Attachment 1 – Office of Research and Development (ORD) File with Relevant Information

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL RESEARCH
AND QUALITY ASSURANCE
WASHINGTON, DC 20460

MAY 19 2000

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Protection of Human Subjects Certification

FROM: Peter W. Preuss, Ph.D. PW Preuss
Director
National Center for Environmental Research (8701R)
EPA Human Subjects Research Review Official

TO: David E. Kleffman, Director
ESRD/NCER (8723R)

I have reviewed this proposal and am satisfied that it complies with EPA Regulation 40 CFR 26 **PROTECTION OF HUMAN SUBJECTS,** and with EPA Order 1000.17, Change A1, **Policy and Procedures on Protection of Human Subjects.**

EPA No: R828017 010 husulg# 00-5 EPA/SPA00-001

APPLICANT: Mississippi State Univ.

PROJECT TITLE: *Assessing Levels of Intermittent Exposures of Children to Flea Control Insecticides from the Fur of Dogs*

PRINCIPAL INVESTIGATOR: Janice E. Chambers, Ph.D.

PROJECT OFFICER: Christopher Saint, Ph.D.

cc. Saint (8723R)

HUMAN SUBJECTS - RSC CHECK LIST

1. Title: *Assessing Levels of Intermittent Exposures of Children to Flea Control Insecticides from the Fur of Dogs*

2. EPA Organization; husulg#; Date: ORD/NCER, 00-5, 00-5-17

3. EPA Contacts: Kleffman, Saint

4. Applicant: Mississippi State Univ.

5. Findings	OK?
a. risk of substantial injury	Yes
b. determination of irreversible effects	Yes
c. foreign study meets "at least equivalent to"	NA
d. rights and welfare adequately protected	Yes
e. risk to subjects is outweighed by the sum of benefit to the subjects plus the value of the knowledge gained	Yes
f. effective informed consent	Yes

6. Documents

a. MPA or equivalent EPA/SPA00-001	Yes
b. IRB approval	Yes
c. procedures for getting informed consent	Yes
d. procedures for recruiting subjects	Yes
e. consent form	Yes

7. Comments, Notes, Caveats, Conditions, etc.

a. Pet dogs will be flea controlled per the label of two kinds of pesticides and the exposure of children, 3 to 12 years old, will be measured from urine samples and t-shirts that they will wear, there being no exposure beyond "normal" antiflea treatment, i.e. zero incremental risk to the children.

b. I recommend approval. Cortes

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C. 20460

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

00-5

DATE: 27

SUBJECT: Request for Human Subjects Approval and Single Project Assurance

FROM: Dave Kleffman, Director
Environmental Sciences Research Division (8723)
National Center for Environmental Research
and Quality Assurance

EPA/SPA 00-001

TO: Roger Cortesi
National Center for Environmental
Research and Quality Assurance (8701)

00-5

This memorandum requests approval for the use of human subjects in a research project sponsored by NCERQA.

Number:	R828017010
Title:	Assessing Levels of Intermittent Exposures of Children to Flea Control Insecticides from the Fur of Dogs
Institution:	Mississippi State University
Investigator:	Janice Chambers
~~MPA number~~:	Need EPA Single Project Assurance

The institutional review board for the institution has reviewed the proposed research and has provided the attached letter of approval. The principle investigator has also provided copies of the informed consent forms they intend to use during the course of the study. A Single Project Assurance application is attached.

Attachments

Mississippi State University

Assurance of Compliance with EPA Regulations for
Protection of Human Research Subjects

Mississippi State University, hereinafter known as the institution, hereby gives assurance that it will comply with the United States Environmental Protection Agency (EPA) regulations for the protection of human research subjects (40 CFR 26) as specified below.

PART 1

Ethical Principles and Institutional Policies Governing Research Involving Human Subjects

1. Applicability

Except for research exempted or waived under the EPA regulations 40 CFR 26. 1 0 1, Part I of this Assurance applies to all research involving human subjects, and all other activities which even in part involve such research, regardless of whether the research is otherwise subject to federal regulation, if:

(a) the research is sponsored by this institution, or

(b) the research is conducted by or under the direction of any employee or agent of this institution in connection with institutional responsibilities, or

(c) the research is conducted by or under the direction of any employee or agent of this institution using any property or facility of this institution, or

(d) the research involves the use of this institutions nonpublic information to identify or contact human research subjects or prospective subjects.

