behind the major portion of material which interferes with the TLC separation and which effects approximately a tenfold increase in concentration.

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Conversion of 1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) to Water-Soluble Products by Microorganisms

Fredrick W. Juengst, Jr.,[1] and Martin Alexander*

Forty-three percent of the bacteria isolated from sea water and marine sediments converted between 5 and 10% of the DDT supplied in vitro to water-soluble products, and 35% transformed less than 5% of the insecticide to water-soluble metabolites. Several water-soluble compounds generated from DDT by *Mucor alternans* were partially characterized and were found to be different from known products of DDT metabolism. However, the insecticide was not converted to water-soluble products in model marine ecosystems supplemented with a large number of organic compounds and incubated aerobically, anaerobically, and with supplemental inorganic nutrients.

DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane) and DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene) are formed from DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) in soil, water, plants, and animals. Although these compounds can be generated by photochemical reactions (Miller and Narang, 1970; Plimmer et al., 1970), microorganisms are apparently important in their formation in certain ecosystems. In addition, 1-chloro-2,2-bis(*p*-chlorophenyl)ethylene, 2,2-bis(*p*-chlorophenyl)ethylene, 1-chloro-2,2-bis(*p*-chlorophenyl)ethane, bis(*p*-chlorophenyl)methane, 4,4'-dichlorobenzophenone (DBP), 4,4'-dichlorobenzhydrol (DBH), and 2,2-bis(*p*-chlorophenyl)acetic acid (DDA) are produced in model freshwater and sewage ecosystems under controlled laboratory conditions (Pfaender and Alexander, 1972). Most of these metabolites have not been isolated from marine waters, but this may result from the minute amounts of the intermediates that accumulate.

DDD and DDE have been reported to accumulate in marine environments (Butler, 1969). Whether these compounds are generated by the activity of microorganisms or by photochemical reactions is not known, but Patil et al. (1972) showed that such products appeared in cultures of marine microorganisms. Some of the isolates were also noted to synthesize 2,2-bis(*p*-chlorophenyl)-ethanol and 2,2-bis(*p*-chlorophenyl)ethane. Products of more extensive degradation of the insecticide have not been reported in marine waters. Considering the environmental problems associated with the use of this insecticide, it is surprising that products representing the removal of only one of the 14 carbon atoms in the DDT molecule have been described. This may partly result from the widespread use of hexane extraction procedures so that only hexane-soluble metabolites are characterized. By contrast, Anderson et al. (1970) observed that *Mucor alternans* converted DDT to water-soluble compounds as well as hexane-soluble products. Miyazaki and Thorsteinson (1972) subsequently reported that *Nitzschia* sp. and an unnamed diatom converted DDT to an unidentified water-soluble product.

Because the water-soluble compounds may represent new groups of DDT metabolites generated by pathways of degradation not heretofore characterized, a study was initiated of the possible synthesis of water-soluble products from DDT by marine microorganisms, and an attempt was made to characterize the water-soluble metabolites formed from the insecticide by *M. alternans*.

MATERIALS AND METHODS

Marine bacteria were isolated and maintained either on a medium containing 2.0 g of yeast extract, 0.05 g of K_2HPO_4, 15 g of agar, and 1000 ml of sea water that was allowed to stand for at least 30 days before use or on a medium described by ZoBell (1946) and containing 2.0 g

Laboratory of Soil Microbiology, Department of Agronomy, Cornell University, Ithaca, New York 14853.
[1]Present address: Calor Agriculture Research, Okemos, Michigan 48864.

of glucose, 1.0 g of peptone, 0.05 g of K_2HPO_4, 15 g of agar, and 1000 ml of the aged sea water. The same media without agar were also employed. *M. alternans* was grown in a medium with 5.0 g of glucose, 1.0 g of NH_4NO_3, 1.0 g of K_2HPO_4, 1.0 g of KH_2PO_4, 0.20 g of $MgSO_4 \cdot 7H_2O$, 0.02 g of $CaCl_2 \cdot 2H_2O$, 0.01 g of $Fe_2(SO_4)_3 \cdot H_2O$, and 1000 ml of distilled water (Anderson and Lichtenstein, 1971). The fungus was grown in the dark for 7 days at 28°C on a rotary shaker operating at 190 rpm, and DDT dissolved in acetone (a maximum of 0.4% acetone, v/v, in the medium) was added 18 hr after the flasks were inoculated with a spore suspension.

