Metabolism of Chlorpyrifos-^{14}C in the Eastern Subterranean Termite[1]

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The organophosphate chlorpyrifos is a potential replacement for the chlorinated hydrocarbons for control of subterranean termites(BEAL and SMITH, 1972). Therefore, we examined the metabolic fate of chlorpyrifos-^{14}C in the eastern subterranean termite, Reticulitermes flavipes (Kollar), an important pest species.

MATERIALS AND METHODS

Eastern subterranean termites were collected in the field in Boone County (Columbia, Missouri), and the colony was maintained in the laboratory. Additional termites of the same species were collected in Mississippi by personnel from the Southern Forest Experiment Station, United States Department of Agriculture Forest Service at Gulfport, Mississippi. The worker caste, which was comprised of undifferentiated larvae beyond the third instar, was used for metabolism studies.

A nonradioactive sample of chlorpyrifos and chlorpyrifos labeled with radiocarbon at ring positions 2 and 6 (specific activity 30.5 mCi/mg) were provided by The Dow Chemical Company, Midland, Michigan. They also provided nonradioactive samples of O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphate (chlorpyrifos oxygen analog), O,O-diethyl O-5,6-dichloro-2-pyridyl phosphorothioate (3-dechlorochlorpyrifos), O,O-diethyl O-3,6-dichloro-2-pyridyl phosphorothioate (5-dechlorochlorpyrifos), O,O-diethyl O-3,5-dichloro-2-pyridyl phosphorothioate (6-dechlorochlorpyrifos), and 3,5,6-trichloro-2-pyridinol (trichloropyridinol).

Thin layer chromatography (TLC) was used for separation and identification of chlorpyrifos-^{14}C and its metabolites recovered from metabolism studies. Glass plates were coated with a 500-µ layer of silica gel GF_{254}. Detection of chlorpyrifos and its potential metabolites was accomplished by dissolving 2 mg of each compound per ml of acetone and spotting 10-µl aliquots of each solution on the TLC plate. After development of the chromatogram in a solvent mixture containing n-hexane:ethyl acetate:acetic acid (85:15:0.3), compounds were observed under short wavelength ultraviolet light (254 nm) as quenched areas on the fluorescent silica gel.

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Organosoluble termite extracts to be chromatographed were spotted along with authentic nonradioactive standards on the thin layer plate, and the chromatogram was developed. After TLC the plate was placed in contact with no screen X-ray film (Eastman Kodak Company, Rochester, New York) and exposed for 5 to 10 days, depending on the amount of radioactivity present. Quantitative estimation of chlorpyrifos and its metabolites was made by scraping the silica gel from each spot corresponding to the Rf of the authentic standards and to the darkened images on the film directly into a scintillation vial and adding 15 ml of scintillation liquid. Each vial was thoroughly swirled to assure mixing of compound with the scintillation liquid, and the mixture was radioassayed.

The radiocarbon content of each sample was measured with a Liquimat 220 liquid scintillation spectrometer (Picker Nuclear, White Plains, New York). Extracted radioactive samples to be assayed were placed in glass scintillation vials, and 15 ml of the appropriate counting solution were added. Calculation of the amount of radioactivity in each sample was accomplished by averaging duplicate 10-min counts taken on each vial. All data were corrected for background, dilution, quenching and counting efficiency.

All samples from organic solvent extracts were analyzed using a scintillation cocktail consisting of toluene-methyl cellosolve (2:1) plus 2,5-diphenyloxazole (0.4%, w/v)and 1,4-bis-2-(4-methyl-5-phenyloxazolyl) benzene (0.004%, w/v)(KNOWLES and SEN GUPTA, 1969). The counting liquid used for analysis of water soluble components consisted of the original scintillation cocktail plus 10% (v/v) BIO-SOLV (BBS-3, Beckman Instruments, Incorporated, Fullerton, California)(SEN GUPTA and KNOWLES, 1970).