II. Ethical Principles Governing Human Subjects Research

This institution is guided by the ethical principles regarding all research involving humans as subjects as set forth in the report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research entitled, Ethical Principles and Guidelines for the Protection of Human Subjects of Research (the Belmont Report) and as specified below.

A. This institution recognizes the principles of respect for persons, beneficence (including minimization of harms and maximization of benefits), and justice as stated in the Belmont Report and will apply these principles in all research covered by this Assurance.

B. This institution acknowledges and accepts its responsibilities for protecting the rights and welfare of human research subjects.

III. Policies

A. This institution acknowledges that it and its investigations bear full responsibility for the performance of all research covered by this Assurance, including full responsibility

for complying with Federal, state and local laws as they may relate to such research.

B. This institution assures that before human subjects are involved in research, proper consideration will be given to:

(1) the risks to the subjects,
(2) the anticipated benefits to the subjects and others,
(3) the importance of the knowledge that may reasonably by expected to result,
(4) the informed consent process to be employed,
(5) the provisions to protect the privacy of subjects, and
(6) the additional safeguards for vulnerable populations.

C. This institution recognizes the need for appropriate additional safeguards in research involving subjects who are likely to be vulnerable to coercion or undue influence such as children, prisoners, pregnant women, mentally disabled persons, or economically or educationally disadvantaged persons.

D. This institution encourages and promotes constructive communication among the institutional officials, research administrators, department heads, research investigators, clinical care staff, human subjects, and all other relevant parties as a means of maintaining a high level of awareness regarding the safeguarding of the rights and welfare of the subjects.

E. This institution will exercise appropriate administrative overview carried out at least annually to assure that its practices and procedures designed for the protection of the rights and welfare of human subjects are being effectively applied.

Part 2
IRB, Institution, and Investigator Compliance with 40 CFR 26

I. Applicability

Part 2 of this Assurance applies to the following research project which is conducted or sponsored by this institution and supported by the United States Environmental Protection Agency (EPA).

Project Title: "Assessing Levels of Intermittent Exposures of Children to Flea Control Insecticides from the Fur of Dogs"

EPA Project Number: R 828017 010 Internal # 00-5

Project Principal Investigator: Janice Chambers

II. Institutional Responsibilities

A. This institution has complied and will continue to comply with the requirements of 40 CFR 26 as specified below.

B. In accordance with the compositional and quorum requirements of 40 CFR 26.107 and 46.108, the Institutional Review Board (IRB) designated in Part 3 and in the attached roster is responsible for the initial and continuing review of this project.

C. This institution has provided and will continue to provide both meeting space for the IRB and sufficient staff to support the IRB's review and record keeping duties.

D. In addition to the review and approval of the IRB, this institution has reviewed and sponsors the project referenced above.

III. IRB Review

A. The IRB shall review, and have the authority to approve, require modification in, or disapprove this research activity or proposed changes in it before human subjects may be involved.

B. The convened IRB reviewed and approved the above project.

C. The IRB determined, in accordance with the criteria found at 40 CFR 26.111, and where applicable, 45 CFR 46 Subparts B, C, and D, that protections for human subjects are adequate.

D. The IRB has the authority to suspend or terminate approval of the above referenced research in accordance with 40 CFR 26.113 for (1) non-compliance with 40 CFR 26, and this Assurance document or the IRB's requirements, and (2) for elimination of unexpected serious harm to subjects.

E. The IRB has determined that legally effective informed consent **[copy of document must be attached unless specified otherwise by EPA]** will be obtained in a manner and method which meets the requirements of 40 CFR 26.116~~and 46.117~~.

F. Certification of IRB approval, at least annually shall be submitted to the EPA awards unit that issued the award, as a condition for receipt of funds for a noncompeting continuation and/or additional involvement of human subjects.

G. Continuing reviews by the IRB shall be conducted at intervals appropriate to the degree of risk, but not less that once per year. (40 CFR 26.109 [e]). The IRB may be called into an interim review session by the Chairperson at the request of any IRB member or Institutional Official to consider any matter concerned with the rights and welfare of any subject.

