RESEARCH PROPOSAL

to the

NORTH CAROLINA STATE BOARD OF SCIENCE AND TECHNOLOGY

for support of research entitled

Enhancement of the Microbial Breakdown of Polychlorinated Biphenyls (PCB's) in Soil by Co-oxidation Techniques

Covering an 18 month period from
15 March 1980 through 15 September 1981.

Requesting Support in the Amount of $12,398.00

SUBMITTED BY

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Birthdate: October 15, 1929
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Education:				
	Penn State	Bacteriology	B.S.	1951
	University of Texas	Microbiology	Ph.D.	1956

Honors and Memberships:

Sigma Xi, AAAS, Society for Microbiology (British), Netherlands Society for Microbiology, American Society for Microbiology, American Academy for Microbiology.

Professional Experience:

Professor	N. C. State University	1971-Present
Associate Professor	N. C. State University	1966-1971
Assistant Professor	N. C. State University	1964-1966
Research Scientist	University of Texas	1961-1964
Asst. Dir. Research	Brooklyn Botanic Garden	1959-1961
Research Scientist	University of Chicago	1958-1959
Research Scientist	The Upjohn Company	1956-1958

Publications: Over 90 in various aspects of microbial metabolism. Only publications pertinent to this research are listed.

Cerniglia, C. E. and J. J. Perry. 1975. Metabolism of n-propylamine, isopropylamine and 1,3 propane diamine by Mycobacterium convolutum. J. Bacteriol. 124:285-289.

Perry, J. J. 1976. Effects on microorganisms, p. 259-262 (Chapter 8) - Interactions and effects on total environment. In Vaporphase Organic Pollutants. Volatile Hydrocarbons and Oxidation Products. National Research Council, Washington, D. C.

Phillips, W. E. and J. J. Perry. 1976. Thermomicrobium fosteri sp. nov., a hydrocarbon-utilizing obligate thermophile. Int. J. Syst. Bacteriol. 26:220-225.

Cerniglia, C. E., W. T. Blevins and J. J. Perry. 1976. Microbial oxidation and assimilation of propylene. Appl. Environ. Microbiol. 32:764-768.

Merkel, G. J. and J. J. Perry. 1977. Increased co-oxidative biodegradation of malathion in soil via co-substrate enrichment. J. Agricul. Food Chem. 25:1011-1012.

Merkel, G. J. and J. J. Perry. 1977. The effect of growth substrate on thermal death of thermophilic bacteria. Appl. Environ. Microbiol. 34:626-629.

Perry, J. J. 1977. Microbial metabolism of cyclic hydrocarbons and related compounds. Critical Reviews in Microbiology 5:387-412.

Markel, G. J., W. H. Underwood and J. J. Perry. 1978. Isolation of thermophilic bacteria capable of growth solely on long-chain hydrocarbons. FEMS Microbiol. Letters 3:81-83.

Durham, D. R. and J. J. Perry. 1978. The amine dehydrogenase in Pseudomonas putida NP and its role in the metabolism of benzylamine. J. Gen. Microbiol. 105:39-44.

Durham, D. R. and J. J. Perry. 1978. Purification and characterization of a heme-containing amine dehydrogenase from Pseudomonas putida NP. J. Bacteriol. 134:837-843.

Perry, J. J. 1978. Co-oxidative processes in the bio-degradation of petroleum refinery effluent. American Institute of Chemical Engineers (Abst.) June 4-8, 1978, p. T-83.

Perry, J. J. 1978. Microbial metabolism of cycloalkanes. In. The role of microorganisms in the recovery of oil. 1976 Engineering Foundation Conferences. Published by the National Science Foundation (RANN). NSF/RA - 770201, p. 45-50.

Durham, D. R. and J. J. Perry. 1978. The amine dehydrogenase of Pseudomonas putida: properties of the heme-prosthetic group. J. Bacteriol. 135:981-986.

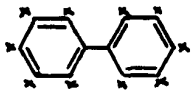
Markel, G. J., S. S. Stapleton and J. J. Perry. 1978. Isolation and peptidoglycan of Gram-negative hydrocarbon-utilizing thermophilic bacteria. J. Gen. Microbiol. 109:187-194.

Perry, J. J. 1979. Microbial cooxidations involving hydrocarbons. Microbiological Reviews 43:59-72.

