POLYCHLORINATED BIPHENYL'S TRANSLOCATION AND ENVIRONMENTAL CONTROL*

BY

Robert S. Reimers, Ph.D.**

BACKGROUND

For the last forty years, polychlorinated biphenyls (PCB's) have been discharged into natural waterways in increasing amounts with truly startling frequency.[1] Moreover, in recent years the hazardous exposure of humans to significant quantities of polychlorinated biphenyls have been noted. A search of the literature indicates that efforts have been inadequate in the development of ecological approaches to reduce or inhibit PCB contamination.

Polychlorinated biphenyls are currently produced in the United States, Great Britain, France, Japan, Germany, USSR, Spain, Italy, and Czechoslavakia. The sole manufacturer of PCB's in the United States is the Monsanto Industrial Chemicals Company which is sold under the trade name "Aroclor" and include Aroclor 1221, 1232, 1242, 1248, 1254, 1260, 1262 and 1268. The latter two digits in the series number indicate the percent chlorine in the formulation; the exception is Aroclor 1016 which contains 41 percent chlorine. The most toxic PCB formulations appear to be isomers containing 3 to 5 chlorine atoms per biphenyls. Polychlorinated biphenyls are heat stable, resistant to acid or base degradation, slightly soluble in water but soluble in lipids and mineral oils.[2] In spite of high molecular weights PCB's show considerable volatility in aqueous solution, (increasing with turbulence).[1] There is some photodecomposition of PCB's following irradiation with sunlight or ultra-violet light with little biodegradation.[3]

A number of studies have been conducted to determine the effects of sublethal and lethal concentrations of PCB's on man and other organisms. Oral doses of PCB's administered to rabbits, guinea pigs, and rats reveal severe liver and kidney damage.[4] Sublethal doses appear to increase susceptibility to infectious agents, occurrence of bladder cancer, and alter the activity of liver enzymes capable of degrading steroids. Occupational or accidental exposure of humans to high concentrations of PCB's causes both severe chloracne and acute liver disease. PCB residues at aqueous concentrations in the range of 0.1 ug/l and 5 ug/l inhibit the reproductivity of fish. This effect has also been noted in rabbits.[5]

Investigations of PCB behavior in the environment have shown bioaccumulation on aquatic species to levels 10^3 to 10^5 times found in fresh or estuarine waters. In the Hudson River, which has had major industrial discharges of PCB's over the last ten years, bioaccumulation has been observed in addition to bioconcentration.[6]

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Since 1969, the Environmental Protection Agency Environmental Research Laboratory, Gulf Breeze, Florida has been engaged in research designed to determine accumulation of PCB and their effects on aquatic organisms. It was observed that the following bioaccumulations (PCB concentrations in tissue related to their concentration in water) under laboratory and field condition existed for Aroclor 1254 were realized:

Organism	Bioaccumulation x 10^5	
	Laboratory	Escambia Bay
Oyster	1.01 - 1.65	1.0
Shrimp	0.26	2.3
Fish	0.37	6.7

The results of the EPA's research indicated four phenomena related to accumulation: (1) oysters bioaccumulate more PCB's than either shrimp or fish in the laboratory but not in the field; (2) shrimp and fish bioaccumulate more in the field than in the laboratory; (3) both sediments and biota accumulate more PCB's than vegetation and (4) the accumulation of PCB's is reversible in shrimp and fish with shrimp showing greater rates of clearance.[7,8]

Between 1969 and 1975 concentrations of PCB's in oysters decreased in Escambia Bay in parallel with the banning of PCB's in waste discharges. In 1976, however, decline in PCB concentration leveled off as a result of an increase in annual aluvial run off which resuspended PCB contaminated sediments.