H. The IRB shall prepare and maintain adequate documentation of its activities in accordance with 40 CFR 26.115.

I. The IRB shall report promptly to institutional officials and the EPA

(1) any serious or continuing noncompliance by investigators with the requirements of the IRB,
(2) any suspension or termination of **IRB** approval,
(3) any unanticipated problems or injuries involving risks to subjects or others, and
(4) any changes in this research activity which are reviewed and approved by the IRB.

J. Where appropriate, the IRB will determine that adequate additional protections are ensured to fetuses, pregnant women, prisoners, and children as required under Subparts B, C, and D of 45 CFR 46. The IRB will notify EPA promptly when IRB membership is modified to satisfy the requirements at 40 CFR 26.304 and when the IRB fulfills its duties under 40 CFR 26.305 (c). not relevant RLC

K. The IRB will comply fully with the requirements of all applicable Federal policies and guidelines, including those concerning notification of sero-positivity, counseling, and confidentiality of subjects.

IV. Research Investigator Reporting Responsibilities

A. Investigators acknowledge and accept their responsibility for protecting the rights and welfare of human research subjects and for complying with all applicable provisions of this Assurance and 40 CFR 26.

B. Research investigators shall reports promptly to the IRB proposed changes in this research activity and the changes shall not be initiated without IRB review and approval except where necessary to eliminate apparent immediate hazards to the subjects.

C. Research investigators shall report promptly to the IRB any unanticipated problems involving risks to subjects and others.

Part 3

Certification of IRB Approval and Institutional Endorsement

Project Title: "Assessing Levels of Intermittent Exposures of Children to Flea Control Insecticides from the Fur of Dogs"

DHHS Project Number _____

Project Principal Investigator: Janice Chambers

Date of IRB Approval: 11/11/99 Date of Next Scheduled IRB Review: 11/2000

The officials signing below assure that the project referenced above was approved by the IRB of the date indicated and that the project will be conducted in accordance with the requirements of Part 26, Title 40 of the Code of Federal Regulations and this Assurance document. A dated roster listing the current membership of the designated IRB is attached.

As appropriate, the officials signing below further assure that for each protocol in this project for which IRB approval was not possible due to delayed onset of subject involvement, the IRB's institution will provide a copy of the IRB-approved protocol, IRB-approved consent language, and documentation of IRB certification (Optional Form 310), including the applicable Assurance number, to for approval prior to accrual of human subjects.

I. **Authorized Official of the Institution Providing this Assurance**

Signature Robert O. Altenkirch Date: 12/13/99

Please type the following items.

Name and Title: Robert Altenkirch, Vice President for Research
Institution: Mississippi State University
Address: P.O. Box 6343
Mississippi State, MS 39762
Telephone: 662-325-3570 Fax: 662-325-8028
E-mail: altenkirch@research.msstate.edu

II. **N/A**

III. **IRB Chairperson**

Signature: Tracy B. Henley Date: 12/15/99

Please type the following items.

Name and Title: Tracy B. Henley, IRB Chairman
Institution: Mississippi State University
Address: P.O. Box 6161
Mississippi State, MS 39762

Telephone: 662-325-7949 Fax: 662-325-7217 E-mail: tbh1@ra.msstate.edu
MPA Number if applicable: N/A

IV. **Responsible Project Investigator at Institution Providing this Assurance**

I have attached copies of all EPA requested and IRB approved Informed Consent Documents to be used in this project unless the designated IRB operates under an OPRR-approved Multiple Project Assurance (MPA) or unless OPRR has indicated otherwise.

Signature: Janice Chambers Date: Dec. 13, 1999

Please type the following items.

Name: Janice Chambers
Title: Professor, College of Veterinary Medicine Research Program
Institution: Mississippi State University
Address: P.O. Box 9825
Mississippi State, MS 39762
Telephone: 662-325-1255 Fax: 662-325-1031 E-mail: chambers@cvm.msstate.edu

****

All parts of this Assurance are in compliance with the requirements of Part 6, Title 4 , of the Code of Federal Regulations.

Approving Official

Signature: P.W. Preuss Date: 5/18/00

Name:
Address: Peter W. Preuss, Ph.D.
Director
National Center for Environmental Research (8701R)
EPA Human Subjects Research Review Official
1200 Pennsylvania Avenue
Washington DC 20460
Telephone: 202 564 6825
Fax: 202 565 2444
Email: preuss.peter@epa.gov

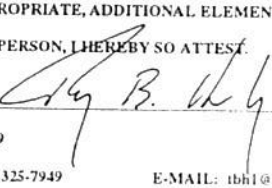
ections
507

ASSURANCE NUMBER S- EPA/SPA 00-001

An application for new or competing support for continuation in which human subjects will be involved will require a new and separate Assurance, unless the activity is exempt under section 40 CFR 26.101 (b).

