APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1990, p. 2347–2353
0099-2240/90/082347-07$02.00/0

Vol. 56, No. 8

Comparative Biodegradation of Alkyl Halide Insecticides by the White Rot Fungus, *Phanerochaete chrysosporium* (BKM-F-1767)

DAVID W. KENNEDY,[1] STEVEN D. AUST,[1,2] AND JOHN A. BUMPUS[1,3]*

Biotechnology Center,[1] Chemistry and Biochemistry Department,[2] and Biology Department,[3] Utah State University, Logan, Utah 84322-4430*

Received 29 December 1989/Accepted 11 May 1990

The ability of *Phanerochaete chrysosporium* to degrade six alkyl halide insecticides (aldrin, dieldrin, heptachlor, chlordane, lindane, and mirex) in liquid and soil-corncob matrices was compared by using ^{14}C-labeled compounds. Of these, only [^{14}C]lindane and [^{14}C]chlordane underwent extensive biodegradation, as evidenced by the fact that 9.4 to 23.4% of these compounds were degraded to $^{14}CO_2$ in 30 days in liquid cultures and 60 days in soil-corncob cultures inoculated with *P. chrysosporium*. Although [^{14}C]aldrin, [^{14}C]dieldrin, [^{14}C]heptachlor, and [^{14}D]mirex were poorly mineralized, substantial bioconversion occurred, as determined by substrate disappearance and metabolite formation. Nonbiological disappearance was observed only with chlordane and heptachlor.

The alkyl halides aldrin, dieldrin, heptachlor, chlordane, lindane, and mirex have been used extensively as insecticides. Although they are very efficient insecticides, their use has been minimized or terminated in the United States owing to their persistence in nature, tendency toward biomagnification (bioaccumulation), and toxicity to higher animals. Their persistence in water, soils, and sediments presents an environmental hazard despite the reduction in their production and use.

The white rot fungus, *Phanerochaete chrysosporium*, has been shown to possess biodegradative capabilities for a broad spectrum of environmentally persistent compounds, including DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane] (4, 6), polychlorinated biphenyls (6, 7), and pentachlorophenol (12). The ability of this fungus to degrade such a diverse group of compounds is thought to be linked to the fact that it is one of relatively few microorganisms known to degrade lignin, a naturally occurring complex biopolymer. Mineralization of xenobiotics appears to be promoted when the fungus is ligninolytic, and lignin peroxidases, which are secreted by this fungus under nutrient-limiting conditions (ligninolytic conditions), have been shown to catalyze the initial oxidation of lignin as well as several environmentally persistent xenobiotics (5, 10, 12, 15, 16).

In addition to being important compounds of environmental concern, the compounds examined in this study may be thought of as being three pairs of structurally related molecules (Fig. 1). For example, heptachlor and chlordane are identical except that heptachlor has a double bond between C-2 and C-3 and is not chlorinated at C-2, whereas chlordane has a single bond between C-2 and C-3 and is chlorinated at C-2. Similarly, dieldrin has an oxygen atom bonded to C-6 and C-7 to form an epoxide, whereas aldrin, otherwise identical to dieldrin, does not contain this epoxide. Mirex and lindane are not as similar to each other as are the above-described pairs of compounds. However, by studying mirex and lindane, the biodegradation of a compound composed solely of C-C and C-Cl bonds (mirex) can be compared with one that is composed of C-C, C-Cl, and C-H bonds (lindane). The present study is designed to compare rates and levels of biodegradation of these compounds by *P. chrysosporium* and to evaluate the feasibility of using this fungus to treat materials contaminated with these chemicals.

* Corresponding author.

MATERIALS AND METHODS

Fungus. *P. chrysosporium* BKM-F-1767 was obtained from the U.S. Department of Agriculture Forest Products Laboratory, Madison, Wis. The fungus was stored at room temperature on malt agar slants prior to use. Subcultures were made routinely every 30 to 60 days.

Chemicals. The trivial names of the six alkyl halide insecticides are used as a matter of convenience. The following names of these insecticides are those recognized by the Chemical Abstracts Service (CAS): mirex, 1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1,3,4-metheno-1*H*-cyclobuta[*cd*]pentalene (CAS no. 2385-85-5); lindane, 1α,2α,3β,4α,5α,6β-hexachlorocyclohexane (CAS no. 58-89-9); aldrin, 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-endo,exo-1,4:5,8-dimethanonaphthalene (CAS no. 309-00-2); dieldrin, 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo,exo-1,4:5,8-dimethanonaphthalene (CAS no. 60-57-1); chlordane, 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan (CAS no. 57-74-9); and heptachlor, 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene (CAS no. 76-44-8).

Radiochemicals. [^{14}C]mirex (37.5 mCi/mmol), [^{14}C]aldrin (1.2 mCi/mmol), [^{14}C]heptachlor (1.8 mCi/mmol), and [^{14}C]chlordane (5.91 mCi/mmol) were purchased from Sigma Chemical Co., St. Louis, Mo. [^{14}C]lindane (54 mCi/mmol) was purchased from Amersham Corp., Arlington Heights, Ill. [^{14}C]dieldrin (85 mCi/mmol) was a gift from Shell Development Corp., Houston, Tex. Mirex and lindane were uniformly labeled. Aldrin and dieldrin were labeled in the 1, 2, 3, 4, and 10 positions, and chlordane and heptachlor were labeled in the 4, 5, 6, 7, and 8 positions. The radiochemical purity of all ^{14}C-labeled insecticides was 97% or greater. When necessary, individual ^{14}C-labeled compounds were purified by thin-layer chromatography or by high-performance liquid chromatography (HPLC).