Since PCB's are insoluble in aqueous solutions and tend to concentrate in sediment, there may be possibilities of reducing PCB contamination by sorption. Hague and Schmedding (1974) investigated varying soil and/or sediments constituents as PCB sorbants and found that sands and silica gel had a low affinity for PCB's. Organics however, added to sediments increased the rate of PCB sediment uptake to levels approaching 85% of the soluble PCB fraction.[9] Recent studies report an increased absorption affinity of PCB's with high chlorine content, by soils and sediments.[10] In the Hudson River investigations, high concentrations of oil emulsions were suspected as one of the means by which the insoluble PCB's were bioaccumulated.[7]

STATEMENT OF PROBLEM AND SPECIFIC OBJECTIVES

Introduction

As previously noted PCB's are bioaccumulated in both fresh water and estuarine environments and sediments (especially silts, clays, and humic organics) concentrate PCB's. However, realistic rates of bioaccumulation through food chain and means of aqueous transport by colloidal sediments are ill-defined.

Thus, there is an immediate need to delineation PCB transport in freshwaters. There should also be an attempt to characterize PCB behavior

so that the feasible methods of PCB decontamination may evolve: The objectives of the proposed study are as follows:

1. To determine the translocation mechanisms and seasonal fluctuations of PCB bioaccumulation in fish collected from a known contaminated aqueous environment (a freshwater river and reservoir).
2. To conduct laboratory investigations on possible PCB decontamination methods.

Methodology

General Approach (two years)

The investigation will be divided into four phases. The initial phase will consist of a literature and information search, development of analytical techniques, completion of a preliminary field survey, and planning of the field investigation. The second phase will include the conduction of a field investigation over four seasons. From the results obtained during the gearing-up and field phase; a laboratory (third) phase will investigate the possibility of microbial degradation of PCB's under field conditions as well as bioaccumulation of PCB's by selected organisms. The final (fourth) phase will entail data analysis and final report preparation.

After anayltical PCB techniques have been perfected, a preliminary contamination survey of the site will be conducted. The site, Anniston, Alabama, has been chosen as a result of previous PCB investigations conducted by Dr. Gunning. This site was the location of a major manufacturer of PCB's which resulted in high quantities being released into the Choccolocco Creek and Coosa River. Collecting sites will be as follows:

1. Shoal Creek, tributary to Choccolocco Creek, 2.3 miles east of White Plains.
2. Choccolocco Creek at Highway 9, 1 mile south of White Plains.
3. Choccolocco Creek, Highway 95, Martha H. Williams Bridge.
4. Choccolocco Creek, Highway 93 Bridge, 3 miles south of I-20.
5. Choccolocco Creek, Highway 77 Bridge.
6. Cane Creek, Tributary to Coosa River, 8.6 miles north of Lincoln.
7. Aker Creek, Tributary to Coosa River, 6.6 miles north of Lincoln.
8. Coosa River at Stemley Bridge (Logan Martin Reservoir), Highway 34.
9. Clear Creek, tributary to Coosa River (Logan Martin Reservoir) Highway 42.

Stations 1,2,6 and 7 are control stations located upstream from the manufacturing plant while stations 3,4,5,8, and 9 are located downstream. Stations 8 and 9 are in Logan Martin Reservoir where PCB's may accumulate in sediments. After completing the preliminary PCB's analysis in soils, sediments, vegetation, waters, fish, crayfish, and plankton from the test area, a final survey will be made in an attempt to correlate water quality and hydrology and climatology data obtained from state, federal, local, and industrial sources.

After completion of the initial survey, a one-year surveilance for PCB's will be conducted. Four sampling periods at seasonal intervals are to be considered. During the summer survey, sediments will be analyzed for organic carbon, organic nitrogen, organic sulfur, acidity, particle size distribution, and humic content. Both soils and vegetation will also be analyzed for total PCB content. For all seasons, water quality will be analyzed for pH, total

dissolved and suspended solids, total organic carbon (filtered and unfiltered), temperature, and estimated flow rates. In order to determine possible mechanisms for the contamination of the aqueous environment, sediments (humic and total), water (soluble and suspended solids), plankton (where possible), and organisms (pertinent fish and specific species of crayfish) at each sampling station, will be analyzed for PCB contamination. From the field survey the primary mechanisms of PCB translocations may be ascertained.

