 ± 1.14bc	1.19 ± 0.55c	22.5 ± 7.43bc	2.54 ± 0.29def	18.0 ± 9.17abc
2, 6-Diisopropylnaphthalene	24.5 ± 12.76b	0.14 ± 0.09c	4.48 ± 1.74bc	35.0 ± 7.04bc	2.75 ± 0.44def	0c
2, 7-Diisopropylnaphthalene	19.5 ± 5.79b	1.80 ± 0.92bc	0.89 ± 0.39c	34.5 ± 8.32bc	3.75 ± 0.41cde	7.0 ± 4.73abc
<i>F; MSD; df; P</i>	95.42; 20.653; 11,108; < 0.0001	5.96; 5.406; 11, 108; < 0.0001	10.01; 6.117; 11, 108; < 0.0001	36.19; 23.770; 11,108; < 0.0001	22.59; 3.307; 11, 108; < 0.0001	3.94; 27.079; 11, 108; < 0.0001

^aData expressed as mean ± SE.

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