INSTITUTIONAL REVIEW BOARD MEMBERS
Mississippi State University

DATE: November 11, 1999

NAME (voting members only[2,3] Last, First	HIGHEST DEGREE EARNED	PRIMARY SCIENTIFIC ORNONSCIENTIFIC SPECIALTY	AFFILIATION WITH INSTITUTION(S) ABOVE (YES/NO; IF YES, WHICH ONE)	ATTESTATIONS OF IRB CHAIRPERSON
* Tracy B. Henley	PhD	Psychology	MSU	FOR ALL RESEARCH CONDUCTED UNDER THIS ASSURANCE, THE IRB CHAIRPERSON HEREBY ATTESTS THAT, EXCEPT WHERE SPECIFICALLY WAIVED OR ALTERED BY THE IRB UNDER 45 CFR 46.116(c), 46.116(d), 46.117(c), THE IRB WILL UPHOLD THE REQUIREMENTS OF 45 CFR 46 FOR WRITTEN INFORMED CONSENT, IN NONEXCULPATORY LANGUAGE UNDERSTANDABLE TO THE SUBJECT (OR THE SUBJECT'S LEGALLY AUTHORIZED REPRESENTATIVE), INCLUDING THE FOLLOWING BASIC ELEMENTS PER 45 CFR 46.116(a/b): (1) IDENTIFICATION AS RESEARCH; PURPOSES, DURATION, AND PROCEDURES; PROCEDURES WHICH ARE EXPERIMENTAL; (2) REASONABLY FORESEEABLE RISKS OR DISCOMFORTS; (3) EXPECTED BENEFITS TO THE SUBJECT OR OTHERS; (4) ALTERNATIVE PROCEDURES OR TREATMENTS; (5) EXTENT OF CONFIDENTIALITY TO BE MAINTAINED; (6) WHETHER COMPENSATION OR MEDICAL TREATMENT ARE AVAILABLE IF INJURY OCCURS (IF MORE THAN MINIMAL RISK); (7) WHOM TO CONTACT FOR ANSWERS TO QUESTIONS ABOUT THE RESEARCH, SUBJECT'S RIGHTS, AND RESEARCH-RELATED INJURY; (8) PARTICIPATION IS VOLUNTARY; REFUSAL TO PARTICIPATE WILL INVOLVE NO PENALTY OR LOSS OF BENEFITS TO WHICH SUBJECT IS ENTITLED; SUBJECT MAY DISCONTINUE AT ANY TIME WITHOUT PENALTY OR LOSS OF BENEFITS TO WHICH SUBJECT IS ENTITLED; (9) WHEN APPROPRIATE, ADDITIONAL ELEMENTS PER 45 CFR 46.116(b). AS IRB CHAIRPERSON, I HEREBY SO ATTEST. SIGNATURE:  DATE: 11/11/99 PHONE: 662-325-7949 E-MAIL: tbh1@ra.msstate.edu ADDRESS: MSU, PO Box 6161, Mississippi State, MS 39762
Joe R. Bumgardner	MD	Medicine	MSU	
Robert K. Collins	MD	Medicine	MSU	
Martin Geisen	PhD	Psychology	MSU	
Abner Harrison	MS	IRB	MSU	
Frank Howell	PhD	Social Science	MSU	
Sam Givhan	PhD	Psychology	MSU	
Ike Ikenberry	PhD	Research	MSU	
Dave Kaber	PhD	Engineering	MSU	
Jay Keehley	PhD	Philosophy/Law	MSU	
Steve Lemay	DBA	Business	MSU	
Eugene Martin	EdD	Education	MSU	
Matthew Ronning	MBA	Research	MSU	
Barbara Spencer	PhD	Business	MSU	
Tracy S. Arwood	MS	IRB	MSU	
Rev. Sarah Burress	MaD	Religion	NONE	

MINOR'S ASSENT FORM

We will specifically obtain assent from the children recruited to our project 'Assessing levels of intermittent exposures of children to flea control insecticides from fur of dogs' for their participation in the project. We are trying to recruit children between the ages of 3 and 12 years of age. We will explain that the child's parent or guardian has given us permission to request his/her help participation in the research project. We will then explain the urine collection protocol and the tee shirt protocol to the children in language appropriate to the age of the child and obtain his/her assent to participate. We will not explain the connection to the pesticide residues on the dog so as not to alter the behavior of the child with the dog. We will obtain the children's assent orally because of the age range of the children involved.