Midway through the field studies, the laboratory phase will commence. The initial objective will be to determine the possibility of microbial degradation of PCB's. Reports from Canada have indicated the capability of certain microbes to degrade PCB's in activated sludge and sonicated sediments.[11] Since the study region has been contaminated for over ten years, the sediments will be analyzed for PCB degrading microbes. An experimental approach will employ lucite columns containing contaminated, uncontaminated, or spiked sediments seeded with selected microbes to simulate natural conditions and PCB samples will be analyzed by GC-MS to determine possible degradation and degradation by-products. If further funding is available after the survey phase, the rate of PCB accumulation will be studied using selected organisms exposed to PCB contaminated colloidal sediments. The design of these experiments will be based on data obtained from the previous phases.

Data obtained from the search, survey and laboratory studies will be analyzed, to determine possible recommendations for in-field inhibition of PCB contamination. A final report will be forwarded to the Sport Fishery Institute.

METHODS:

Field Collections:

Fish and Crayfish: A ten-foot seine (six feet deep) of 3/16 inch Ace mesh will be used for all field collections of fish and crayfish. Daily samples from each station, which will have been previously packed in ice in an ice-chest, will be identified according to species. All specimens of a given species will be placed together in a sub-packet; the subpackets for all species collected at a given station will then collectively be placed into a single large packet. Upon return to the laboratory, all packets will be frozen for future PCB analysis.

Water Quality Analyses: Routine water quality tests will be conducted in the field or at a nearby portable laboratory. Analyses will include temperature, dissolved oxygen, pH total and bicarbonate alkalinity, total solids, turbidity, color, and other tests to be determined as the study progresses. A Yellow Springs Instrument Company dissolved oxygen meter and a Hach Chemical Company Portable laboratory will be employed. Analyses will be performed according to procedures and methods approved by federal agencies or listed in Standard Methods for the Examination of Water and Wastewaters.

Characterization of Soils and Sediments: The sediments and soil samples will be characterized for organic soluble acidity, humic acid, organic nitrogen and organic sulfur content; and particle size distributing of the sediments and soils. Procedures will be conducted according to Black [14,15] or Chapman.[16]

Methods of PCB Analysis:

Extraction: Tissue samples will be ground in a Danill tissue blender with anhydrous sodium sulfate until the mixture appears dry. The dried solids will then be extracted in warm hexane and ethyl ether (1:1 V/V) for four hours in a Soxhlet extractor. The resulting hexane-ethyl ether mixture will be evaporated in a Kaderna-Danish concentrator.[17,18] For soils analysis, the extraction will be carried out with acetone or acetonitrile instead of hexane-ethyl ether mixture as PCBs are more strongly bonded in soils.[19]

Removal of Interferences (DDT, DDE, etc.: The evaporated solution (ethyl ether in hexane 1 percent V/V) will be eluted through a size "B" chromaflex column packed with Florisil (approximately 100 mesh) and topped with anhydrous sodium sulfate. If high concentrations of DDE and DDT isomers are present, a separation from PCBs may require further elution through a silicic acid column. In addition, the silicic acid will be characterized to insure against contaminants.[20]

Gas Chromatography Analysis with an Electron Capture Detector: The operating conditions for the GC are the following:

a. The column - 2.0 m x 1.8 (T.D.), glass packed with OV-101/QF-1 (3/4 5 percent) on Gas Chrom Q (120/140 mesh) and held at 180°C.
b. The nitrogen carrier gas will flow at a rate of 21 ml/min.
c. The optimum temperatures of the injection port and Tritium. detector are 240°C and 220°C respectively.[17]

Confirmation of PCB's Analysis: Rather than averaging all PCBs as one Aroclor, recent literature suggests the use of multicomponent standards (obtainable from Ron Webb, EPA lab, Athens, Ga.) and comparison of these traces with that of the sample analysis to determine concentration.[21]

III. Facilities, Personnel, and Equipment

Personnel

Supervisory Professor:

a. Dr. Robert S. Reimers will be the project director. Dr. Reimers is currently an Assistant Professor in the Department of Environmental Health Sciences at Tulane's School of Public Health and Tropical Medicine. He has considerable experience in the area of water quality management, and has consulted for various firms for the translocation of trace contaminants (See attached curriculum vitae found in Appendix A).