Soil. The soil used in these experiments was classified as a silt loam, comprising 19% sand, 54% silt, and 27% clay. The content of organic matter in the soil was 3.62%, the organic carbon content was 2.10%, and the total nitrogen was 0.19%. The soil pH was 6.4, and the cation exchange capacity was

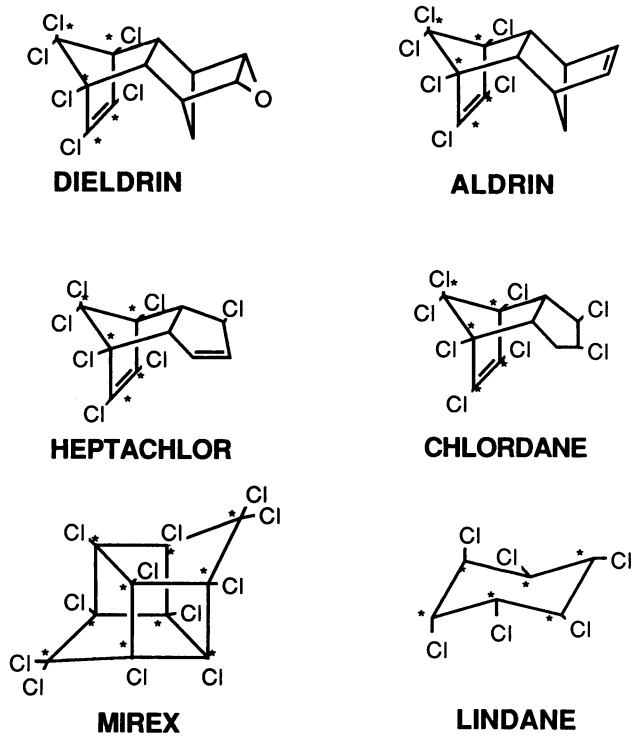

FIG. 1. Structures of alkyl halide insecticides used in this study. Asterisks indicate ^{14}C-labeled carbon atoms.

23.6 meq/100 g. Concentrations of selected extractable cations were as follows: Na^+, 0.01 meq/100 g; K^+, 0.99 meq/100 g; Ca^{2+}, 16.85 meq/100 g; and Mg^{2+}, 3.59 meq/100 g. Concentrations of divalent cations extractable with diethylenetriamine pentaacetic acid (DTPA) were as follows: Zn^{2+}, 2.3 mg/kg; Fe^{2+}, 35.1 mg/kg; Cu^{2+}, 1.2 mg/kg; Mn^{2+}, 29.4 mg/kg; Cd^{2+}, 1.4 mg/kg; Pb^{2+}, 0.86 mg/kg; Ni^{2+}, 0.47 mg/kg; and Co^{2+}, 0.16 mg/kg. Soil was sterilized by autoclaving at 121°C and 15 lb/in^2 for 20 min.

Experimental conditions. Biodegradation studies in aqueous cultures contained 1.2 mM ammonium tartrate, 56 mM (wt/vol) glucose, trace elements (11), and thiamine (1 mg/liter) in 20 mM dimethylsuccinate (sodium) buffer, pH 4.2 (9). Cultures (final volume, 10 ml) were inoculated with 1 ml of a spore suspension (optical density of 0.5 at 650 nm) in culture medium. Spore suspensions were prepared as described by Kirk et al. (11). The incubation temperature for all experiments was 39°C. Controls received 1 ml of formaldehyde (36%, wt/wt) instead of spore suspension to prevent microbial growth. Prior to the addition of media and spore suspensions, [^{14}C]aldrin, [^{14}C]dieldrin, [^{14}C]heptachlor, [^{14}C]chlordane, [^{14}C]mirex, or [^{14}C]lindane in acetone was adsorbed onto cellulose filters (5.5 cm in diameter; no. 1 filters; Whatman, Inc., Clifton, N.J.) and inserted into culture bottles.

Biodegradation studies with soil contained 1 g of soil and 4 g of corncobs (processed corncobs; Mt. Pulaski Products, Inc., Mt. Pulaski, Ill.) that had been previously inoculated with *P. chrysosporium* BKM-F-1767 and allowed to grow for 21 days. [^{14}C]aldrin, [^{14}C]dieldrin, [^{14}C]heptachlor, [^{14}C]chlordane, [^{14}C]mirex, or [^{14}C]lindane was added directly to 1 g of sterilized soil in 100 μl of acetone. The acetone was then allowed to evaporate in the hood under ambient conditions. Sterile water (0.67 ml) was added to achieve 40% (wt/wt) moisture content with 4 g of *P. chrysosporium*-inoculated corncobs. Control cultures received 1 ml of formaldehyde to prevent microbial growth and 4 g of sterile corncobs.

