Drywood Termite Metabolism of Vikane Fumigant as Shown by Labeled Pool Technique

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The mode of action of Vikane fumigant (sulfuryl fluoride) in destroying the Western drywood termite was investigated using the labeled pool technique. The results strongly indicate that inorganic fluoride is the primary poison. This conclusion was arrived at through a consideration of the disturbances in intermediary metabolism. These disturbances were deduced from variations in the paper chromatographic spectra of the labeled metabolites isolated from fumigated and check termites.

SULFURYL FLUORIDE, Vikane (trademark of The Dow Chemical Co.) fumigant, is a compound which has shown considerable promise as a structural fumigant (12). Since nothing was known concerning the mechanism of action of this compound in destroying termites (*Kalotermes minor* Hagen), the work described in this report was undertaken to shed some light on this issue.

Previous work with this fumigant and flour (14) suggested that the compound could be expected to react with proteinaceous material and, thus, should be nonspecific with respect to the sites of attack.

The most expedient way to determine the disturbance in intermediary metabolism was felt to be to use the labeled pool technique (20). This technique provides a sensitive, quantitative method for the determination of relative amounts or concentrations of related metabolites in a given biochemical system. The method's sensitivity makes it especially suitable for the study of effects of pesticides on insects when only limited amounts of biological material are available.

Briefly, the labeled pool technique consists of the following steps. Labeled metabolites, manufactured by the insect kept on a radioactive diet, can be extracted and then resolved by paper chromatography. This, then, provides what might be called a spectrum of the labeled metabolite which existed in the biological system at the instant of extraction. Any differences in the spectra derived from treated and untreated systems would be strongly indicative of the site of the metabolic disturbances.

Experimental

Fumigation with Sulfuryl-S[35] Fluoride. The Western drywood termite (*Kalotermes minor* Hagen) was fumigated with sulfuryl-S[35] fluoride (7) at the rate of 1 mg. per liter, 20° C. for 24 hours. This is 40% of the LD_{100} (12). The insects were then removed from the fumigation chamber, and the pellets were collected after 1 week. Extraction of the pellets with 5% trichloroacetic acid (TCA) solution resulted in the removal of 93% of the radioactivity. The radioactivity in the TCA solution was not retained by Dowex-50 ion exchange resin (hydrogen form) but was held up by Dowex-1 resin (bicarbonate form). The radioactivity was precipitated as barium sulfate and was also cochromatographed with authentic radioactive sulfate, thus establishing its identity as inorganic sulfate. No other radioactive entity was detected.

Labeling of Termites. Fifty Western drywood termites were allowed to feed on 70 mg. of paper toweling which had been impregnated with 200 μc. (5 mg.) of sodium acetate-1-C[14] and in a second experiment, with 83 μc. of phosphate-P[32] from a pH 7.0 solution. The insects were allowed to feed on this diet for 7 days at 70° F. and a relative humidity of 70 to 90%. At the end of this time, the termites and pellets from both labeling experiments were highly radioactive.

Fumigation of Termites. The radioactive paper was replaced by untreated paper, and the termites were allowed to feed 1 day on this diet under the conditions described above. Twenty-five termites from each labeled group were then fumigated with Vikane at the rate of 1 mg. per liter as described above.

Sacrifice and Extraction of Termites. At the end of the fumigation period, all the termites were frozen with dry ice. Each group of 25 termites, fumigated and checks, carbon-14 and phosphorus-32 labeled, was ground separately under 35-mesh borosilicate glass and 3 ml. of 80% ethanol at room temperature for 10 minutes. The solids were separated by centrifugation, washed with 80% ethanol, and the extracts and washings were combined. The solutions were concentrated to about 0.5 ml. by lyophillization. The distillate contained no radioactivity.