Advisory Professors:

a. Dr. Gerald E. Gunning will be in charge of field surveys and correlation of ecological data. Dr. Gunning is a Professor in the Department of Biology, Tulane University. He has over twenty years experience in ecological studies concerning limnology and water quality management. As a consultant to Monsanto, he has already twice conducted

surveys in the proposed site for possible PCB contamination of fish and sediments (See Appendix A for curriculum vitae.).

b. Dr. J. Walter Mason will be an advisor on both the field surveys and laboratory analysis. Dr. Mason is currently Associate Professor in Department of Environmental Health Sciences. He has considerable experience in translocation studies of trace contaminants especially pesticides. (See Appendix A for Curriculum vitae).

Principal Investigators:

a. Ms. Mary Wilke is a Ph.D. candidate in the Department of Biology. Her background is quite strong in both ecology and environmental chemistry. She is presently reviewing the available literature.

b. Mr. Henry S. Gardner,Jr. is a Masters Candidate in the Department of Environmental Health Sciences. Mr. Gardner has been reviewing the PCB literature. He has had previous research and field experience in environmental science before coming to Tulane. He has a strong background in both ecology and chemistry.

General Research Team:

Biographical sketches of Project personnel are listed in Appendix A. Personnel were selected on the basis of experience and interest in the proposed work. Table 1 shows the proposed research team.

TABLE 1

GENERAL RESEARCH TEAM

Sport Fishery Institute
Mr. Richard Stroud

Principal Supervisor
R.S. Reimers, Ph.D.

Field Survey	Analysis	Laboratory Studies
M.Wilke, Ph.D. Candidate	M.Wilke, Ph.D. Candidate	H.S.Gardner,M.S.Candidat
H.S. Gardner,M.S. Candidate	H.S. Gardner,M.S.Candidate	R.S. Reimers, Ph.D.
G.E. Gunning, Ph.D.	J.W. Mason, D.Sc.Hyg.	J.W. Mason, D.Sc.Hyg.
J.W. Mason	R.S. Reimers, Ph.D.	

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Recent Projects:

Recent work at Tulane University (specifically in the Department of Environmental Health Sciences and Biology) in this general area of environmental engineering and chemistry are listed in the following paragraphs.

ARSENIC IN SEDIMENTS
METHYLATION OF MERCURY IN BOTTOM SEDIMENTS
PREDICTION OF SELECTED ORGANIC REMOVAL BY CARBON ADSORPTION
DISTRIBUTION AND TOXICITY OF MSMA APPLIED TO ROADSIDE RIGHTS OF WAY
UPTAKE AND FATE OF MSMA IN CRAYFISH AND BLACKBERRIES
STUDY OF SO_2 AND SULFATION RATES IN CALI, COLOMBIA
BIOLOGICAL TREATABILITY STUDY OF A REFINERY WASTEWATER
PERFORMANCE EVALUATION OF EXISTING COMBINED AERATED/FACULTATIVE LAGOON SYSTEM AT NORTH GULFPORT, MISSISSIPPI
WASTEWATER MANAGEMENT STUDY FOR DELTA REGIONAL PRIMATE CENTER
PREDICTIONS OF EFFLUENT VARIABILITY FROM COMPLETE-MIX ACTIVATED SLUDGE WASTE TREATMENT FACILITIES
WASTE TREATMENT STUDY AT A PETROCHEMICAL PLANT
UPTAKE OF ATMOSPHERIC FLUORIDE BY SOYBEANS

Facilities:

The Department of Environmental Health Sciences acquired a new building in January 1969 located in the F. Edward Hebert Research Center in Belle Chasse, Louisiana approximately ten miles from the Tulane Medical Center. The department facility is fully equipped for teaching and research and includes offices, classrooms, and laboratories for air pollution, water, and waste, radiological health, environmental microbiology, and general research. A total of 7,000 square feet is currently being utilized, with 3,000 square feet available for future expansion. A partial list of the equipment is in Appendix B for the Riverside Laboratory.

The Department of Biology has both field equipment and additional laboratory facilities on the main campus. Two vehicles and boats are available for the project. The vehicles can transport all necessary instrumentation for water quality to the field sites.

Tentative Schedule and Budget

This proposed project will require approximately 24 months to complete. The start-up phase will require 2 to 3 months (the initiation of the information search, the development of the analytical techniques, the conduction of preliminary survey, and the planning of survey). The field phase will be conducted after the initial phase and completed in a year. Halfway through the field survey, a laboratory study will be initiated and continued for one year. After completion of the laboratory phase, date will be analyzed and a final report will be completed within three months. A Gannt diagram for the projected time frame of the proposed investigation is presented in Figure 1.