To isolate DDT-metabolizing microorganisms, sea water and sediment were collected in sterile containers from seven sites along the Connecticut coast. The sites included four distinct habitats: brackish river water, salt marsh, and waters with bottoms consisting of fine sand or gravelly, coarse sand. The samples were shaken vigorously and streaked onto the two agar media within 2 hr of collection. The plates were incubated at about 23°C. Different colony types were selected, and pure cultures were obtained by standard techniques.

To measure the ability of the isolates to transform DDT to water-soluble products, each bacterium was grown in the yeast extract–sea water liquid medium in the presence of 0.5 μg of ^{14}C-labeled DDT/ml (sp act. 2.25 μCi/mg). The cultures were incubated for 7 to 18 days at 23°C, after which the cells were removed by centrifugation, the supernatant fluids were extracted three times with 50-ml portions of hexane, and the hexane extracts were discarded. The water phase was then examined for its radioactivity.

The effect of organic compounds on DDT degradation was determined by placing 250 ml of surface sea water from the Sagamore Terrace site, 50 ml of a suspension of gravelly coarse sand from the upper 10 cm of sediment from the same site, and 25 mg of the test compounds into 500-ml Erlenmeyer flasks. After 24 hr at 23°C, ^{14}C-labeled DDT (0.14 μCi/mg) was introduced to a concentration of 2 μg/ml, and the flasks were incubated in the dark. The test substrates were: acetate, alginate, 4-aminopyridine, *m*-aminophenol, *m*-aminobenzoate, anthranilate, ascorbate, casamino acids, catechol, chitin, 4-chlorodiphenylmethane, 4-chlororesorcinol, citrate, coumalate, *o*-cresol, crotonate, diphenylmethane, α,α-dipyridyl, *m*-ethoxybenzoate, gelatin, glucose, L-glutamate, glutarate, glycerol, guaiacol, gum arabic, *p*-hydroxybenzoate, *trans*-β-hydroxymuconate, 4-hydroxybutyrate, 4-hydroxydiphenylmethane, kojic acid, kynurenate, laurate, lauryl sulfate, L-leucine, a lignin preparation, linoleate, maleate, mannitol, *m*- and *o*-methylbenzoate, 3- and 4-methylcatechol, *m*-methoxyphenol, methylmalonate, mucic acid, α-naphthol, oleate, palmitate, pectin, L-phenylalanine, 4- and 2-phenylbutyrate, α-phenyl-*o*-cresol, *p*-phenylphenol, phenylurea, phloroglucinol, phthalate, picolinate, pyruvate, quinaldate, resorcinol, ribose, rutin, sebacate, shikimate, starch, succinate, syringate, tannate, tartrate, *p*-toluate, 2,4,6-trihydroxybenzoate, tyrosine, urea, urethan, vanillin, and yeast extract. In addition the following conditions were established to test the effect of carbon sources and simulated environmental factors: (a) flasks containing no amendments, 0.1% K_2HPO_4 plus 0.1% NH_4NO_3, or the two salts plus 25 mg of catechol, diphenylmethane, glucose, 4-methylcatechol, palmitate, 4-phenylbutyrate, or rutin, these solutions being incubated at 23°C in the dark under a layer of mineral oil to minimize O_2 entry; (b) flasks containing no amendments or 0.1% K_2HPO_4 plus 0.1% NH_4NO_3 and incubated at 27°C under continuous illumination with a layer of mineral oil over the solution; and (c) flasks containing no amendments, K_2HPO_4 plus NH_4NO_3, or the two salts plus 25 mg of catechol, diphenylmethane, 4-methylcatechol, palmitate, or 4-phenylbutyrate and incubated at 28°C in the dark on a rotary shaker operating at 190 rpm.