Chromatography and Radioautography. The techniques described by Benson *et al.* (1) were followed. It was not found necessary to submit the termite extracts to desalting. Aliquots of the solutions, containing an equal number of total counts for each pair of check and fumigated termites, were applied on 15 × 18 inch Whatman No. 1 filter paper sheets, and two-dimensional chromatograms were developed in an ascending-descending manner as described by Block (2), first in water-phenol and then in butanol–propionic acid–water. The sheets were dried at 30° to 35° C. for 6 hours in the case of the phenol solvent and at room temperature overnight in the case of the butanol solvent.

No-Screen x-ray film (14 × 17 inches) was used for the preparation of radioautographs. Exposure time was 15 days for carbon-14 extracts and 8 days for phosphorus extracts.

Table I. C[14]-Labeled Metabolites from *Kalotermes Minor* Hagen

	Per Cent of Total Activity[a]	
Metabolites	Check samples	Fumigated samples
Lipide	9.1 ± 2.4	14.4 ± 3.7
Glyceric acid	0.5 ± 0.3	0
Succinic acid	0	0.5 ± 0.2
Fumaric acid	0	0.4 ± 0.2
Proline	28.0 ± 1.6	23.3 ± 1.2
Alanine	2.2 ± 0.5	1.8 ± 0.3
Glutamine	6.2 ± 1.1	3.6 ± 0.6
Serine	8.1 ± 0.5	6.5 ± 0.5
Glutamic acid	11.6 ± 0.5	13.1 ± 0.4
Aspartic acid	3.3 ± 0.6	2.3 ± 0.4
Unknowns		
1	15.6 ± 0.5	11.4 ± 1.2
2	1.1 ± 0.5	0
3	0	1.7 ± 0.8
4	0.8 ± 0.1	<1
5	0.3 ± 0.4	<1
6	7.9 ± 0.4	6.1 ± 0.2
7	1.5 ± 0.1	1.4 ± 0.2
8	0	2.7 ± 0.8
9	3.8 ± 0.6	5.3 ± 0.5
10	0	5.2 ± 0.9
Total counts	3431	3144

[a] ± Standard error. Results are average of three experiments.

Table II. P³²-Labeled Metabolites from *Kalotermes Minor* Hagen

Metabolites	Check samples	Fumigated samples
	Per Cent of Total Activity[a]	
P-lipide	12.1 ± 0.5	11.5 ± 0.4
Hexose mono-P	27.0 ± 0.9	29.5 ± 0.6
Hexose di-P	18.8 ± 0.2	20.5 ± 0.4
Pentose di-P	9.6 ± 0.5	10.3 ± 0.6
Triose-P ⎫ Pentose-P ⎭	0.7 ± 0.3	0.4 ± 0.2
Dihydroxy-acetone-P	0.4 ± 0.2	0.4 ± 0.2
P-glyceric acid	10.7 ± 1.0	11.0 ± 0.8
P-pyruvic acid	0	0.9 ± 0.3
Adenosine tri-P	15.1 ± 1.7	10.0 ± 1.3
Adenosine di-P	1.2 ± 0.3	1.0 ± 0.1
Adenosine mono-P	0.1 ± 0.2	0
Agrinine-P	0.1 ± 0.1	2.3 ± 0.6
Unknowns		
1	2.2 ± 0.6	1.1 ± 0.5
2	0	0.3 ± 0.5
3	0	0.06 ± 0.1
4	0.4 ± 0.4	0
5	0.1 ± 0.1	0
6	0.1 ± 0.1	0.2 ± 0.1
7	0.5 = 0.6	0
8	0.04 ± 0.1	0
9	0.02	0.1
10	0.07	0.03
11	0.5 = 0.4	0.2 ± 0.1
Total counts	18417	23506
Total counts in inorganic phosphate	17689	14745

[a] ± Standard error. Results are average of three experiments.