The cost of the proposed project is shown in the itemized budget in Table 2.

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TABLE 2

RESEARCH BUDGET

		First Year	Second Year
1.	Personnel		
	Research Personnel Support	4,400	4,400
2.	Travel		
	Surveys	2,200	800
3.	Equipment		
	GC revitalization	1,000	
	Electron Capture Detectors	2,000	0
	Field Equipment (Hach kits, pumps, Coolers, etc.)	1,500	0
	Lab Equipment	1,000	400
			1,000
4.	Supplies		
	(Chemicals reagents, gases for GC, and misc. supplies)	4,000	1,400
5.	Other		
	Computer time	200	300
	Xeroxing and report preparation	100	300
6.	Total Cost Per Year	16,400	8,600
7.	Total Cost of Project		25,000

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Figure 1

GANNT DIAGRAM

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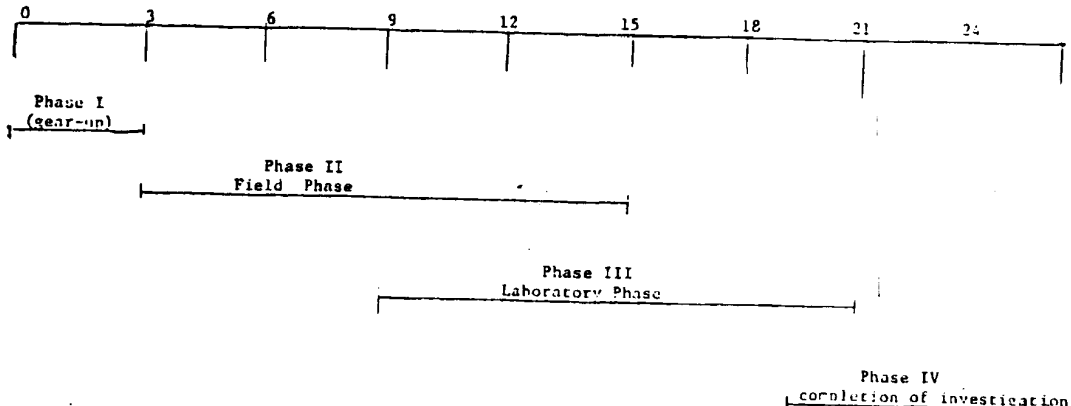

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REFERENCES

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XI

Appendix B

An Addendum to the Research Grant

"POLYCHLORINATED BIPHENYL'S TRANSLOCATION AND ENVIRONMENT CONTROL"

Submitted to the Sport Fishery Research Foundation

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Introduction

Recently there has been a great deal of public concern over PCB contamination in the aquatic environment especially in the Great Lakes region (Illinois and Wisconsin). Interest arose due to the banning of fishing in various Wisconsin rivers and in portions of Lake Michigan because of high PCB concentrations noted in fish. In response, the Sport Fishery Research Foundation funded a project at Tulane University to investigate PCB translocation mechanisms in freshwater ecosystems, to determine PCB bioaccumulation fluctuations in fish and to explore possible PCB decontamination mechanisms. In the first year of this project several interesting observations have been noted regarding the contaminated aquatic ecosystem under study: (1) the drop in PCB concentration in a ten year period appears to be substantially faster than the half-life projected in laboratory studies; (2) aqueous translocation does not appear to be due to soluble PCB's; (3) the major sinks for PCB appear to be the colloidal portions of the sediment and benthos organisms; and (4) some of the benthos organisms appear to exhibit a detoxication mechanism for PCB's.

Upon the request of the Sport Fishery Research Foundation, this addendum is submitted to ask for funding for a second year and request a third year extension. In the second and third years, we propose to: (1) validate gas chromatography analysis by the use of mass spectroscopy to better confirm the results obtained in both field and laboratory; (2) investigate biodegradation; (3) develop bioaccumulation studies; (4) study the possible removal of both

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soluble and bound PCB by vascular aquatic plants; and (5) determine PCB half-life by obtaining permission to utilize industrial data and continuing the field monitoring program.

