Laboratory model chamber and field studies are underway to establish sensitive biomarkers of exposure to toxic chemicals found at Air Force study sites. In the first year of the grant a protein important to the toxicity of TCE (a neurotoxic chemical found at Air Force study sites) was highly purified, long-term inhibitors of blood enzymes in birds were studied, and a model chamber was designed and constructed. In the coming year, chemical distribution and stress proteins in bacteria and other organisms will be studied in the model chamber and compared to findings at field sites.
Annual Technical Report

"Biomarkers of Exposure: Molecules to Ecosystem"

Barry W. Wilson and James N. Seiber

Summary

Laboratory, model chamber and field studies are underway to establish sensitive biomarkers of exposure to toxic chemicals found at Air Force study sites. In the first year of the grant a protein important to the toxicity of TOCP (a neurotoxic chemical found at Air Force study sites) was highly purified, long-term inhibitors of blood enzymes in birds were studied, and a model chamber was designed and constructed. In the coming year, chemical distribution and stress proteins in bacteria and other organisms will be studied in the model chamber and compared to findings at field sites.

Research Objectives/Statement of Work

Specific Objectives:

Animal Models, Contaminant Mixtures and Neurotoxicity

Animal models of exposure to environmental contaminants are under development using birds and mammals that can be studied both in the laboratory and in the field. The model compound is TOCP, end points are organophosphate induced delayed neuropathy (OPIDN), blood and tissue cholinesterase depressions, and liver mixed function oxidases. Possible synergisms between TOCP, oil and organochlorine agents will be examined. A highly purified supply of CBDP, the active metabolite of TOCP, has been synthesized by Dr. James Sanborn. Studies of the neurotoxicity of TOCP and other organophosphates (OPs) to pigeons and shrews are in progress. One important finding is that dermal exposures of OPs to the feet of pigeons lead to a long-lasting, severe depression of blood cholinesterases, more than occurs with oral exposures or injections, as if there is some depot on or under the skin for the toxic agents. Study sites, including Air Force sites, are being selected, and examination of contaminants found in the field will soon begin.

Field biomarkers under study are stress proteins (in collaboration with Dr. Larry Harshman and Dr. Bruce Hammock) and, due to its availability on another project, the Microtox device. There is evidence that proteins such as heat shock proteins rise when animals are exposed to stress and that the luminescence of the bacterial preparations in the Microtox device is reduced in the presence of many toxins. Such approaches provide non-specific ways to examine the physiological state of animals in the field and of soil and water samples at sites suspected of creating environmental stresses. They do not substitute for the more specific protein assays and residue analyses.
2. **Blood Markers**

Emphasis is on developing serum protein markers of environmental stress to wildlife. Specific goals include (a) improving acetyl- and butyrylcholinesterase (AChE, BChE) assays in wildlife species, (b) developing enzyme and other protein markers for environmental stress, and (c) validating the assays in indicator species. Experiments to improve field testing for AChE and BChE are in progress using a field kit developed by Dr. Magnotti (U. Cincinnati) for human exposures known as the TEST-MATE. Results from the kit are being compared with our automated, optical plate reader enzyme assays. Studies on experimental animals and humans are in progress, work on animals in the field will soon begin. Plans are to establish a breeding colony of field mice in collaboration with Dr. W. Lasley (UCD Primate Center). Oxime reaction methods of OP-ChE enzyme complexes have been developed and tested on experimental animals. A student is completing her M.S. thesis examining the usefulness of the carboxyesterase activity in the blood of birds as biomarkers of exposure to OPs using the pigeon and blood from captive raptors kept at the UCD Raptor Center.

Field work focused on Red-Tailed Hawks and American Kestrels in an orchard study site in the Central Valley of California supported in part by a consortium of orchard growers and chemical companies to assess the risk of dormant sprays to raptors and other birds. Radiotransmitters have been used to show that the home range of raptors in the winter is only a few square miles, and residues on the birds, blood cholinesterase depressions and pesticide metabolites in the excreta of the birds indicate the extent of their exposures to the organophosphates in the sprays. Whether or not mixtures of OPs and the light oils used in dormant sprays will be more toxic than the compounds alone is under study, first with pigeons and then with mammals.

One of the goals of this part of the project is to develop antibodies to OP-protein complexes for use in sensitive immunoassays to detect exposure. The first protein under study is Neuropathy Target Esterase (NTE), the enzyme found in neural tissue, lymphocytes and testicles implicated in the initiation of OPIDN. The protein has been highly purified using affinity columns and is almost ready for sequencing. Another project that will soon begin is an attempt to obtain an antibody to the OP-AChE enzyme complex working with biochemists Josef Seifert, beginning a sabbatical leave in our laboratory, and B.P. Doctor (Walter Reed).

3. **Laboratory Models of Exposure**

The research objectives for this portion of the project include the determination and prediction of the volatilization rates of chemicals from soil and the investigation of the chemical movement through the air to non-target biota. The specific chemicals being studied to date are triorthocresyl phosphate (TOCP), tributyl phosphate, and trichloroethylene. Other non-pesticide phosphoric acid esters such as dibutyl ester, diethyl ester, 2-ethylhexyl diphenyl ester, triethyl ester, trimethyl ester, triphenyl ester, and tris(2-methylpropyl) ester are being considered for inclusion in the study at this time. An extensive literature search is currently underway to determine previous research efforts associated with environmental release, movement, persistence, and monitoring of the chemicals of interest.