Radioactivity Assay of Paper Chromatograms. The radioactive content of each compound on the paper sheet was assayed with a 1-inch thin window Geiger tube placed directly on top of the spot. This was then expressed as a percentage of the total activity. Each spot was counted for a total of 1000 counts with a background count rate of about 40 counts per minute. When duplicate samples were chromatographed, good agreement in the radioactivity distribution among the various compounds was obtained. Three experiments were carried out, and the mean values ± standard error are reported in Tables I and II.

Identification of Radioactive Spots. The compounds on the chromatograms were first detected by spraying with ninhydrin for amino acids, bromcresol green for organic acids, and aniline oxalate for reducing substances (*16*). The spots were provisionally identified by reference to a prepared chromatographic map of known compounds.

For final identification, the spots were cut out, the activity was eluted, and the active compounds were rechromatographed unidimensionally in several different solvents in the presence of added

Table III. Respiration Rates of Fumigated and Check Western Drywood Termites

	Check Samples	Fumigated Samples
Eq. of curve describing[a] µl. of oxygen consumed	$0.66T - 2.96$	$0.90T - 4.75$
Eq. of curve describing µl. carbon dioxide produced	$0.32T - 1.01$	$0.94T - 4.93$
µl. of oxygen at 60 min.	36.6	49.0
µl. of carbon dioxide at 60 min.	18.2	49.0
Respiratory quotient	0.50	1.00

[a] Equations were derived by applying the method of least squares to respiration data; T = minutes.

carrier. Although the coincidence between the area of radioactivity on the radiogram and the area detected by spraying cannot be taken as absolute proof of identity, it was considered tentatively established when coincidence was observed with at least three solvents of different types.

Measurement of Respiration and Determination of Respiratory Quotient. The procedure described by Umbreit *et al.* (*15*) was used to measure the respiration rate of fumigated and check termites. Termites were fumigated as previously described; paper toweling was placed in the Warburg flasks along with the termites. Table III gives the results of this experiment.

Effect of Fluoride on Oxidative Phosphorylation. The experiments on oxidative phosphorylation were carried out using the procedure described by Colowick and Kaplan (*3*). Termite homogenates were used as the enzyme source and succinate as the substrate. Two experiments were conducted: first, the effect of fluoride on esterification of
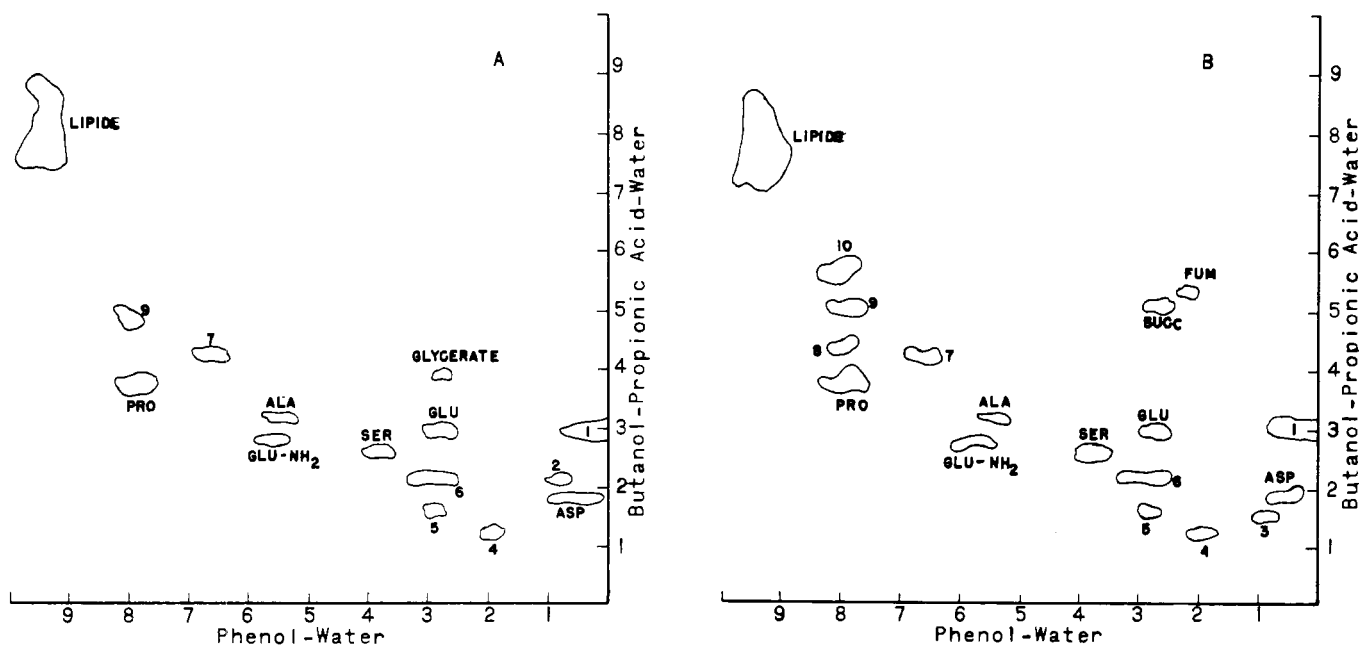