Each conversation will include (paraphrased):

Your parent knows we are going to ask you to help us with a project. We want to understand how you play. You will get a tee shirt to play in and you will need to urinate in a bottle on certain days that you parents/guardians say. Your name will not be written anywhere, and no one will know that the samples came from you personally.

If you don't want to participate, you can stop at any time. There will be no bad feelings if you don't want to do this. You can ask questions if you do not understand any part of the project and we will try to explain them.

Do you understand? Is this OK?

Thank you.

Name (Please print):_____

Date: _____

Investigator's Signature: _____ Date:_____

AUTHORIZATION FOR PARTICIPATION IN RESEARCH PROJECT (permethrin)

Participant: _____

To: "Assessing Levels of Intermittent Exposures of Children to Flea Control Insecticides from the Fur of Dogs." Project

I hereby authorize my participation or the participation of _____ _____, my minor child, in the above named research project. I understand that I or my child will be asked to provide 16 urine and 16 tee-shirt samples each during a 64 day period. Each participating household will receive $150. I understand that records resulting from this project will be coded and that the information will not be identified by name. I understand that the urine sample will be used only for the analysis of a metabolite of the insecticide permethrin, and for no other purposes. No risks are anticipated to the participants. This research will allow estimates to be made regarding the levels of insecticide exposure which might occur in humans in contact with pet dogs treated with a commercial permethrin spot treatment.

I hereby authorize you to furnish me with a copy of the records of myself or _____, my minor child, compiled during participation in the aforementioned research project, or to allow those records to be inspected or copied by myself or my authorized representative.

I understand that I, or my minor child, may withdraw from the research project at any time. I further understand any services I, or my minor child, may receive from the Mississippi State Department of Health, or from other state agencies, will not be affected, in any way whatsoever, by my participation, failure to participate, or withdrawal from the research project.

Information about this research project can be obtained from Dr. Janice Chambers, College of Veterinary Medicine, Mississippi State University, 662-325-1255. Information regarding the MSU Institutional Review Board for the protection of Human Subjects in Research can be obtained from Ms. Tracy S. Arwood, 662-325-3994.

This is the _____ day of _____, 19____.

_____

Participant or Guardian of Research Participant

_____

Relationship to Participant

AUTHORIZATION FOR PARTICIPATION IN RESEARCH PROJECT (collar)

Participant: _____

To: "Assessing Levels of Intermittent Exposures of Children to Flea Control Insecticides from the Fur of Dogs." Project

I hereby authorize my participation or the participation of _____ _____, my minor child, in the above named research project. I understand that I or my child will be asked to provide 22 urine and 22 tee-shirt samples each during a 4 month period. Each participating household will receive $150. I understand that records resulting from this project will be coded and that the information will not be identified by name. I understand that the urine sample will be used only for the analysis of a metabolite of the insecticide chlorpyrifos or tetrachlorvinphos, and for no other purposes. No risks are anticipated to the participants. This research will allow estimates to be made regarding the levels of insecticide exposure which might occur in humans in contact with pet dogs wearing commercial flea collars.

I hereby authorize you to furnish me with a copy of the records of myself or _____, my minor child, compiled during participation in the aforementioned research project, or to allow those records to be inspected or copied by myself or my authorized representative.

I understand that I, or my minor child, may withdraw from the research project at any time. I further understand any services I, or my minor child, may receive from the Mississippi State Department of Health, or from other state agencies, will not be affected, in any way whatsoever, by my participation, failure to participate, or withdrawal from the research project.

Information about this research project can be obtained from Dr. Janice Chambers, College of Veterinary Medicine, Mississippi State University, 662-325-1255. Information regarding the MSU Institutional Review Board for the protection of Human Subjects in Research can be obtained from Ms. Tracy S. Arwood, 662-325-3994.

This is the _____ day of _____, 19____.