The environmental chamber that will be used to determine vapor capture rates of water, soil, and biota is currently under construction (see attached figure). It is patterned after the chambers
we are currently using to study air emissions from hazardous waste sites and pesticide-treated fields, which are now producing useful information on volatilization rates in both field and laboratory experiments.

**Significant Accomplishments/Progress**

Significant accomplishments this year include (a) the purification of NTE, (b) discovery of long-term inhibition of cholinesterases of pigeons after exposure to OPs, (c) application of methods to detect exposure in humans successfully to birds, (d) initiation of combined field laborator y studies of environmental residues and effects in animals. Next steps include examination and choice of study sites, including an Air Force site, initiation of work on TOCP in pigeons and small mammals, and testing of the laboratory chamber. Work will continue to purify NTE and obtain antibodies to it, and work will begin on finding antibodies to OP-inhibited cholinesterases.

**Publications**


Wilson, B.W. and J.D. Henderson. Blood esterase determinations as markers of exposure. *Critical Reviews of Toxicology,* in press


Participating Professionals

Barry W. Wilson

B.A. 1950 Liberal Arts University of Chicago, Chicago IL
B.S. 1957 Biology Illinois Institute of Technology, Chicago IL
M.S. 1957 Zoology Illinois Institute of Technology, Chicago IL
Ph.D. 1962 Zoology UCLA, Los Angeles CA

Dr. Wilson has begun working on standardizing cholinesterase assays with the EPA and the State of California. He chaired a meeting in December, 1991 on Cholinesterase Methodologies for the EPA, is coordinating a round-robin testing of several laboratories to standardize clinical testing for ChE activity in animal studies, and will be a member of an EPA panel reviewing neurotoxicity guidelines for the agency. Several members of the EPA cholinesterase panel are from DOD (David Lenz, B.P. Doctor). Dr. Wilson was participant in a Conference of Esterases Inhibited by Organophosphates in Parma, Italy in April 1992 with a session on NTE for which he was a coorganizer. He is cochair of setting of a Conference on Ecotoxicology in 1993 to signal the formation of an EPA Center on Ecotoxicology.

James N. Seiber

A.B. 1961 Chemistry Bellarmine College, Louisville, KY
M.S. 1964 Chemistry Arizona State University, Tempe, AZ
Ph.D. 1966 Chemistry Utah State University, Logan, UT

Dr. Seiber was appointed member of the National Academy of Sciences Committee on Risk Assessment for Hazardous Air Pollutants and also a member of the California Air Resources Board Scientific Review Panel. He attends monthly meetings of each committee, which primarily deal with exposure-risk issues. He has also been recently appointed Director of the Center for Environmental Sciences and Engineering at the University of Nevada, Reno, which he will begin July 1, 1992, while on approved Leave of Absence from UC Davis.

Josef Seifert

M.S. 1964 Biochemistry Prague Institute of Chemical Technology, Czechoslovakia
Ph.D. 1973 Biochemical Toxicology Prague Institute of Chemical Technology, Czechoslovakia

Dr. Seifert is an Associate Professor of Biochemistry at the University of Hawaii. He is an expert toxicologist interested in the mechanism of action of pesticides, and is interested in organophosphates and neurotoxicities.
Interactions

Meeting Presentations


California Almond Board Research Meeting (December, 1990, Fresno, CA) Oral and poster presentation by B.W. Wilson


Society of Toxicology (February, 1991, Dallas, TX) B.W. Wilson, students K. Funk and T. Kellner attended and presented posters.

Northern California SETEAC (May, 1991, Sacramento, CA) Student Julie Yamamoto attended and presented poster.


b. Recent Invited Talks

2nd International Meeting on Esterases Hydrolyzing Organophosphorus Compounds (Salsomaggiore, Italy, April, 1992), Session on Neuropathy Target Esterase. Scientific Committee: Martin K. Johnson (UK), Marcello Lotti (Italy) and Barry Wilson (USA); Coorganizer, presented an invited paper.


American Chemical Society Toxicology Course (April, 1992, Newport Beach, CA) Invited lecture on neurotoxicology.

Abstracts


Thomas, T.C., Szekacs, A., Hammock, B.D., McNamee, M.G. and Wilson, B.W. 1992. Purification and characterization of neuropathy target esterase. 2nd International Meeting


Thomas, T.C., Szekacs, A., Hammock, B.D., McNamee, M.G., and Wilson, B.W., 1990. Affinity chromatography of neuropathy target esterase. 199th ACS meeting, #116


Seiber, J.N. Weisskopf, C.P., McChesney, M and Wilson, B.W. 1990, Atmospheric route to pesticide exposures in agroecosystems. 199th ACS meeting #134.


Thomas, T.C., Szekacs, A., Rojas, S., Hammock, B.D., McNamee, M.G. and Wilson, B.W. 1990, Characterization of neuropathy target esterase using trifluromethyl ketone transition state analogs. The Toxicologist 10(1), #737


New Discoveries, Inventions, Patent Disclosures
None

AFOSR Program Manager Special Information
I am sorry and embarrassed for having missed the report deadline.
Environmental Chamber