Increased Cooxidative Biodegradation of Malathion in Soil via Cosubstrate Enrichment

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Malathion is biodegraded in soil at an increased rate when suitable cosubstrates are present. Cosubstrates that are initially attacked by a molecular oxygenase, e.g., *n*-heptadecane, are the most effective substrates for cooxidation.

The phenomenon of cooxidation was first described by Leadbetter and Foster (1959) and the role of cooxidation (cometabolism) in the biodegradation of relatively recalcitrant halogenated pesticide compounds has been reviewed by Horvath (1972). Other studies have led to the proposal (van Ravenswaay Claasen and van der Linden, 1971; Beam and Perry, 1973, 1974) that some hydrocarbons, e.g., cyclohexane, cannot act as an inducer for the oxygenase necessary for initial oxidative attack on the hydrocarbon moiety. The addition of an inducer for the molecular oxygenase, e.g., propane, or *n*-hexadecane, will lead, in the case of the cycloalkane, to rapid transformation to the cycloalkanone which is readily utilized by soil microorganisms (Beam and Perry, 1974; Shaw, 1966). Incorporation of *n*-hexadecane as cosubstrate in soil along with [^{14}C]cyclohexane resulted in rapid mineralization of the cycloalkane as measured by the generation of [^{14}C]-carbon dioxide (Beam and Perry, 1974). Conversely, addition of equal amounts of glucose, which is oxidized by pathways not involving an oxygenase, did not result in a significant increase in [^{14}C]carbon dioxide production in this system. The induction of the oxygenase for propane in an *Arthrobacter* sp. by *o*-phthalate has also been described by Perry and Scheld (1968). Using as a model the system devised for the induction of cyclohexane oxidation (Beam and Perry, 1974), a study has been conducted on the effect of various cosubstrates on pesticide mineralization. This report is concerned with the effect of *n*-alkanes, 1-alkenes, and other cosubstrates on the rate of malathion [*O*,*O*-dimethyl-*S*-(1,2-dicarbethoxy) ethyl phosphorodithionate] biodegradation in intact soil.

EXPERIMENTAL SECTION

General. Soil samples were collected in eastern North Carolina from tobacco fields and from Core Creek, an estuary of the Neuse River.

One-gram quantities of the soil as collected was added to 50 mL of the sterile L-salts basal medium of Leadbetter and Foster (1958). The pH of the soil suspension ranged from 6.8 to 7.0 at the beginning of the experiment. The soil suspension was continuously mixed at a speed of 100 rpm on a rotary shaker.

$^{14}CO_2$ Detection. Detection of metabolically produced carbon dioxide was achieved by passing a continuous stream of CO_2 -free air over the surface of the soil suspension and the effluent air was bubbled through 50 mL of saturated barium hydroxide to collect the evolved CO_2. Traps for volatile organic compounds were not necessary as control flask indicated that malathion remained stable under the conditions of these experiments.

All cosubstrates were added to a final concentration of 0.1% w/v or v/v. The [^{14}C]malathion (labeled in the 2 and 3 positions of the maleate group) had a sp act. of 4.6 mCi/mmol and was added to each soil, after dissolution in a minimal amount of acetone, to a final concentration of 0.06–0.1 μCi/flask.

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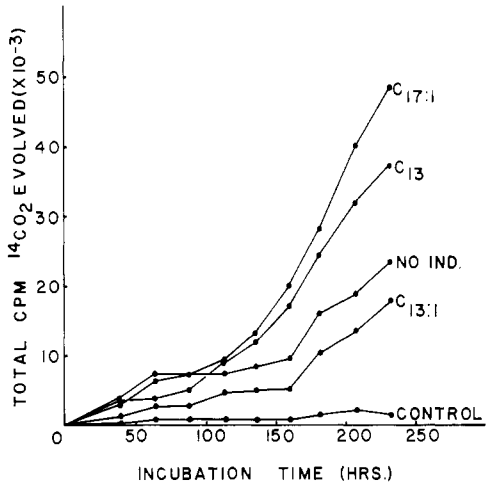

Figure 1. Evolution of $^{14}CO_2$ from Core Creek soil suspensions amended with [^{14}C]malathion. (0.1 μCi of [^{14}C]malathion added to each flask at beginning of the experiment. NO IND = no inducer added; $C_{17:1}$ = 1-heptadecene; C_{13} = *n*-tridecane; $C_{13:1}$ = 1-tridecene. Control was 1 g of soil/50 mL of basal medium autoclaved for 50 min on three consecutive days.)