_____

Participant or Guardian of Research Participant

_____

Relationship to Participant

Chris Saint

11/09/1998 12:28 PM

To: Roger Cortesi/DC/USEPA/US
cc:
Subject: Single Project Assurance Number for R828017-01-0 Mississippi State University

Roger:

Back in January you raised several questions about the Consent for project R828017-01-0 Mississippi State University (see attached). I have discussed this project at some length with the investigators and I feel that the consent for does cover all the relevant aspects covered in the Common Rule.

Your Note mentioned:

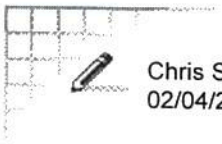
116 (a) (1) - Both the parent and child forms cover this.
116 (a) (2) - The parents form covers this directly with a statement that no riks are anticipated.
116 (a) (3) - There are no real benefits from participation except the incentive fee (covered in parents form).
116 (a) (4) - There are no alternative treatments since this is not a disease related study.

I recommend that we issue a Single Project Assurance Number for the R828017-01-0 project at Mississippi State University as soon as possible using the existing consents form which has been approved by the IRB.

Thank you

Dr. Chris Saint
Assistant Director, NCERQA
1300 Penn Ave Room 51179
Washington DC 20004
202-564-6909
Fax: 202-565-2448
saint.chris@epamail.epa.gov

HUMAN SUBJECTS FOLLOW-UP



Chris Saint
02/04/2000 05:13 PM

To: chambers@cvm.msstate.edu
cc: Roger Cortesi/DC/USEPA/US, Dave Kleffman/DC/USEPA/US, Robert Menzer/DC/USEPA/US

Subject: Human subjects

Jan:

I have processed you funding for the project titled assessing levels of intermittent exposures of children to flea control incecticies from the fur of dogs (EPA Number R828017). I have run into a problem with the human subjects material submitted with your request for an EPA single project assurance number. The problem relates to your informed consent form. The Section 116 of the common rule (40 CFR part 26) for the protection of fhuman subjects lays out the requirements for informed consent. Our human subjects official feels that your informed consent form does not meet these requirements. The basic requirements include:

YES **(1) A statement that the study involves research, an explanation of the purposes of the research and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any procedures which are experimental;**

YES **(2) A description of any reasonably foreseeable risks or discomforts to the subject;**

(3) A description of any benefits to the subject or to others which may reasonably be expected from the research; NONE

(4) A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject;

(5) A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained;

(6) For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be obtained;

(7) An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject; and

(8) A statement that participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

[Note: The sections in **Bold** do not seem to be addressed at all in the form.]

We feel that your informed consent form should be revised to incorporate all of these features and then be reviewed agian by your IRB. We feel that these revisions must be completed before we can approve your human subjects and issue your single project assurance number. The funding package for the project will be put on hold until this issue is resolved. If you have any prolems or questions please contact me at 202-564-6909. I have attahced the full text of 40 CFR part 26.116 for your information. I look forward to your reply.

40cfr26.116.w

Chris
Chris Saint

Assistant Director, NCER
Project Officer

chambers@cvm.msstate.edu on 02/07/2000 11:45:35 AM

To: Chris Saint/DC/USEPA/US@EPA
cc: Roger Cortesi/DC/USEPA/US@EPA, Dave Kleffman/DC/USEPA/US@EPA, Robert Menzer/DC/USEPA/US@EPA

Subject: Re: Human subjects

Chris-
Thanks for your message. I will start revising it. It had been approved by our IRB so thought it covered all the essentials. Your message did not come across with any bold character, so I would appreciate your letting me know which points you considered deficient. Would it be useful for your human subjects officers to look at my revision before I submit it to our committee here?
Thanks.
Jan

Please note new area code

Janice E. Chambers, Ph.D., D.A.B.T.
William L. Giles Distinguished Professor
Director, Center for Environmental Health Sciences
College of Veterinary Medicine
Mississippi State University
Box 9825
Mississippi State, MS 39762-9825
662-325-1255; fax 662-325-1031

C. ABSTRACT

Sorting Code: 99-NCERQA-B1

Title: Assessing levels of intermittent exposures of children to flea control insecticides from the fur of dogs.

Investigators:

Janice E. Chambers, Ph.D, Principal Investigator, College of Veterinary Medicine.
J. Scott Boone, M.S., Ph.D., Co-Investigator, College of Veterinary Medicine.
John W. Tyler, D.V.M., Co-Investigator, College of Veterinary Medicine.
Carolyn R. Boyle, Ph.D., Biostatistical Consultant, College of Veterinary Medicine.