Cultures were grown in sealed 250-ml Wheaton bottles equipped with gas exchange manifolds. During the first 3 days of incubation, cultures were grown under ambient atmosphere. Every 3 days thereafter, cultures were flushed with oxygen for 25 min, the evolved CO_2 was trapped in an ethanolamine-containing scintillation cocktail, and the radioactivity was quantitated by liquid scintillation spectrometry. All $^{14}CO_2$ evolution studies were performed in triplicate or quadruplicate. Details of this mineralization assay have been previously described (6, 11).

Mass balance studies. Mass balance analyses were performed on cultures of *P. chrysosporium* that had been incubated with [^{14}C]aldrin, [^{14}C]dieldrin, [^{14}C]heptachlor, [^{14}C]chlordane, [^{14}C]mirex, or [^{14}C]lindane for 30 or 60 days. Liquid cultures (four for each compound) were extracted by adding 30 ml of methylene chloride to each bottle and shaking the bottles vigorously for 2 min. Cultures were pooled, after the first extraction, in a 250-ml separatory funnel. Pooled cultures were extracted two additional times with 30 ml of methylene chloride. Particulate material (mycelium and filter paper) was separated from the remaining aqueous fraction by filtration through glass wool. Samples of the particulate material were placed in 10 ml of Safety Solve (Research Products International Corp., Mount Prospect, Ill.) in scintillation vials for quantitation of radioactivity by liquid scintillation spectrometry. Samples (2 ml) of the methylene chloride and aqueous fractions were also added to 10 ml of Safety Solve for quantitation of radioactivity by liquid scintillation spectrometry.

Cultures containing soil and corncobs were extracted by addition of 30 ml of ether-ethyl acetate-acetone (5:5:1) to each culture bottle (except for aldrin and dieldrin, which were extracted with 30 ml of hexane-acetone [1:1]) followed by sonication in a Branson ultrasonic cleaner bath (Branson Cleaning Equipment Co., Shelton, Conn.) for 10 min. The phases were allowed to separate, and the solvent (top phase) was decanted and saved. This extraction was performed three times. After the third extraction the contents of four cultures were filtered through glass wool to remove particulate material, and the solvent extracts were pooled. The solvent fractions were dried over anhydrous sodium sulfate, and 2 ml was added to 10 ml of Safety Solve for quantitation of radioactivity by liquid scintillation spectrometry. Particulate fractions were dried, a portion was oxidized in a Harvey Biological Oxidizer 0400 (R. J. Harvey Instrument Co., Hillsdale, N.J.), and CO_2 was collected in an ethanolamine-containing scintillation cocktail for subsequent quantitation of radioactivity. Radioactivity in all fractions was determined by liquid scintillation spectrometry.

HPLC and thin-layer chromatography. Analytical and preparative thin-layer chromatography of [^{14}C]lindane (in cyclohexane-chloroform [4:1]), [^{14}C]mirex (in chloroform-acetone [9:1]), and [^{14}C]dieldrin (in hexane-diethyl ether [1:1]) was performed by using precoated silica gel 60 G F-254 plates (5 by 20 cm [aluminum backed] or 20 by 20 cm [glass backed]; thickness, 250 μm; E. Merck AG, Darmstadt, Federal Republic of Germany).

Analytical and preparative HPLC of [^{14}C]aldrin, [^{14}C]heptachlor, and [^{14}C]chlordane was performed by using an HPLC system (System Gold Gradient; Beckman Instru-

TABLE 1. Mass balance of ^{14}C radioactivity from *P. chrysosporium* 60-day soil and 30-day liquid cultures incubated with radiolabeled insecticides

Compound	Culture matrix[a]	% Recovery	Mass balance data (% per fraction of total radioactivity added)						
			Solvent		Particulate	Aqueous	$^{14}CO_2$	Manifold caps	% Disappearance[b]
			% Original	% Metabolites					
Mirex	Soil (5.2)	85	70	9.6	0.2	1.2	4.0		15
Mirex	Liquid (5.0)	76	56.2	3.6	13.6	0.6	2.0		19.8
Lindane	Soil (4.7)	62	27.3	6.5	0.9	4.5	22.8		34.7
Lindane	Liquid (5.3)	79.5	26.0	14.6	1.2	14.3	23.4		53.5
Aldrin	Soil (27.8)	90.2	4.5	68.7	4.0	12.2	0.8		85.7
Aldrin	Liquid (33.4)	99.2	2.1	31.7	1.5	2.7	0.6	60.6	36.5
Dieldrin	Soil (0.5)	86.4	58.4	1.0	22.3	4.5	0.2		28.0
Dieldrin	Liquid (0.4)	100	31.5	6.1	5.5	3.4	0.5	53.0	15.5
Heptachlor	Soil (27.0)	91.3	71.8	9.8	6.9	0.6	2.2		19.5
Heptachlor	Liquid (37.6)	81.5	45.4	27.0	6.3	2.3	0.5		26.1
Chlordane	Soil (5.2)	85	57	9.4	0.8	2.9	14.9		28
Chlordane	Liquid (7.6)	105.6	28.3	2.7	8.7	16.0	9.4	40.5	36.8

[a] Numbers in parentheses indicate mass (in nanomoles) of radiolabeled compounds added per culture.
[b] The percent disappearance represents the amount of radioactivity recovered which was not parent compound.

ments, Inc., Fullerton, Calif.) equipped with a reversed-phase column (VYDAC 201 TP5; 4.6 by 150 mm; The Sep/a/ra/tions Group, Hesperia, Calif.). Isocratic elution was performed with acetonitrile-water (55:45).