Figure 1. Chromatograms of extracts from Western drywood termite labeled with carbon-14

[ASP, aspartic acid; FUM, fumaric acid; SUCC, succinic acid; GLU, glutamic acid; SER, serine; ALA, alanine; GLU-NH₂, glutamine; PRO, proline; 1–10, unidentified compounds; (A) check termites; (B) fumigated termites]
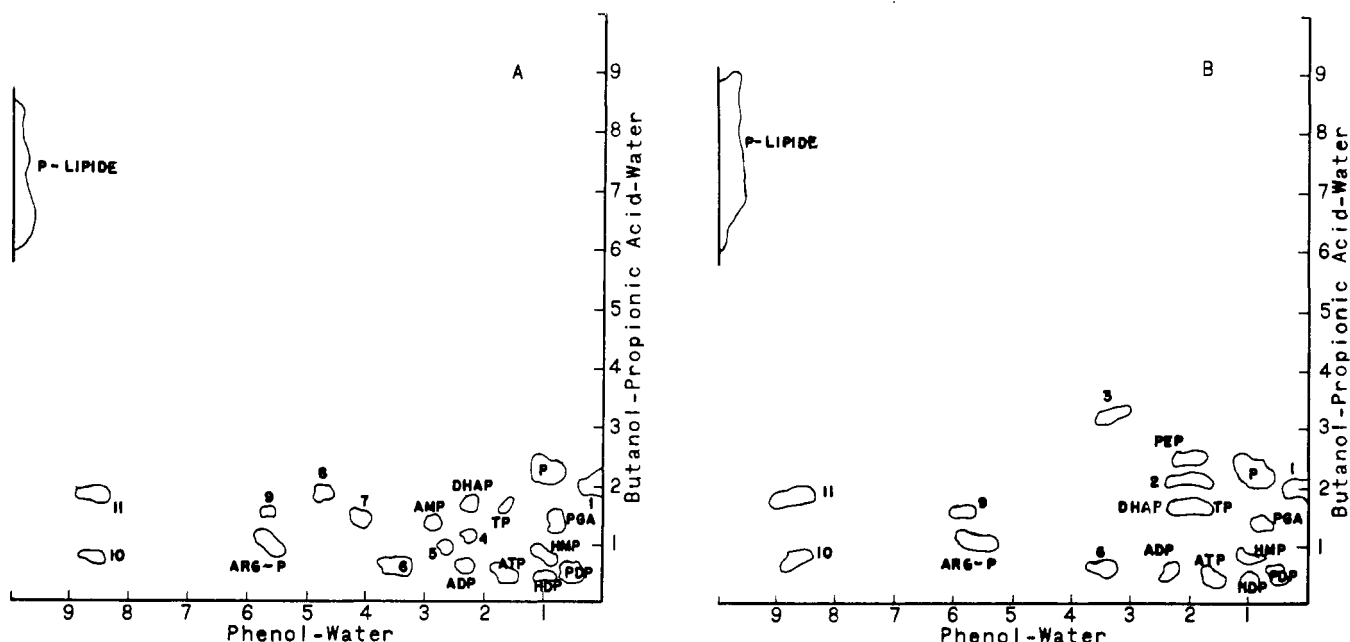
Figure 2. Chromatograms of extracts from Western drywood termite labeled with phosphorus-32