After 5 weeks, a 50-ml portion was removed from each flask and filtered through Whatman no. 42 filter paper. The filter paper was dried in air and extracted with three 25-ml quantities of hexane. The ^{14}C content of the pooled hexane phases was measured with a liquid scintillation counter.

For gas–liquid chromatography, a Varian Model 200 gas chromatograph equipped with a flame ionization detector was employed. When a 10% DC 200 column on Chromosorb W (AW, DMCS) was used, the operating temperatures were 185, 215, and 195°C for the column, injector, and detector, respectively. When the column was 15% SE 30 on Chromosorb W (AW, DMCS) the operating temperatures were 90, 185, and 215°C for the column, injector, and detector, respectively. The flow rate of N_2 through both columns was 45 ml/min.

The method of Schlenk and Gellerman (1960) was used to esterify organic acids and a fungal metabolite prior to gas chromatography. The diazomethane generator resembled the one described by these authors. As soon as excess diazomethane was evident by the yellow color of the sample, the sample tube was disconnected, and its contents were evaporated to dryness immediately with a flow of N_2. The esterified acids were then dissolved in chloroform or ether and examined directly by thin-layer or gas–liquid chromatography.

For thin-layer chromatography, the chemicals were spotted on silica gel (Eastman chromatogram sheet 6060) bearing a fluorescent indicator. After development, the spots were visualized under ultraviolet light (254 nm) with a UVS 12 Mineralite (Ultraviolet Products, Inc., San Gabriel, Calif.). Chromatograms were cut into 4-cm strips, and their radioactivity was counted with an Actigraph III strip counter (Nuclear-Chicago Corp., Chicago, Ill.). To measure the radioactivity of liquids, 0.1 to 0.5 ml samples were placed into scintillation vials, and 10 to 15 ml of scintillation fluid (Bray, 1960) was added. The radioactivity in the vials was counted with a liquid scintillation counter (Nuclear-Chicago Corp., Model Mark I) which automatically subtracted the background counts.

For mass spectrometry, samples were adsorbed onto silica gel, and the gel was placed into a capillary tube. The samples were analyzed with a low-resolution mass spectrometer (Model MS 902, AEI, Manchester, England). The operating conditions were: electron energy, 500 eV; accelerating voltage, 3 kV; ion source, 170°C; and probe, 135°C.

DDT, DDA, DBP, DBH, and *p*-chlorophenylacetic acid (PCPA) were obtained from Aldrich Chemical Co., Milwaukee, Wis. 2-Chlorosuccinic acid was purchased from K and K Laboratories, Plainview, N.Y. Ring-labeled [^{14}C]DDT was provided by New England Nuclear, Boston, Mass. (17.9 μCi/mg) or Tracerlab Inc., Waltham, Mass. (2.25 μCi/mg), and it was found to be free of labeled water-soluble impurities. Hexanes used for extractions were pesticide grade from Fisher Scientific Co., Fairlawn, N.J. Column packing materials were purchased from Applied Science Laboratories, State College, Pa.