Solvent extracts of the cultures were analyzed by HPLC with the apparatus described above. Gradient elution was performed in a solvent system composed of acetonitrile and water beginning with 10% acetonitrile for 5 min followed by a linear increase to 100% acetonitrile over 40 min for all compounds except aldrin and dieldrin, which were separated by using a linear gradient from 40 to 80% acetonitrile over 40 min. The flow rate was 2 ml/min. In a typical experiment, the methylene chloride, ether-ethyl acetate-acetone (5:5:1), or hexane-acetone (1:1) extracts were concentrated to about 1 ml by evaporation under nitrogen. Portions (20 to 100 μl) of these concentrated extracts were used for HPLC. Fractions (2 ml) from the HPLC were collected in scintillation vials. Safety Solve (10 ml) was then added to each fraction, and the radioactivity was determined by liquid scintillation spectrometry.

For the conversion of aldrin to dieldrin, the metabolite (dieldrin) was identified by comigration with an authentic standard during gas chromatography (model 700 gas chromatograph [Varian, Palo Alto, Calif.] equipped with an electron capture detector and an integrator [model 3390A; Hewlett-Packard Co., Palo Alto, Calif.]). A glass column (1.8 m by 2 mm [inner diameter]) packed with 3% OV-17 on Gas Chrom Q 100/120 mesh was used with a column temperature of 200°C, a injection port temperature of 220°C, and a detector temperature of 340°C. Nitrogen was the carrier gas.

Disappearance of insecticides. The disappearance of insecticides incubated in cultures of *P. chrysosporium* was calculated from mass balance analyses. Radioactivity in the aqueous, CO_2, solvent, and particulate fractions was determined and expressed as a percentage of the total disintegrations per minute recovered. Radioactivity in the solvent fractions was further divided into percent metabolites and percent original compound by comparing the radioactivity representing the pesticide peak with the radioactivity in other HPLC fractions. The total radioactivity recovered in aqueous, particulate, CO_2, and solvent metabolite fractions was divided by the total recovery to obtain the percent disappearance.

RESULTS

The ability of *P. chrysosporium* to degrade [^{14}C]lindane, [^{14}C]mirex, [^{14}C]aldrin, [^{14}C]dieldrin, [^{14}C]heptachlor, and [^{14}C]chlordane is illustrated in Table 1 and Fig. 2 through 4. Of the six compounds tested, only [^{14}C]lindane and [^{14}C]chlordane underwent extensive biodegradation, as evidenced by the fact that substantial amounts (9.4 to 23.4%) of these compounds were degraded to $^{14}CO_2$ in liquid and soil cultures containing *P. chrysosporium*. The amount of $^{14}CO_2$ evolved from cultures containing [^{14}C]heptachlor, [^{14}C]dieldrin, [^{14}C]aldrin, or [^{14}C]mirex was generally between 0.5 and 2.2%. Because the amount of radiochemical impurities in these chemicals ranged from 1 to 3%, it is impossible to state with certainty that the $^{14}CO_2$ evolved was from the radiolabeled compounds of interest rather than from associated radiochemical impurities. An exception was observed for [^{14}C]mirex in soil cultures of *P. chrysosporium*, in which 4.0% of the [^{14}C]mirex was recovered as $^{14}CO_2$. Since the radiochemical purity of [^{14}C]mirex was 98%, it seems that the fungus does have the ability to degrade small amounts of this compound to $^{14}CO_2$.

Although only [^{14}C]lindane and [^{14}C]chlordane underwent extensive biodegradation as judged by the ability of *P. chrysosporium* to degrade them to $^{14}CO_2$, all of the compounds studied underwent considerable biodegradation when compound disappearance and metabolite formation were used as a measure of biodegradation (Table 1). HPLC analysis of solvent extracts from [^{14}C]heptachlor and [^{14}C]chlordane control flasks revealed additional peaks in the vicinity of the chlordane (between fraction 13 and 15) and heptachlor (between fraction 10 and 13) peaks (data not shown). The presence of these peaks in the controls suggests that some physical or chemical alterations of chlordane and heptachlor can occur under sterile conditions. Nonbiological disappearance did not occur in cultures containing [^{14}C]lindane, [^{14}C]mirex, [^{14}C]aldrin, or [^{14}C]dieldrin. Unrecovered radioactivity and radioactivity extracted from gassing manifold caps were regarded as undegraded compound in calculating disappearance data. It is important to note that disappearance of [^{14}C]aldrin is largely due to the formation of [^{14}C]dieldrin, which is also an important and persistent environmental pollutant.