P, ortho phosphate; PGA, 3-phosphoglyceric acid; HMP, hexose monophosphate; HDP, hexose diphosphate; PDP, pentose diphosphate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; TP, trios phosphate; DHAP, dihydroxyacetone phosphote; ARG-P, arginine phosphate; 1-11, unidentified compounds; (A) check termites; (B) fumigated termites
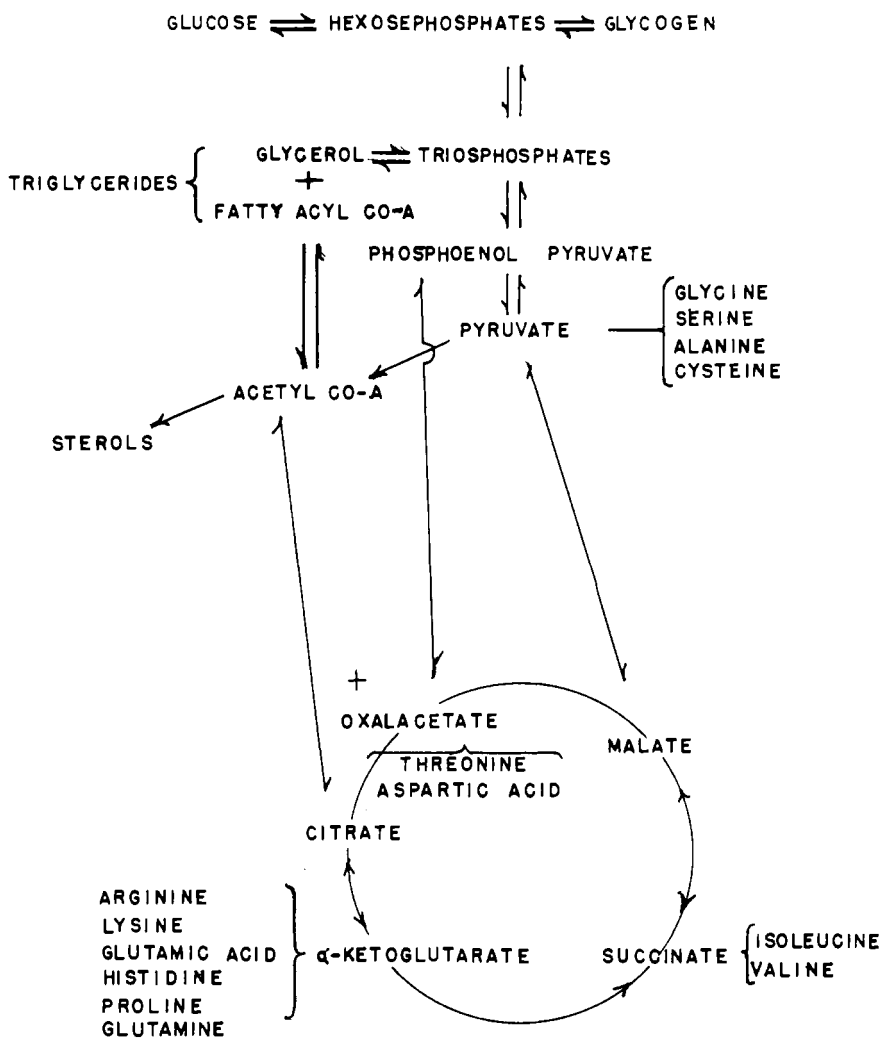

Figure 3. Outline of intermediary metabolism

phosphate by termite homogenates was determined, and second, a comparison was made of the ability to esterify phosphate between homogenates from fumigated and check termites. The results of these experiments show that fluoride has no effect on the reaction—if anything it increases the rate; there was no significant difference in the esterification rates of the two termite homogenates.