RESULTS

Isolation of Microorganisms Degrading DDT. The isolated marine bacteria were tested individually for their activity on DDT. An isolate capable of forming water-

Table I. The Ability of Marine Bacteria to Form Water-Soluble Products from DDT

Sample site	Surface water: No. of bacteria tested	Surface water: No. of active bacteria	Sediment: No. of bacteria tested	Sediment: No. of active bacteria
Connecticut River at Saybrook, Conn. (brackish water)	16	5	11	4
Saybrook salt marsh	8	1	7	2
Coast, 1 mi. from Conn. River	14	6		
Coast, 2 mi. from Conn. River	9	4		
Back River (brackish water)	10	7	5	2
Kelsey Point (fine black sand)	3	2	11	4
Sagamore Terrace (course gravel)	5	3	11	7

soluble products was considered as one converting more than 5% of the labeled DDT to water-soluble products. The results in Table I show that a large percentage of the isolates had the ability to form water-soluble compounds from DDT and presumably to initiate its degradation. A total of 47 of the 110 isolates were found to have converted more than 5% of the [^{14}C]DDT to water-soluble compounds, but none solubilized more than 10% of the radioactivity. Of the 110 organisms, moreover, 38 converted less than 5% of the [^{14}C]DDT to water-soluble metabolites, the remaining 25 being inactive under the test conditions. Because water-soluble compounds were not detected in 25 cultures, it seems unlikely that the water-soluble substances found in the active cultures were present in the original DDT or were derived from nonbiological reactions.

Effect of Organic Compounds on DDT Degradation. The addition of a particular organic substrate to natural microbial communities may enhance DDT metabolism because it allows a minor population having the requisite enzymes to become a dominant species in the community or it induces an enzyme system which, in addition to transforming the added substrate, catalyzes an initial phase in the degradation of DDT. Since the enzymes involved in the cometabolism of DDT are unknown, it is impossible to predict which substrates might stimulate the cometabolism of DDT. Consequently, 78 organic preparations or compounds were tested to see if their addition to 100 separate model marine communities might enhance the conversion of DDT to water-soluble products.

Water and sediment from the Sagamore Terrace site were used since they apparently contained many DDT-metabolizing bacteria. After 5 weeks of incubation, the solutions initially containing DDT and the various organic supplements were passed through Whatman no. 42 filter paper. All of the radioactivity was removed from 84 of the 100 test flasks by this filtration, and none was detected in the filtrate. The remaining 16 flasks contained some ^{14}C in the filtrate, and these filtrates were extracted with three 50-ml portions of hexane. The resulting aqueous phase was analyzed for its ^{14}C content, but in no instance was it noted to contain detectable amounts of radioactivity. The average recovery of ^{14}C from all 100 flasks was 90%. Thus, no water-soluble DDT metabolites were apparently formed in these model ecosystems.

Water-Soluble Products Formed by *Mucor alternans*. Since the reported rate of conversion of DDT to water-soluble products by *M. alternans* (Anderson et al., 1970) is considerably faster than that of the marine bacteria, the identities of the products formed by the fungus were investigated. A culture of the fungus was grown in 2-l. Erlenmeyer flasks containing 500 ml of the glucose–salts medium and 2 μg of [^{14}C]DDT/ml. The radioactive DDT was mixed with unlabeled insecticide to give a final specific activity of 1.79 μCi/mg. After 7 days, the hyphae were removed by filtration, and the filtrate was extracted with three 500-ml portions of hexane. About 15% of the radioactivity from DDT was found among the water-soluble metabolites. The extracted filtrate was taken to dryness by flash evaporation at 30°C, and the residue was extracted with methanol and then ether, as described by Anderson et al. (1970). The ether solution was concentrated to 5 ml by flash evaporation. The liquid was then examined by thin-layer and gas–liquid chromatography.

The retention time of the methylated fungal product by gas chromatography on a 10% DC 200 column was 130 sec, while the retention times of the methyl esters of DDA and PCPA were 34.0 min and 132 sec, respectively; hence, the compound is not DDA although it could be PCPA. However, evidence that it was not identical with PCPA was obtained by thin-layer chromatography using a hexane–acetone (90:10) solvent system; in this solvent system, the R_f values of diazomethane-treated DDA, PCPA, and the fungal metabolites were 0.82, 0.90, and 0.00, respectively. The unknown also was not DBP or DBH because the R_f values of DBP, DBH, PCPA, DDA, and the *M. alternans* product when a hexane–ether–acetic acid (100:1:1) solvent system was employed were 0.74, 0.14, 0.19, 0.09, and 0.00, respectively. Its identity as 2-chlorosuccinic acid was ruled out because the methyl derivative of the unknown and of chlorosuccinate had different retention times by gas chromatography on a 15% SE 30 column.