Perry, J. J. 1980. Introduction to Environmental Toxicology. (Editor with F. E. Guthrie) approximately 600 pages in press. Elsevier-North Holland, N.Y.

A. PROPOSED RESEARCH

Polychlorinated biphenyls (PCB's) have been manufactured since 1929 and between the years 1948 and 1973 over 400,000 tons were produced in the United States. This amount represented about 1/2 the worldwide production and all in the U.S. was synthesized by Monsanto Chemical Company. The physical properties of PCB's (thermal stability, resistance to acid/base, inertness) have rendered them an important industrial chemical. The general formula is as follows:



X marks the carbon atom where a chlorine or hydrogen atom may be substituted.

There are 210 possible combinations of chlorine and hydrogen on biphenyl but probably about 102 actually exist if one assumes five to eight chlorines per molecule. They have a low vapor pressure, are of low water solubility and miscible with most organic solvents. Their physical state ranges from liquid to resinous to crystalline as the degree of chlorination increases.

PCB's have had wide use in capacitors, as plasticizers, hydraulic fluids, adhesives, printing products and numerous other applications. Unfortunately, PCB's are toxic and have caused acute toxicity problems in workers involved in their manufacture--jaundice, nausea, edema, liver lesions--and may well be carcinogenic. Acute toxicity is probably of less consequence than chronic toxicity as these compounds have been widely dispersed and are poorly degraded by indigenous microbial populations. As a result they have become involved in intricate food web dynamics and have been isolated from wildlife and both marine and fresh water animals.

The process of manufacture and careless use of PCB's has resulted in significant amounts of these chemicals accumulating in relatively small areas. General Electric manufactured PCB containing capacitors in the upper reaches

of the Hudson River for over 25 years, and during this time period dumped 1,300 tons of PCB which now contaminates the Hudson River Basin. A significant part of this PCB (690 tons) is present in landfills, dumps and dredge spoils. PCB was unfortunately dumped along 210.9 miles of road in North Carolina by an irresponsible New York firm. This amounts to an estimated 40,000 cubic yards of PCB contaminated soil.

The most satisfactory and permanent solution to environmental contamination by toxic material, such as PCB's, is the total mineralization or biodegradation of the contaminant. All naturally occurring compounds are biodegraded by the indigenous microbial population of soil or water with the return of organic carbon to CO_2 in the atmosphere, nitrogen to ammonia, sulfur to sulfate, etc.

Generally, the rate at which a man-made pesticide or other unnatural compound can be mineralized is related to the similarity of the compound to naturally occurring components of living cells or the enzymatic capacity in soil microflora to convert the contaminant to a compound amenable to microbial degradation. The degree of resistance to biodegradation is described as the level of recalcitrance. DDT with a half life of 30 years is considered to be a very recalcitrant molecule while dalapon (2,2 dichloropropionic acid) with a half life of less than six months has little recalcitrance.

The degradation of recalcitrant molecules in nature generally occurs by the concerted action of several organisms. The insertion of O_2 in a molecule, removal of chlorine, or other oxidative changes can yield a molecule that is more readily attacked by other microorganisms in an environmental niche. Since dehalogenation would not be an energy yielding reaction and halogenated compounds are not commonly produced in natural systems, the removal of such substituent groups from molecules is generally not associated with a single species of microbe utilizing that compound. Most dechlorination occurs in nature

by co-oxidative means, whereby an organism while growing on one substrate will remove a Cl^- from a molecule without further change in the product of the dehalogenation.

There is ample evidence in the literature that dechlorination of a number of halogenated compounds (herbicides, insecticides, etc.) occurs only when another growth substrate is available. Some appropriate references are as follows:

Burge, W. E. 1969. Populations of dalapon-decomposing bacteria in soil as influenced by additions of dalapon or other carbon sources. Appl. Microbiol. 17:545-550.

Chacko, C. I., J. L. Lockwood and M. Zabik. 1966. Chlorinated hydrocarbon pesticides: Degradation by microbes. Science 154:893.

Focht, Dennis and M. Alexander. 1971. Aerobic co-metabolism of DDT analogues by Hydrogenomonas sp. J. Agr. Food Chem. 19:20-22.