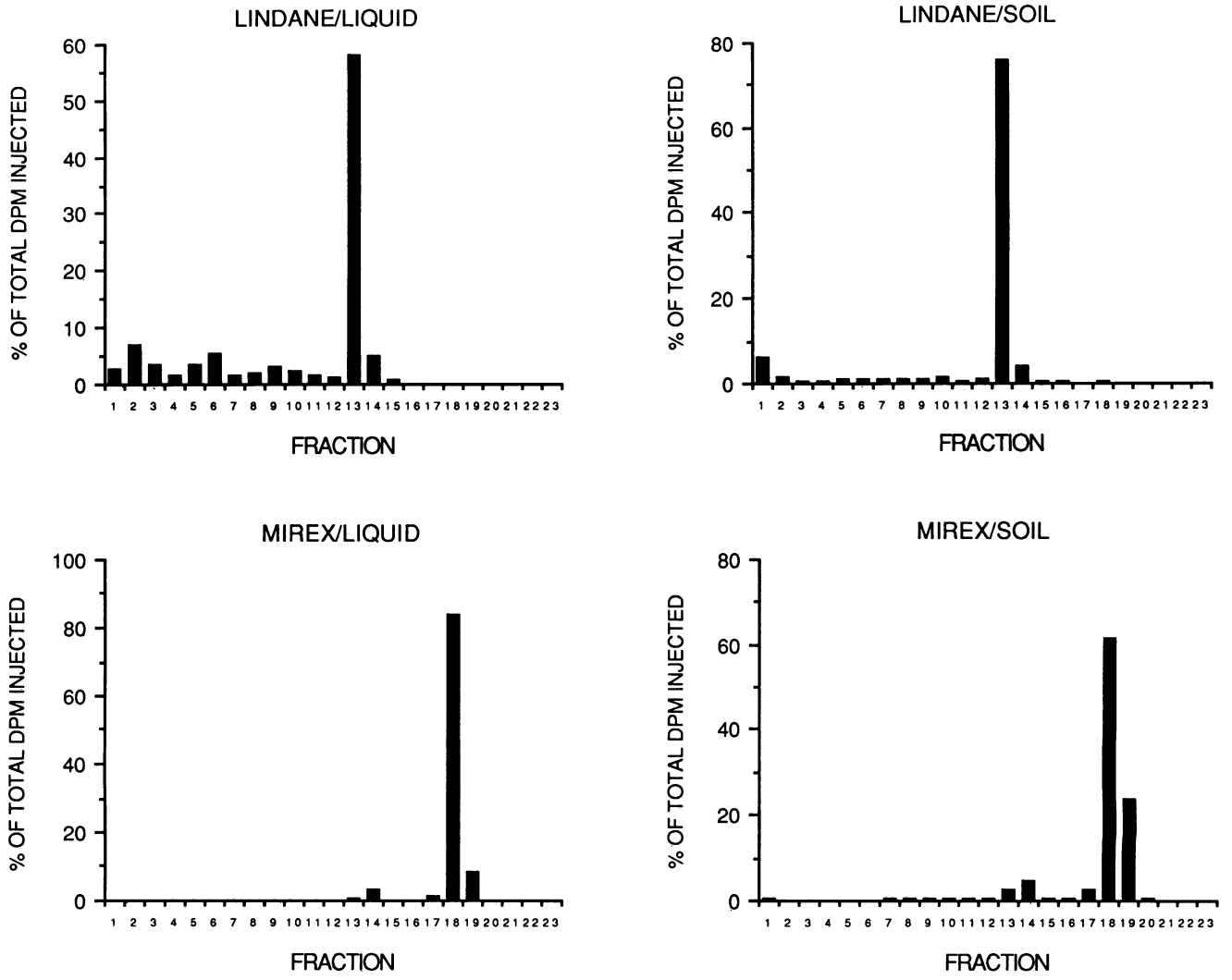

FIG. 2. Radioactivity in HPLC fractions of solvent extracts from liquid and soil-corncob *P. chrysosporium* cultures incubated with [^{14}C]lindane (top) and [^{14}C]mirex (bottom). The tallest bar in each graph represents unaltered original compounds.

DISCUSSION

Data collected in this study demonstrate that substantial differences exist in the ability of *P. chrysosporium* to degrade the alkyl halide insecticides lindane, mirex, heptachlor, chlordane, dieldrin, and aldrin. Results show the difficulty of degrading the uniformly ^{14}C-labeled hexachlorocyclopentadiene rings of aldrin and dieldrin and the similar cagelike perchloromethenocyclobutapentalene (mirex).

Chlordane and heptachlor were also uniformly ^{14}C labeled in the hexachlorocyclopentadiene ring. However, chlordane was much easier to degrade than heptachlor, as evidenced by the fact that ^{14}C-labeled atoms in chlordane were degraded to $^{14}CO_2$. Heptachlor has a double bond between C-2 and C-3 and is not chlorinated at C-2, whereas chlordane has a single bond between C-2 and C-3 and is chlorinated at C-2. Since this is the only difference between these two molecules, it is likely that initial oxidation of chlordane by fungal enzymes occurs at either C-2 or C-3 in such a manner that the stable hexachlorocyclopentadiene portion of the molecule is subject to enzymatic attack and further degradation to CO_2.