Validation of PCB Analysis

The PCB measurement will be periodically confirmed by GC-MS analysis in order to further validate field observations. In biodegradation studies, metabolites resulting from PCB degradation will be carefully monitored by frequent GC-MS analysis. Finally, all other laboratory investigations should be monitored periodically in order to substantiate the chromatograph technique.

Decontamination Studies

By obtaining further support, in-field decontamination techniques may be determined. Field investigations and recent unpublished findings[1,2], give strong evidence for microbial degradation of PCB. The proposed decontamination study will require three phases: (1) an initial microbial acclimitization study; (2) sonications experiments (causing the stable PCB's to be altered to a more degradable form); and (3) a final microcosm study for final varifications of decontamination technique.

A possible technique for PCB removal from contaminated environments is the utilizing and harvesting of vascular aquatic plants which have a high affinity to remove soluble PCB's or PCB's bonded to soils, sediments and colloidals. Since it has been noted that plants tend not to accumulate PCB's (as readily as sediments, soils, and biota), this investigation will study the efficiencies of specific vascular plants in removing harmful quantities of PCB's from soil and sediment sinks.

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Bioaccumulation Studies

The bioaccumulation studies and the destabilization of contaminated colloidal sediments (clays and humic acids) will involve two phases: (1) the determinations of PCB's affinity for various sediment constituents and (2) the determination of PCB bioaccumulation by fish from contaminated clays and humates.

By chance, it was noted that while colloidally bonded PCB's are not as toxic as soluble PCB, the bound PCB's affect the behavior of various fish. Therefore, in this proposed bioaccumulation study sublethal toxicity and more realistic values for PCB's bound to colloidal sediments can be ascertained.

The Determination of PCB's Half-Life

The first year of study has shown that even though PCB's are still above contaminated levels in fish tissue, there has been a dramatic drop in PCB concentrations in soils, sediment, water and fish tissue in the area of study. Additional funding support would enable us to obtain permission to use industrial data and to continue monitoring the Alabama site; to thereby determine a realistic PCB half-life for the southeastern portion of the United States.

Tentative Schedule and Budget

This project will require approximately 24 months to complete. The gearing-up phase will require two to three months (the planning stage and preliminary experiments). The experimental phase will be conducted after the gear-up phase and be completed in a year. A final refined experimental phase will be completed during the subsequent six months. After completion of the laboratory phases, the data will be analyzed and a final report will be prepared with a three month time frame.

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The cost of the proposed project is shown in Table I and the tentative research team is listed in Table II.

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References

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TABLE I

RESEARCH BUDGET

		First Year	Second Year
1.)	Personnel	$ 15,600	$ 15,600
2.)	Travel		
	Surveys		800
3.)	Equipment		
	Maintenance	1,000	1,000
	Field Equipment	500	500
	Recorder	1,500	0
	Lab Equipment	1,000	1,000
4.)	Supplies		
	(chemical reagents, gases for GC and misc. supplies)	2,000	2,000
5.)	Other		
	Computer Time		300
	Xeroxing and report preparation		300
6.)	GC-MS Analysis	7,500	7,500
7.)	Total Cost Project	$ 31,100	$ 29,000

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TABLE II

GENERAL RESEARCH TEAM

SPORT FISHERY INSTITUTE

Mr. Richard Stroud

PRINCIPAL SUPERVISOR

R.S. Reimers, Ph.D.

Field Survey	Analysis	Microbial Degradation	Bioaccumulation	Plant Harvesting
J.E. Gunning, Ph.D.	R.S. Reimers, Ph.D.	A.C. Anderson, Ph.D.	A. Abdelghani, D.Sc.	A.J. Englande, Jr., Ph.D.
M. Wilke, Ph.D. Cand.	A. Abdelghani, D.Sc.	A. Abdelghani, D.Sc.	R.S. Reimers, Ph.D.	S. Tridech, D.Sc. Cand.
L.S. Gardner, M.S. Cand.	Research Assistant	Graduate Student	A.C. Anderson, Ph.D.	R.S. Reimers, Ph.D.
Graduate Student			Graduate Student	

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