To obtain large amounts of the products, *M. alternans* was grown for 7 days in eight 2-l. Erlenmeyer flasks each containing 500 ml of the glucose–salts medium plus 1.0 mg of [^{14}C]DDT. Radioactive DDT was mixed with unlabeled insecticide to give a final specific activity of 1.81 μCi/mg. Two-liter quantities of the medium were extracted twice with 1-l. portions of hexane, the hexane was discarded, and the residual medium (containing about 960 μg of water-soluble products, based on ^{14}C recovery) was extracted twice with 200-ml quantities of ether. The ether-extracted medium was then acidified to pH 1.6 and extracted twice with 400-ml quantities of ether, the solvent phase being concentrated to 10 ml in a flash evaporator. The yield was 370 μg/2 l. of medium based on the ^{14}C remaining.

A portion of the concentrated ether extract was applied in a narrow band on two silica gel thin-layer chromatography plates, and the plates were developed in methanol–water (75:25) and then dried in air. A strip was cut from the plate, and the ^{14}C-containing areas were located using an Actigraph III stripscanner. At least three components were present since three radioactive peaks were found. The procedure employed separated the radioactive compounds from yellow substances which were found between 7.5 and 8.5 cm from the origin.

The area of the silica gel showing greatest radioactivity (3.7 to 7.5 cm from the origin) was extracted with water, and the liquid was filtered to remove the silica gel, acidified to pH 1.5, and extracted with ether. This ether extract was concentrated to 5 ml by flash evaporation, the recovery of ^{14}C material being about 50%. The losses presumably occurred during the evaporation. The entire extract was

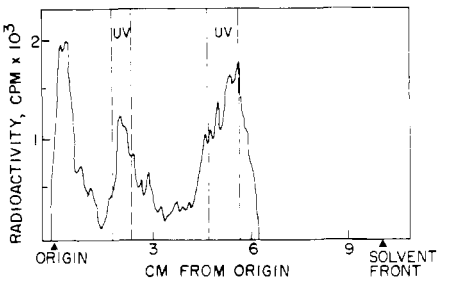

Figure 1. Separation of metabolites in fraction S prior to mass spectrometry.

spread in a thin band on a second silica gel plate and developed in ethanol–NH_4OH–water (20:1:4). All the radioactivity was found in the area from 0 to 4.7 cm from the origin. Some nonradioactive uv-absorbing material was evident near the solvent front, but it was completely separated from the labeled products. Two separate radioactive peaks, designated S and F, were evident at 0.0 to 1.8 cm and 1.8 to 4.7 cm from the origin. These were removed separately from the plate. The material so removed was extracted with water, and the water phase was filtered, acidified, and extracted with ether. The recovery of ^{14}C material after concentration of the ether extract was 68%. At this stage of purification, about 24 μg of component S and 22 μg of component F remained.

One-half of extract S was placed in a small spot on a strip of silica gel and developed in methanol–water (75:25). The strip was then examined for radioactivity. The results are shown in Figure 1. Two small spots that weakly absorbed uv light were observed, and these were in an area of the gel containing the ^{14}C activity. These spots and also a portion of gel free of radioactivity were placed into small capillary tubes and analyzed by mass spectrometry. The mass spectrum of the control gel showed no peaks above m/e 57. If these products contain chlorine, as do all reported products of DDT degradation, the parent ion and its fragments should show distinctive chlorine couplets resulting from the natural ratio of ^{35}Cl and ^{37}Cl. No such chlorine couplets were found for the two compounds in fraction S, and no parent ion was detected.