Lindane was readily mineralized by *P. chrysosporium* in soil and liquid matrices. When disappearance was used as a criterion of biodegradation, lindane was the most extensively degraded compound of all the alkyl halides studied if aldrin disappearance is not counted, since its primary conversion product is dieldrin and this is not regarded as biodegradation or detoxification since one hazardous compound is merely exchanged for another. Since [^{14}C]lindane was uniformly ring labeled, the production of $^{14}CO_2$ implies that ring cleavage and subsequent degradation occurred. [^{14}C]lindane mineralization by *P. chrysosporium* has been previously demonstrated (6). Other studies of microbial degradation of lindane demonstrated that degradation was more rapid under anaerobic conditions (13, 14) and occurred via reductive dehydrodechlorination. Anaerobic degradation of lindane, however, can result in less polar products such as chlorobenzenes (8) or in undesirable isomerization to α-hexachlorocyclohexane (3).

Mirex was mineralized very slowly (≤4% in 30 days) by *P. chrysosporium*. Previous studies have shown some biodegradation of mirex by organisms in sewage sludge under

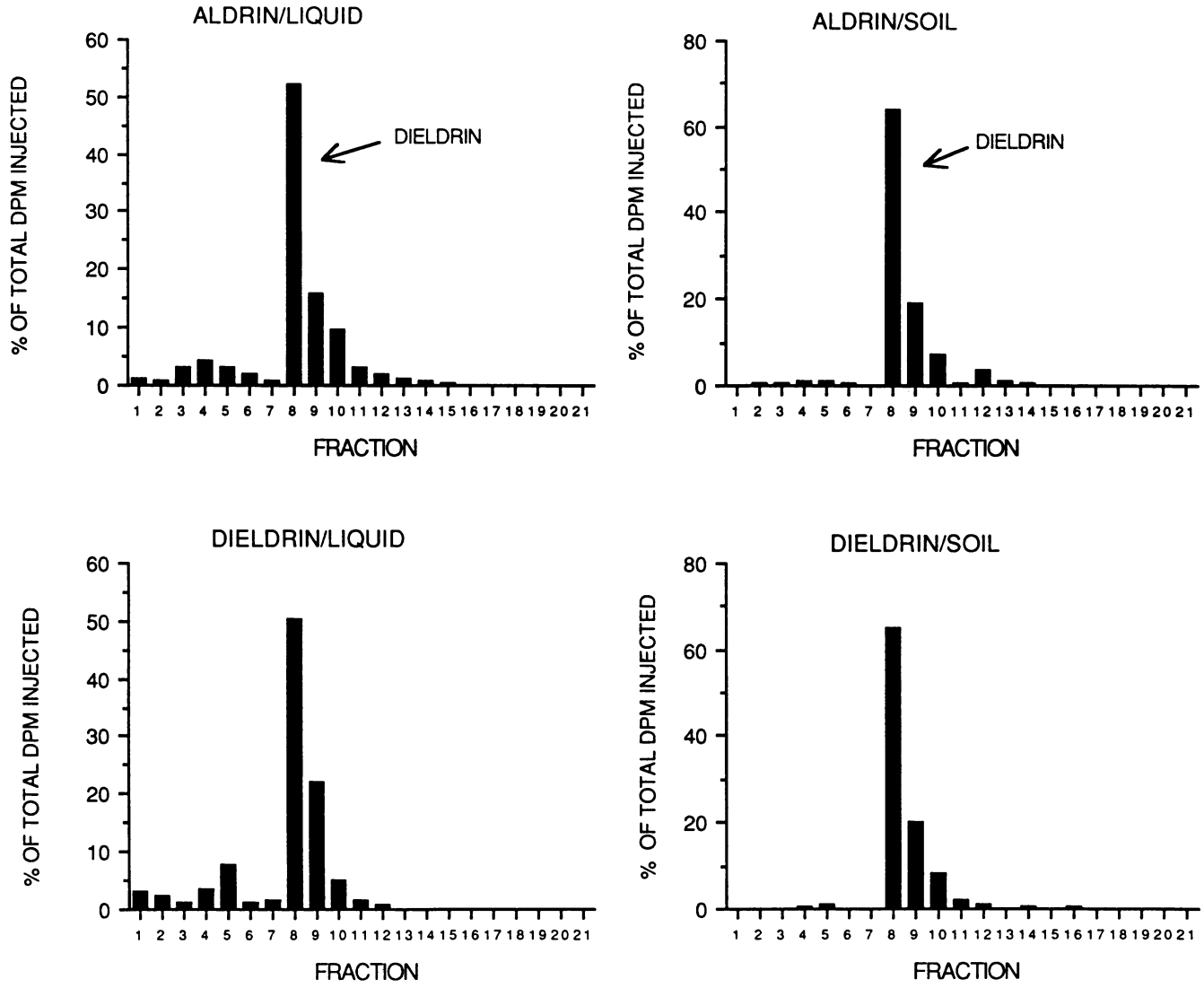

FIG. 3. Radioactivity in HPLC fractions of solvent extracts from liquid and soil-corncob *P. chrysosporium* cultures incubated with [^{14}C]aldrin (top) and [^{14}C]dieldrin (bottom). The tallest bar in each graph represents dieldrin. Aldrin elutes between fraction 11 and 13 in this system.