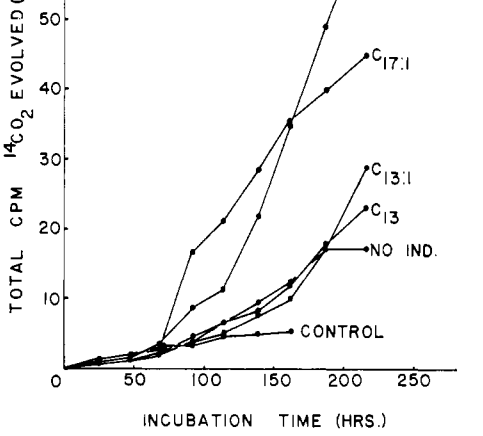

Figure 2. Evolution $^{14}CO_2$ from tobacco field soil suspensions. Conditions same as Figure 1 with C_{17} = *n*-heptadecane also added.

RESULTS AND DISCUSSION

The $^{14}CO_2$ evolution profile obtained with soil from Core Creek is illustrated in Figure 1. Similar results were obtained with tobacco field soil (Figure 2). The addition

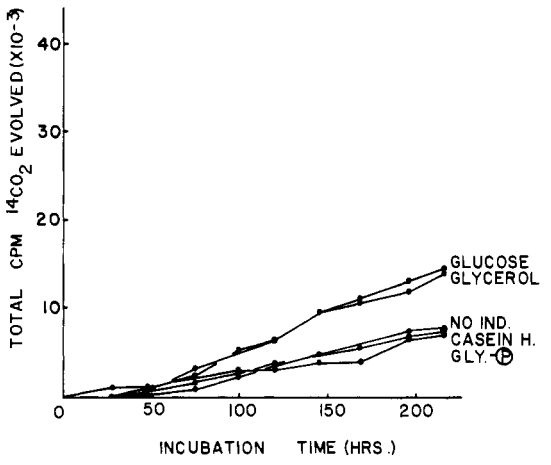

Figure 3. Evolution of $^{14}CO_2$ from tobacco field soil suspensions. 0.06 μCi of [^{14}C]malathion added. Casein H = pancreatic digest of casein; Gly-P = DL-glycerophosphate; NO IND = no inducer added.

of 1-heptadecene as cosubstrate resulted in a twofold increase in metabolically produced $^{14}CO_2$ over that generated in the unsupplemented system. With *n*-heptadecane as cosubstrate, the amount of $^{14}CO_2$ produced by the end of the experiment was nearly three times that produced in the uninduced culture.

Glucose, glycerol, glycerophosphate, and a mixture of amino acids and peptides (casein hydrolysate) did not have an appreciable effect on the rate of [^{14}C]carbon dioxide evolved from malathion in tobacco field soil (Figure 3). Preliminary results also indicate that acetate, succinate, pyruvate, and citrate are not effective as cosubstrates for malathion oxidation in these soils.

The same cosubstrates used in these experiments were tested for the effect on oxidation of radiolabeled DDT, dieldrin, and lindane in both the tobacco field soil and the Core Creek soil. The cultures were incubated for up to 2 months; however, no evidence of $^{14}CO_2$ production from these recalcitrant molecules was observed for any of the inducers effective in the oxidation of malathion.

We cannot speculate from these results on the pathway(s) by which malathion is degraded in the two soil types; however, it is possible that the oxidative enzymes involved with utilization of certain alkenes and alkanes might be associated with the initial biodegradative attack on the malathion molecule and thus increase its rate of biodegration. There is also the possibility that the esterase (Forney and Markovetz, 1970) implicated in the biodegradation of *n*-alkane substrates might also be involved. These results lend support to the supposition that the rate at which recalcitrant molecules disappear from soil may not be solely dependent on the organisms present but depend also on the presence of selected organic compounds.

ACKNOWLEDGMENT

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