The remaining 12 μg of fraction S was also analyzed by mass spectrometry. The resulting spectrum showed no distinctive chlorine couplets, and no parent ion could be identified. The major peaks were at m/e 177, 149, 147, 131, 129, 121, 105, 103, and 89.

Fraction F was analyzed by placing the entire extract on one 80 mm^2 spot on a silica gel plate. A sample of the spot was placed in a capillary tube and examined by mass spectrometry. No parent peak or chlorine couplets were found, however. The major peaks were at m/e 147, 145, 119, 103, and 89.

DISCUSSION

The lack of microbial degradation of DDT in nature is not the result of the absence of species capable of destroying the insecticide because microorganisms able to degrade the molecule extensively have been isolated from both soil and sewage (Pfaender and Alexander, 1972, 1973). Moreover, marine isolates have been found that act on the two nonring carbon atoms of DDT (Patil et al., 1972), but the production of ring-cleavage products in marine waters owing to extensive enzymatic attack has not been demonstrated. The formation of water-soluble metabolites from DDT may be taken as an index of extensive degradation of DDT, although admittedly conjugation, hydroxylation, or other reactions might also yield soluble products. The present findings show that a high percentage of randomly chosen marine isolates converted the insecticide to water-soluble products.

Despite the variety of treatments imposed on the model ecosystems, on the other hand, no water-soluble DDT metabolites were apparently formed. In most instances, all the ^{14}C was recovered in the particulate fraction (i.e., it failed to pass through the filter paper), suggesting that DDT, or products formed from it, was either sorbed to particles or formed nonfilterable clumps as suggested by Bowman et al. (1960). Nevertheless, water-soluble metabolites may have been generated and then sorbed by the particulate matter.

The observation that so many microorganisms are potentially able to perform at least certain steps in the degradation raises the question of why DDT is so long-lived in the oceans. A number of reasons for the persistence of compounds in nature have been proposed (Alexander, 1973). Among the possibilities are that the enzymes metabolizing DDT or its products are not induced because of the low concentration of chemicals in solution or that, because of the low level of substrate, the rate of enzyme reaction is extremely slow. Another possibility is that microorganisms capable of degrading DDT are physically separated from it. Thus, the accumulation of the pesticide in lipids of living organisms may protect it since the tissues are free of microorganisms, and even though the tissues undergo decomposition, the pesticide may be reincorporated into lipids of other organisms before appreciable destruction occurs. A third possibility is that the insecticide, which is retained by microorganisms (Rice and Sikka, 1973; Woodwell et al., 1971), is shielded from attack when retained by the cells of nondegrading species. Nonliving organic material such as simple organic molecules (Tinsley et al., 1971) and dead algae (Rice and Sikka, 1973) also can bind DDT, but the availability of DDT bound to nonliving organic material is not presently known.

Because of the presence of para chloro substituents on the rings of DDT, a 2,3-dihydroxy-4-chloro derivative may be generated in DDT metabolism. Cleavage of this postulated intermediate between the two hydroxyl groups, in the so-called ortho cleavage, would probably give rise to 2-chlorosuccinic acid or a closely related compound. However, 2-chlorosuccinic acid was not found in culture fluids of *M. alternans*. Meta cleaving oxygenases are less common than ortho cleaving enzymes but are not as seriously affected by ring substituents (Dagley, 1971). A meta type of ring cleavage would probably produce 3-chloro-substituted acids from DDT following cleavage of the 2,3-dihydroxy-4-chloro compound, as suggested by Focht (1972). However, meta cleavage typically yields intensely colored muconic acid intermediates (Goldman, 1972), but no such muconic acids have been demonstrated to result from DDT metabolism.