Institution: Mississippi State University.

Project Period: June 1, 1999-May 31, 2002.

Research Category: Children's Vulnerability to Toxic Substances in the Environment.

Project Summary:

Objectives/Hypothesis: There are reported insecticide residues present in food, water, and surfaces such as on carpets treated for flea control. However, no studies (except those we currently have in place) have quantified the dislodgable flea control insecticide residues which occur on pets (the majority of which are dogs) that could be transferred to children. These dermal exposures could easily become oral exposures when children place their contaminated hands in their mouths. Organophosphorus insecticides or synthetic pyrethroids are among the most common types of insecticides used for flea control. Our calculations have estimated that transfer of these dislodgable residues could result in exposure levels exceeding the adult reference dose (R_fD), which does not account for the greater sensitivity of children. There are a very large number of dog-owning households in the United States (about 37%) and about half of pet-owning households have children in them. The opportunity for large numbers of children to contact flea control insecticides on pets is high. Because of this lack of information and the likelihood of appreciable insecticide residues being present on pet fur, we propose to test the following hypothesis: *The residues of insecticides available for intermittent transfer to children from the fur of dogs treated by either a spot treatment or a collar for flea control will be appreciable and of a magnitude necessitating inclusion in cumulative risk assessments of pesticides to children; secondly, that the fur rubbing procedure developed to quantify dislodgable residues provides a useful estimate of insecticide residues which could be transferred from the fur of dogs to children.*

Approach: This project will generate unique and much needed information by determining the amount of residues which may be obtained from pets which are treated with flea control insecticides. Treatment of the dogs with either the synthetic pyrethroid permethrin in a spot treatment, or the organophosphates tetrachlorvinphos (TCVP) or chlorpyrifos in a flea collar will be performed according to label directions; all three products are over-the-counter, and readily available to the public. Dogs will be sampled before and periodically after treatment by rubbing the fur with

white cotton gloves using a standardized protocol which we currently have in place in our laboratories. The current fur rubbing protocol will be expanded to include the analysis of residues of tee shirts worn by children aged 4-10 in the households of these dogs to determine the amount of these insecticides actually dislodged by the children's interaction with their pets. These gloves and shirts will be extracted with organic solvents by standard methods used for pesticide residue analysis, and the extract will be analyzed by gas chromatography with electron capture detection. Correlation analysis will be conducted on the glove and tee shirt data to determine whether the rubbing protocol is an effective surrogate for the children's exposure. Additionally, urinary metabolites will be quantified at these same sampling times from the child and from one adult in the household to determine how these dislodgable residues compare to estimates of internal exposure. This approach will be conducted in three specific aims: 1) To determine the exposure of children to permethrin resulting from residues from the fur of dogs from a permethrin spot treatment; 2) To determine the exposure of children to TCVP resulting from residues from the fur of dogs treated with a TCVP flea collar; and 3) To determine the exposure of children to chlorpyrifos resulting from residues from the fur of dogs treated with a chlorpyrifos flea collar. These exposure estimates will indicate whether appreciable residues are available from common flea control strategies which could yield significant intermittent exposures of children to pesticides.

Expected Results: It is our prediction, based on the dislodgable residues we are currently measuring from flea dips and collars, which are in the mg range, that these exposure estimates will be substantial and will warrant being included in cumulative risk assessments of pesticides with respect to the safety of children. We expect that, based on our current data on flea control dips, that initial residues will be very high shortly after application of the flea control product, but that these residues will dissipate relatively quickly. Thus, we predict that flea control products will yield intermittent exposures of children to insecticides at levels which must be considered in cumulative risk assessments.

Improvements in Risk Assessment/Risk Management: The 1996 Food Quality Protection Act mandates that cumulative risk assessments be performed on multiple insecticides from multiple sources (dietary and non-dietary) if they display a common mechanism of toxicity. The flea control insecticides are very likely to fall under this mandate along with others in their chemical classes when concurrent exposures occur. At present, there is no information, except what we are generating, which indicates how great insecticide exposure from flea control insecticides on pets would be. This project proposes to obtain information on dislodgable residues from dog fur together with concurrent biomonitoring of of urine from children and adults in contact with the dog to give much needed information of the levels of intermittent exposure. This information will fill a prominent data gap in the field of pesticide exposure assessment and will enable future cumulative risk assessments to be appropriately and more accurately conducted.