anaerobic conditions but not under aerobic conditions (2). An anaerobic dechlorination product was identified as the 10-monohydro derivative of mirex (1). HPLC analysis of solvent extracts from soil-corncob and liquid *P. chrysosporium* cultures show the presence of a possible mirex metabolite representing 3.6% of the total radioactivity added in liquids and 9.6% of the radioactivity in soils (Fig. 2). Sixty-day soil-corncob experiments yielded twice as much mineralization (4%) as that in liquid cultures (2%), and very little radioactivity remained bound to particulate material in soils, whereas 13.6% was bound to the filter paper-mycelium particulate fraction in the liquid cultures. Whether the radioactivity in the particulate fraction represents unaltered mirex is unclear, but, regardless, it was very tightly bound.

Aldrin is converted to dieldrin by common soil microorganisms (17). *P. chrysosporium* was also able to carry out the epoxidation of aldrin to dieldrin (Fig. 3), as demonstrated by the recovery of [^{14}C]dieldrin from solvent extracts from *P. chrysosporium* cultures incubated with [^{14}C]aldrin. In solvent extracts from liquid cultures a more polar metabolite of the aldrin was formed in addition to dieldrin. Solvent extracts from liquid cultures of *P. chrysosporium* incubated with [^{14}C]dieldrin also contained a more polar metabolite of dieldrin. Mineralization rates of both [^{14}C]aldrin and [^{14}C]dieldrin were higher in liquid cultures than in soils, but mineralization was still less than 1% and could be due to impurities in the [^{14}C]aldrin and [^{14}C]dieldrin. Mass balance analysis of liquid cultures of *P. chrysosporium* incubated with [^{14}C]dieldrin and [^{14}C]aldrin resulted in recoveries of less than 50% until gassing manifold caps and tubing were also extracted. Radioactivity adsorbed in the gassing manifold caps was probably exacerbated by flushing with oxygen during $^{14}CO_2$ collection, which aided in volatilizing these ^{14}C-compounds, allowing them to adsorb to the caps and tubing.