Two hundred termites were treated topically on the thorax with chlorpyrifos-^{14}C. Each termite received 500 cpm of chlorpyrifos-^{14}C dissolved in 0.5 μl of acetone. The treated termites were placed in a 50-ml beaker covered with a moistened section of tissue paper. Termites treated in this way were kept in the dark at 26^{o}C for periods of 1, 4, 8, and 16 hr.

At the designated time interval all treated termites were removed from the beaker and were rinsed twice with 10-ml aliquots of acetone (termite rinse). The beaker also was rinsed twice with 10-ml aliquots of acetone (metabolism chamber). Aliquots from the termite rinse and metabolism chamber were radioassayed to determine total radioactivity. Following the acetone rinse, the termites were homogenized in 10 ml of acetone. After centrifugation at low speed the supernatant was decanted, and the precipitate was homogenized again in 10 ml of acetone. This homogenate was centrifuged and the acetone was decanted. This left the particulate termite residue (termite residue). The total radioactivity in the termite residue was determined by oxygen flask combustion and subsequent radioassay of the trapped-$^{14}CO_2$, (SEN GUPTA and KNOWLES, 1969).

The two acetone extracts were combined and were dried over anhydrous sodium sulfate. The acetone was evaporated almost to dryness on the rotary evaporator, and the residue was dissolved in 25 ml of chloroform and transferred to a separatory funnel. The chloroform fraction was extracted twice with 25-ml aliquots of distilled water. The two water extracts were combined and concentrated to a volume of 10 ml (water extract). An aliquot was counted to determine total radioactivity. The chloroform fraction was concentrated to 10 ml, and an aliquot was radioassayed (chloroform extract). The remaining chloroform extract was concentrated almost to dryness, and the residue was partitioned between 25 ml of n-hexane and 25 ml of acetonitrile. The acetonitrile was evaporated to a volume of 0.5 ml and subjected to TLC, radioautography, and radioassay. The hexane and water fractions were not further analyzed.

RESULTS AND DISCUSSION

Table 1 lists the distribution of radioactivity following topical application of chlorpyrifos-^{14}C to termites. It was previously determined using radioautography and liquid scintillation spectrometry that the radiochemical purity was greater than 99%. The radioactive materials in the external termite rinse and in the metabolism chamber decreased with time, indicating that the termites were absorbing the chlorpyrifos. This was accompanied by a corresponding increase in internal radioactive materials (Table 1). The percentage of chloroform-soluble radioactive materials decreased with time. Concurrently there was an increase in water-soluble radioactive materials and in radioactive materials unextractable from the termite residue. This suggested that chlorpyrifos was converted to water-soluble metabolites and that certain of its metabolites were bound to the termite residue.

TABLE 1

Distribution of Radioactivity Following Topical Application of Chlorpyrifos-^{14}C to Termites[a]

Time After Treatment	Chloroform Extract	Water Extract	Termite Residue	Termite Rinse	Metabolism Chamber
1 Hour	46.4	7.0	11.5	25.8	9.3
4 Hours	44.6	12.7	19.0	16.6	7.1
8 Hours	38.2	20.5	24.5	11.2	5.6
16 Hours	31.4	26.0	32.4	6.0	4.2

[a]Data are expressed as relative percentage of recovered radioactive materials. Recovery of applied radioactivity averaged 86.5% of theory.