Discussion

Termites fumigated with lethal doses of Vikane show no unusual or characteristic symptoms of poisoning other than the usual expulsion of fluid. Because observation of the behavior pattern of the fumigated termite failed to give any clue as to the mode of action of the fumigant, the present study was undertaken to find the more subtle changes in intermediary metabolism which might be expected to take place. A systematic consideration of these changes, then, should lead to an understanding of the mechanism by which the toxicant works.

The first experiment with termites involved a fumigation with sulfuryl-S^{35} fluoride. This led to the discovery that the pellets left by the insects contained radioactive sulfate, thus showing without any question that Vikane was metabolized.

Two additional experiments were carried out, one with carbon-14 labeled and the other with phosphorus-32 labeled termites. Half of each group was fumigated with a sublethal dose of Vikane, and the other half acted as unfumigated checks. Extraction and

two-dimensional chromatography led to the radioautographs shown in Figures 1 and 2.

Soluble carbon and phosphate pools become rapidly labeled when a biological system is fed acetate-C^{14} and phosphate-P^{32}, and the termite is no exception.

The quantitative distribution of the identified metabolites serves to point out the metabolic disturbances which seem most likely to be a factor in causing the observed changes. In the following discussion, it will be assumed that in general, the broad outlines of intermediary metabolism in insects is similar to that in vertebrates (9) as shown in Figure 3.

Fats serve as the storage form of carbon chains derived from protein and carbohydrate. The pyruvate which is formed from either carbohydrate or amino acids can be converted into acetyl Coenzyme A, which in turn can be built up to long-chain fatty acids by successive addition of two-carbon units. Likewise, fatty acids can be degraded into acetyl Co-A. These reactions constitute the fatty acid cycle which is outlined in Figure 3. Fats, like other body constituents, are in dynamic exchange with the pool of fatty acids. The mechanism of this exchange can be accounted for by the reactions of the fatty acid cycle and that of lipoprotein lipase. If the level of lipide in the unfumigated termites at the moment of sacrifice is considered as the steady state level, then obviously somewhere in the fumigated termite there is a block in the dynamic exchange system which results in a buildup of the radioactive lipide as recorded in Table I.

Any conceivable reaction involving Vikane, whether hydrolysis or an acylation, will yield inorganic fluoride; therefore, it is perfectly tenable to assume that fluoride will be present as a result of Vikane metabolism. Fluoride has been shown to be inhibitory toward the enzyme lipase (4, 18, 19).

Carbohydrate foodstuffs, cellulose in the case of the termite, are hydrolyzed to monosaccharides and carried to the fat bodies where they are converted to hexose- and pentose-phosphates. It is generally thought that the major site for the interconversions among proteins, fats, and carbohydrates in insects are the fat bodies, which may be considered to possess certain of the functions of the vertebrate liver, as well as serving as a fat depot. The phosphate esters are maintained in dynamic equilibrium with glycogen, and these substances serve as a reservoir for the operation of the glycolosis pathway through which they are converted to lactate, pyruvate, and particularly α-glycerophosphate in insects. In muscle, this process is considered as one of the major sources of energy. Most tissues have little storage ability for these end products, and when

they are produced at a rate faster than they can be oxidized through respiratory reactions, they are released from the organism. Glycolosis, therefore, serves as a drain on the glycogen supply, and under ordinary conditions the equilibria favor glycolosis.

Hexose mono- and di- and pentose-diphosphates are all higher in concentration in the fumigated termite than in the checks as shown in Table II. In addition, phosphoenol pyruvate was not detectable in the checks although there was a substantial amount present in the fumigated insects. Again, it seems clear that there is a block in the glycolosis pathway causing these compounds to build up in the fumigated termites.