In summary, *P. chrysosporium* has the ability to degrade

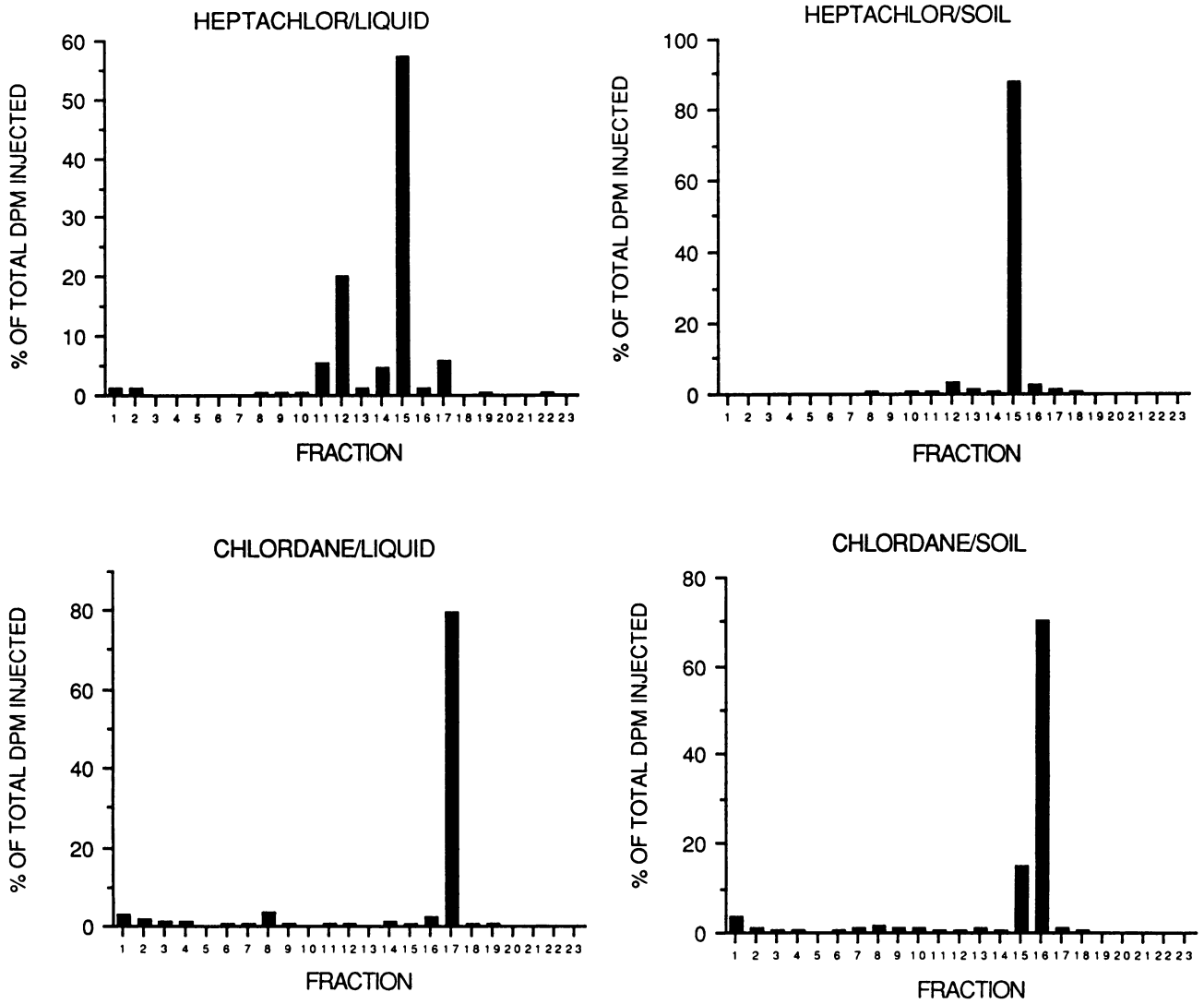

FIG. 4. Radioactivity in HPLC fractions of solvent extracts from liquid and soil-corncob *P. chrysosporium* cultures incubated with [^{14}C]heptachlor (top) and [^{14}C]chlordane (bottom). The tallest bar in each graph represents unaltered original compounds.

alkyl halide insecticides, but, of those studied, only lindane and chlordane are degraded extensively enough to warrant the use of this fungus for bioremediation.

ACKNOWLEDGMENTS

This research was supported in part by Public Health Service grant ES 04922 from the National Institute of Environmental Health Sciences.

We thank Terri Maughan for her expert secretarial assistance during the preparation of the manuscript. We also thank Andra Waddoups, Tom Clark, Steven Harich, and Nate Spear for technical assistance.

LITERATURE CITED

1. **Andrade, P., Jr., and W. B. Wheeler.** 1975. Identification of a mirex metabolite. Bull. Environ. Contam. Toxicol. **14**:473–479.
2. **Andrade, P. S. L., Jr., and W. B. Wheeler.** 1974. Biodegradation of mirex by sewage sludge organisms. Bull. Environ. Contam. Toxicol. **11**:415–416.
3. **Benezet, H. J., and F. Matsumura.** 1973. Isomerisation of γ-BHC to α-BHC in the environment. Nature (London) **243**: 480–481.
4. **Bumpus, J. A., and S. D. Aust.** 1987. Biodegradation of DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane] by the white rot fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **53**:2001–2008.
5. **Bumpus, J. A., and B. J. Brock.** 1988. Biodegradation of crystal violet by the white rot fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **54**:1143–1150.
6. **Bumpus, J. A., M. Tien, D. Wright, and S. D. Aust.** 1985. Oxidation of persistent environmental pollutants by a white rot fungus. Science **228**:1434–1436.
7. **Eaton, D. C.** 1985. Mineralization of polychlorinated biphenyls by *Phanerochaete chrysosporium*: a ligninolytic fungus. Enzyme Microb. Technol. **7**:194–196.
8. **Engst, R., W. Fritsche, R. Knoll, M. Kujawa, R. M. Macholz, and G. Straube.** 1979. Interim results of studies of microbial isomerization of gamma-hexachlorocyclohexane. Bull. Environ. Contam. Toxicol. **22**:699–707.
9. **Fenn, P., and T. K. Kirk.** 1979. Ligninolytic system of *Phanerochaete chrysosporium*: inhibition by ophthalate. Arch. Microbiol. **123**:307–309.
10. **Hammel, K. E., B. Kalyanaraman, and T. K. Kirk.** 1986.

Oxidation of polycyclic aromatic hydrocarbons and dibenzo[p] dioxins by *Phanerochaete chrysosporium* ligninase. J. Biol. Chem. **261**:16948–16952.

11. **Kirk, T. K., E. Schultz, W. J. Connors, L. F. Lorenz, and J. G. Zeikus.** 1977. Influence of culture parameters on lignin metabolism by *Phanerochaete chrysosporium*. Arch. Microbiol. **177**: 277–285.
12. **Mileski, G. J., J. A. Bumpus, M. A. Jurek, and S. D. Aust.** 1988. Biodegradation of pentachlorophenol by the white rot fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **54**: 2885–2889.
13. **Ohisa, N., and M. Yamaguchi.** 1978. Gamma BHC degradation accompanied by growth of *Clostridium rectum* isolated from paddy field soil. Agric. Biol. Chem. **42**:1819–1823.
14. **Sethuran, N., E. M. Bautista, and T. Yoshida.** 1969. Degradation of benzene hexachloride by a soil bacterium. Can. J. Microbiol. **15**:1349–1354.
15. **Tien, M., and T. K. Kirk.** 1983. Lignin degrading enzyme from the hymenomycete *Phanerochaete chrysosporium* Burds. Science **221**:661–663.
16. **Tien, M., and T. K. Kirk.** 1984. Lignin-degrading enzyme from *Phanerochaete chrysosporium*: purification, characterization and catalytic properties of a unique H_2O_2 requiring oxygenase. Proc. Natl. Acad. Sci. USA **81**:2280–2284.
17. **Tu, G. M., J. R. W. Miles, and C. R. Harris.** 1968. Soil microbial degradation of aldrin. Life Sci. **7**:311–322.