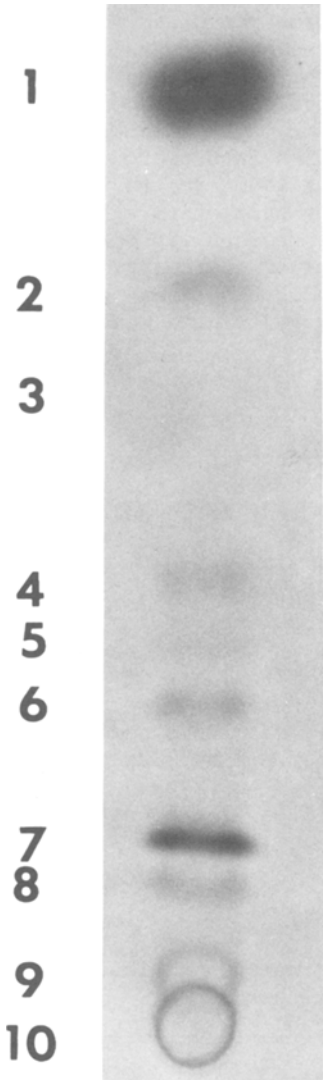

FIGURE 1. Radioautograph of organic extract from termites exposed to chlorpyrifos-^{14}C for 8 hr. Legend: 1 = chlorpyrifos, 2 = 3 dechlorochlorpyrifos (tentative), 4 = trichloropyridinol, 7 = chlorpyrifos oxygen analog; 3,5,6, 8,9, and 10 are unknowns.

Table 2 gives the nature and relative concentration of organosoluble radioactive materials isolated from termites treated topically with chlorpyrifos-^{14}C. The parent compound, chlorpyrifos,decreased from 85.9% in 1 hr to 54.5% at 16 hr. The major metabolite cochromatographed with chlorpyrifos oxygen analog. It increased from 1.6% at 1 hr to 17.7% at 16 hr.Unknown I also increased with time; it cochromatographed with 3-dechlorochlorpyrifos. There also were minor amounts of the trichloropyridinol and several unknown metabolites.

Fig.1 is a radioautographic presentation of the organic extract from termites exposed to chlorpyrifos-^{14}C for 8 hr. Compound 1 cochromatographed with chlorpyrifos, compound 2 cochromatographed with 3-dechlorochlorpyrifos, compound 4 cochromatographed with trichloropyrinol, and compound 7 cochromatographed with chlorpyrifos oxygen analog. Compounds 3,5,6,8,9,and 10 have not been identified; they may be oxygen analogs of dechlorochlorpyrifos derivatives and/or dichloropyridinols.

Based on these studies, it seemed that the major path for chlorpyrifos metabolism in termites was oxidative desulfuration to chlorpyrifos oxygen analog. There apparently was some dechlorination to 3-dechlorochlorpyrifos; however,identification of this compound is tentative. It is noteworthy that 3-dechlorochlorpyrifos has not been reported in previous studies of chlorpyrifos metabolism in plants, rats, and fish (SMITH et al., 1966,1967a, 1967b, 1967c). The presence of trichloropyridinol provided evidence for cleavage of the anhydride bond. Whether this occurred via the oxidative or hydrolytic mode is not certain.

TABLE 2

Nature and Relative Concentration of Organosoluble Radioactive Materials Isolated from Termites Treated Topically with Chlorpyrifos-^{14}C

Compound	Rf Value for TLC	% Radioactivity recovered at indicated time			
		1 Hour	4 Hours	8 Hours	16 Hours
Chlorpyrifos	0.80	85.9	76.0	65.5	54.5
Unknown I[a]	.60	0.3	0.4	3.6	4.1
Unknown II	.50	0.5	1.2	1.4	0.1
Trichloropyridinol	.36	2.5	0.7	1.0	2.2
Unknown III	.31	1.8	2.1	0.9	0.7
Unknown IV	.26	1.9	0.9	2.8	3.4
Chlorpyrifos oxygen analog	.18	1.6	10.4	12.8	17.7
Unknown V	.13	1.4	2.2	2.4	5.3
Unknown VI	.04	1.5	1.3	2.2	3.5
Origin	.00	2.6	4.8	7.4	8.6

[a]Cochromatographed with 3-dechlorochlorpyrifos.

CONCLUSIONS

Termites treated topically with chlorpyrifos-^{14}C readily absorbed the material and metabolized it to chlorpyrifos oxygen analog. There also was evidence for some dechlorination of chlorpyrifos to 3-dechlorochlorpyrifos. Subsequent metabolism of chlorpyrifos and chlorpyrifos oxygen analog to water-soluble metabolites proceeded slowly. The efficacy of chlorpyrifos as a termiticide is obviously related to the rapid formation and relatively slow degradation of the toxic chlorpyrifos oxygen analog in the eastern subterranean termite.

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