Fluoride is known to be generally inhibitory to the process of glycolosis, and, certainly so, to many of those enzyme systems which depend upon magnesium ion for their activity. The strong inhibition of enolase by fluoride (17) is an appropriate example of this. Many enzymes of the glycolosis pathway are of this type. Both phosphopyruvate carboxylase and pyruvate kinase require magnesium ion as a cofactor and are quite likely to be inhibited by fluoride although this has not been reported in the literature to the authors' knowledge. These latter two enzyme systems, along with enolase, are the systems acting on phosphoenol pyruvate; inhibition of these systems could well account for the high level of this compound in the fumigated termites.

A consideration of the relative amino acid concentrations in Table I indicates that the level in the fumigated termites is substantially lower than in the checks. Ordinarily, the citric acid cycle and glycolosis provide for synthesis and energy by utilizing fats and carbohydrates. It seems reasonable that if the operation of these systems is interfered with, the animal's metabolism will adjust itself in such a way as to overcome this stress. Now, it is well established that the amino acids participate in transamination and deamination reactions. In these processes, they are converted into certain members of the citric acid cycle—α-ketoglutaric, oxalacetic, and pyruvic acids. The operation of this cycle in cells permits the conversion of all but three amino acids into pyruvic acid. These can readily be converted to glucose and glycogen, and to fatty acids via acetyl Co-A. Thus, either carbohydrate or fat can be synthesized from carbon chains derived originally from protein; the former process is called gluconeogenesis—the formation of carbohydrate from noncarbohydrate precursors—and the latter might similarly be termed liponeogenesis. For example, it has been known for a long time that the blood glucose concentration in animals is maintained relatively constant over fairly long periods of starva-

tion. During this interval the total protein of the organism decreases. This process of gluconeogenesis is accomplished primarily by means of those enzyme systems which catalyze the conversion of amino acids to members of the citric acid cycle. The enzymes are the transaminases and dehydrogenases. It is well established that the transaminases are unaffected by fluoride (13). Neither is fluoride mentioned in the literature as an inhibitor of dehydrogenases involved in amino acid metabolism.

To account for the low level of amino acids in the fumigated insects, it is suggested that the amino acids are led into the citric acid cycle at an abnormally high rate in an attempt to maintain an energy level sufficient to carry out body processes; it has already been pointed out that the normal channels for maintaining the energy balance, the fatty acid cycle and glycolosis, appear to be blocked to some extent.

The process of oxidative phosphorylation is vital in the economy of aerobic cells. Since most of the enzyme systems controlling this process require magnesium, they would appear to be vulnerable to the action of fluoride. The adenosine triphosphatases are quite important in insects; most of these require magnesium ion as a cofactor and are inhibited by fluoride (10). Under conditions of inactive phosphorylation, as induced by pretreatment with fluoride, adenosine triphosphate (ATP) is known to be reduced in concentration (11). In Table II this is the case; the check termites show a significantly higher level of this metabolite. However, the authors were unable to show that oxidative phosphorylation was inhibited by fluoride or by homogenates of termites fumigated with Vikane.

Arginine phosphate is present in substantially higher concentration in the fumigated termites. A study by Pettersson (8) correlated nicely the reciprocal concentration of phosphagens with that of ATP, and in the present work, the same reciprocal relationship seems to be in operation. There is substantial evidence that in the living cell, phosphagens serve as a reserve of energy-rich phosphate bonds which are utilized for the rephosphorylation of adenosine diphosphate (ADP) through the Lohman reaction. This mechanism is believed to function where the energy demands are such that the rate of energy expenditure exceeds the rate at which new energy-rich bonds can be regenerated. The presence of this high level of arginine phosphate suggests, in itself, that there is a particularly rapid expenditure of energy occurring in the fumigated termites.