Gibson, D. T., J. R. Koch, C. L. Schuld and R. E. Kallio. 1968. Oxidative degradation of aromatic hydrocarbons by microorganisms. II. Metabolism of halogenated aromatic hydrocarbons. Biochemistry 7:3795-3802.

Haider, K., G. Jagnow, R. Kohner and S. U. Lim. 1974. Abbau chlorierter benzole, phenole und cyclohexan-derivate durch benzol und phenol verwertende bodenbakterien unter aeroben bedingungen. Arch. Microbiol. 96:183-200.

Horvath, R. S. 1970. Microbial co-metabolism of 2,4,5 trichlorophenoxy-acetic acid. Bull. Environ. Contam. Toxicol. 5:537-541.

Horvath, R. S. 1971. Co-metabolism of the herbicide 2,3,6-trichlorobenzoate. J. Agr. Food Chem. 19:291-293.

Horvath, R. S. 1973. Enhancement of co-metabolism of chlorobenzoates by the co-substrate enrichment technique. Appl. Microbiol. 25:961-963.

Jensen, H. L. 1963. Carbon nutrition of some microorganisms decomposing halogen-substituted aliphatic acids. Acta Agr. Scand. 13:404-412.

Skryabin, G. K., L. A. Golovleva, B. P. Strekozov, and N. V. Pribudko. 1974. Microbial transformation of pesticides under co-oxidation conditions. Izv. Akad, Nauk. SSSR Ser Biol. 3:353-360.

We have had considerable experience with studies on the role of co-oxidation in the biodegradation of recalcitrant molecules. Recent publications include results of our work on malathion, cyclohexane and other relatively recalcitrant compounds. With the knowledge that dalapon biodegradation occurs more rapidly with repeated soil application (see references on page 5) we theorized that adding this compound to soil must result either in an increased number of organisms capable of dehalogenation or the level of dehalogenases in the resident population is markedly stimulated. If so, it would follow that adding dalapon or lindane (1,2,3,4,5,6 hexachlorocyclohexane), which also has a relatively short half life, to soil containing recalcitrant chlorinated compounds would result in a more rapid dehalogenation and mineralization of the recalcitrant compound. Preliminary experiments indicate that this postulation is indeed correct.

Addition of lindane to a mixture of soils together with ^{14}C-radiolabeled 4-chlorobiphenyl resulted in a significantly accelerated release of ^{14}C-carbon dioxide (period of 10 days) than occurred in controls without added lindane. The total CO_2 released was also considerably enhanced. The object of this proposed research is to examine in greater detail the results thus far obtained and assess the possibility that addition of an appropriate co-substrate and a compound that will stimulate the potential for dehalogenation will result in a more rapid mineralization of PCB. Among the major factors that will be investigated are:

(1) Test a number of halogenated compounds to ascertain the relative ability of these to stimulate the dehalogenation process. These should be added to mixtures of soil and to individual soils from a variety of sources in order to find a soil containing microbes with the greatest potential.

(2) Examine a variety of co-substrates, e.g., sugars, animal waste, sawdust, etc., to select a growth substrate that might be most

effective. This would be done in conjunction with (1) and especially after an effective augmentor (chlorinated compound or otherwise) of dehalogenation was found.

(3) Examine soil extracts, treated with PCB + "stimulator" + co-substrate for products of PCB biodegradation other than carbon dioxide. This will be done by thin layer chromatography (TLC) and by gas-liquid chromatography.

(4) Isolate microbial cultures, preferably mixed populations, that can effect the biodegradation of PCB and test these in a soil system. Such a "consortium" of organisms has been found effective in the biodegradation of DDT and other halogenated aromatic compounds.

(5) If populations are found that can effectively biodegrade PCB, examine parameters necessary for maximum activity.

B. EXPECTED RESULTS AND BENEFIT TO NORTH CAROLINA

North Carolina has a problem in the disposal of the PCB's that were dumped in the eastern part of the state. Movement of the contaminated soil to land fill sites offers a temporary solution but is not permanent. The best long term solution is mineralization of the PCB by microorganisms to yield carbon dioxide or dehalogenated products that are non-toxic.

BUDGET

Personnel	
Graduate Research Assistant (18 months)	$ 8,665.00
Overhead (20%)	1,733.00
Chemicals*	2,000.00
Total (18 months)	$12,398.00

*Radio-labelled polychlorinated biphenyls are not routinely available.

MONS 014258