The evidence presented here suggests that the water-soluble compounds produced by *M. alternans* are distinct from known DDT metabolites. In addition, at least three components are apparently present, and they may all be acids. If chlorine is in fact absent from these compounds, they may be generated by a pathway involving the most extensive degradation of DDT so far reported for a single organism, since all products described to date contain chlorine. However, since fractions S and F were not extensively purified, these products may in fact have contained chlorine, which might have been obscured in the mass spectrum by impurities. Further work is thus clearly required to establish the identities of the metabolites.

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Metabolism of Aldicarb by Five Soil Fungi

Alice Stevens Jones

Pure cultures of five common soil fungi grown in Czapek-Dox Broth were tested for degradation of aldicarb, and three of the five were tested against aldicarb sulfoxide, the major toxic metabolite. The fungi were, in decreasing order of their effectiveness in degrading the pesticide: *Gliocladium catenulatum* > *Penicillium multicolor* = *Cunninghamella elegans* > *Rhizoctonia* sp. > *Trichoderma harzianum*. Although there was considerable variability among the fungi in the amounts of organo- and water-soluble products from both aldicarb and aldicarb sulfoxide, the major organosoluble metabolites were aldicarb sulfoxide and the oxime and nitrile sulfoxides, with much smaller amounts of the sulfones. Aldicarb was stable under sterile conditions, but some breakdown of aldicarb sulfoxide was found in sterile media after the 28-day incubation.

The metabolism of Temik aldicarb pesticide [2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime] has been studied extensively in plants (Bartley et al., 1970; Andrawes et al., 1973) and in animals (Andrawes et al., 1967; Dorough and Ivie, 1968). Coppedge et al. (1967) reported that aldicarb had a half-life of 9–12 days in soils under laboratory conditions. Other studies of aldicarb in soils (Bull et al., 1970) showed that aldicarb disappeared rapidly from moist soils, but it was relatively stable in dry soils. It was found to be stable in moist or dry sand, with a half-life exceeding 56 days. Spurr and Sousa (1966, 1974) tested aldicarb and its metabolites against pathogenic and saprophytic microorganisms. They found no direct disease control and no adverse effects against the saprophytes tested at dosages considerably higher than the recommended usage levels. They also reported that some of the microorganisms tested appeared to be able to utilize aldicarb as a carbon source.

The studies reported here were initiated to determine the ability of five soil fungi to degrade aldicarb and its major toxic metabolite, aldicarb sulfoxide. The various soil fungi chosen are found frequently in cultivated and forest soils (Hodges, 1962) and are capable of rapid growth in synthetic media. The group included three genera which are early colonizers or decomposers of organic materials, i.e. *Cunninghamella*, *Penicillium*, and *Trichoderma*, and two (*Gliocladium* and *Rhizoctonia*) which are often associated with root surfaces (Dickinson and Pugh, 1974).

USDA Forest Service, Southeastern Forest Experiment Station, Forestry Sciences Laboratory, Research Triangle Park, North Carolina 27709.

MATERIALS AND METHODS

Radiolabeled aldicarb (methylthio-^{14}C labeled, sp act. 7.1 mCi/mmol) was obtained from Mallinckrodt Chemical Works, St. Louis, Mo. The purity was determined to be 98% by thin-layer chromatography (TLC) with 3:1 methylene chloride–acetonitrile and liquid scintillation counting (lsc). Nonlabeled standards of aldicarb and its known metabolites (Table I) were synthesized and authenticated by Union Carbide Corp., Research and Development Dept., South Charleston, W.Va.

Radiolabeled aldicarb sulfoxide was recovered from the organic extracts of fungal cultures by Florisil column chromatography (Andrawes et al., 1967). The radiochemical purity of the sulfoxide was determined to be 98% by TLC with 6:1 chloroform–methanol and lsc.

For the metabolic studies, flasks capped with aluminum foil and containing 50 ml of Czapek-Dox Broth (Difco Laboratories, Detroit, Mich., pH 7.3) were autoclaved and allowed to cool. Then 0.5 ml of an ethanol solution of [^{14}C]aldicarb was aseptically added to each flask from a