Since most insecticides, and almost any form of stress for that matter, bring about an intensification of the oxygen uptake in insects (5), it seemed of inter-

est to measure the respiration rates of the fumigated and unfumigated termites. The results of this experiment showed a definite increase in the rate of oxygen uptake in the case of the fumigated insect. The respiratory quotient was increased from 0.5 to 1.0. The fluoride probably disrupts the basic energetics of the cell in some manner and increases the oxygen consumption by disturbing the interrelationships between inorganic phosphate, ADP, and ATP (6).

In the case of Vikane, then, the only path open to the fumigated termite for maintaining the necessary energy balance appears to be one involving the utilization of protein and amino acids. Since this source of internal food is probably not capable of increasing the metabolic rate sufficiently to keep up with or overcome the stress, the insect dies.

The general procedure described here has yielded a sizable amount of information which is readily susceptible to interpretation leading to an understanding of the mechanism of action of a toxicant in a biological system. The procedure is flexible and of general utility since it requires only labeled acetate and phosphate and not necessarily labeled toxicant.

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SOIL ADSORPTION OF HERBICIDES

Adsorption of Several Pre-emergence Herbicides by Hawaiian Sugar Cane Soils

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The equilibrium adsorptions of fenuron, linuron, pentachlorophenol, simazine, and atrazine on Hawaiian sugar cane soils were compared to monuron and diuron. The adsorptive pattern for different soils was similar for the different herbicides; the major differences were in the quantitative adsorptivity level of a given chemical and were in the order pentachlorophenol $\geq$ linuron $>$ diuron $>$ simazine $>$ monuron $>$ atrazine. Empirical conditions for Freundlich isothermal adsorption were followed at most concentrations. Adsorptive behavior of these soils was found to be a property of soil mineral fraction (minor for most soils), easily oxidized organic fraction, and carbon arising from cane leaf burning. The latter two were considered major factors.

IN A PREVIOUS COMMUNICATION (4), a study was made of the equilibrium adsorption and desorption characteristics of a number of Hawaiian sugar cane soils for the two pre-emergence herbicides: monuron, 3-(p-chlorophenyl)-1,1-dimethylurea, and diuron, 3-(3,4-dichlorophenyl)-1,1-dimethylurea. The data obtained established that Hawaiian topsoils were generally highly adsorptive and that the isothermal equilibrium adsorption obeyed the Freundlich equation.

The present study extends the previous work to the comparison of monuron and diuron with two other substituted urea herbicides: fenuron, 3-phenyl-1,1-dimethylurea, and linuron, 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea [the Du Pont Co. has proposed formally to the K-62 Committee of the American Standards Association that the term linuron be established as the approved common (generic) name]; with two substituted triazine herbicides now in common use in Hawaii—simazine, 2-chloro-4,6-bis-(ethylamino)-s-triazine, and atrazine, 2-chloro-4-ethylamino-6-isopropylamino-s-triazine; and with pentachlorophenol (PCP). While PCP has not been considered a pre-emergence herbicide in the usual sense, its excellence as a contact weed seedicide has led to its use in irrigation water prior to sugar cane emergence. In this application, soil becomes important to the extent that it removes the PCP from available soil solution and that it is a major factor in protecting the crop root system from contact with the chemical.

Experimental

Adsorption of Substituted Urea Herbicides by Soils. An aqueous solution, 100 ml., containing from 5 to 10 μg. per ml. of the respective herbicides, purified by repeated crystallization, and 2.5 grams of K_2SO_4 per liter as a flocculent, was shaken with 50 grams of air dry soil for 15 minutes. The soil had been ground and screened to pass a 2-mm. mesh screen. The suspensions were filtered through Whatman No. 12 paper, and the clear filtrates were analyzed by hydrolysis to the substituted aniline followed by steam distillation, diazotization, and coupling with N-1-naphthylethylenediamine according to the method of Young and Gortner (3).

Adsorption of Substituted Triazine Herbicides by Soils. SIMAZINE. An