Supplemental Keywords: health effects, human health, infants, children, age, sensitive populations

D. Project Description:

1. Objectives

A. Pesticide risk to infants and children

One of the most pressing issues in the field of risk assessment is the issue of assessing the risk of pesticide exposures to infants and children. Several years ago, the National Research Council (NRC, 1993) compiled much of the information which was available on pesticide exposure and toxicity to immature humans and animals. These scientists concluded that insufficient information existed at that time to confidently estimate risk to infants and children because of the differences in physiology, anatomy, dietary composition and behavior which will lead to differences in exposure, absorption, metabolism and toxic responses. The main theme of this report was potential dietary exposure to pesticides from residues in foods, even when the residues were within the approved tolerances. The typical estimates of exposure and toxicity, which are used in the risk estimate calculations, have been generated for adults, and have not taken into account the special differences of the immature, such as typical dietary composition and preferences, the greater vulnerability of developing organ systems, and the different behavior patterns.

This concern regarding pesticide risk in children is clearly warranted. Using the published literature or from FDA drug registration information, Goldenthal (1971) found that 235 drugs were more toxic to newborn animals than adults, whereas only 46 were more toxic to adults than newborns. Similar results were obtained with pesticides; 15 of 16 anticholinesterase pesticides were more toxic to weanlings than to adults (Brodeur and DuBois, 1963). The organophosphorus (OP) insecticide chlorpyrifos was substantially more toxic following cutaneous exposure to newborn piglets than to 3 day old piglets (Long *et al.*, 1986). Chlorpyrifos was also found to be absorbed more effectively by young rats than by adults rats (Shah *et al.*, 1987). The fungicide triadimefon entered the blood following dermal exposure faster in young rats than adult rats (Knaak *et al.*, 1984). Early studies suggested that the greater sensitivity of weanling rats to the two OP insecticides, parathion and methyl parathion, was related to lower hepatic detoxication mechanisms (Gagné and Brodeur, 1972; Benke and Murphy, 1975). However, in contrast to these weanlings, neonates had a substantially more permeable brain, which contributed to their greater sensitivity to morphine (Kupferberg and Way, 1963). Additionally, the greater sensitivity of newborn rats to two antihistamines was related to greater oral absorption in newborns than in adults (Lee, 1966).

Data from our own laboratories have indicated that the two OP insecticides, parathion and chlorpyrifos, are more acutely toxic to juvenile than to adult rats (Dorough, 1992; Burnett, 1994; Atterberry *et al.*, 1997). This greater sensitivity appeared to be related to differences of cytochrome P450 and esterase-mediated detoxication reactions, which were very low in newborns and increased with age. Therefore, the immature appeared to have an appreciably smaller protective capacity than adults, allowing the target molecule, acetylcholinesterase, to be more vulnerable to the toxic effects. Data from the laboratories of others have generated similar information (Mortensen *et al.*, 1996; Pope *et al.*, 1991). It is logical to assume that young humans also have relatively little detoxication potential, which will make infants and children considerably more susceptible to OP insecticide toxicity. It is logical to assume that infants and children would also be more vulnerable to other classes of insecticides, such as the synthetic pyrethroids, which are readily detoxified in adults.

While most of the available information is from animal studies, there is some very convincing evidence that immature humans are also more sensitive to pesticide toxicity. In a group of 79 humans poisoned by ingestion of parathion-contaminated flour in Jamaica in 1976, the case-fatality ratios were highest in the newborn to 4-year age group compared to older groups (Diggory et al.,

1977). There are other instances of children being poisoned by exposure to such items as clothing, bed linens, and burlap sacks which were used as swings for play (Eitzman and Wolfson, 1967; Warren et al., 1963; Woody, 1984). In 37 case histories of infants and children displaying moderate to severe poisoning to OP and carbamate insecticides, 6 of the cases were from contaminated surfaces and 76% were in children younger than 3 years (Zwiener and Ginsberg, 1988), indicating appreciable absorption through the child's skin.

Thus, infants and children are probably more sensitive to pesticide-induced toxicity because of potentially greater absorption, poorer detoxication, a less-developed blood-brain barrier, and vulnerable developing organ systems. Children have a larger surface area to volume ratio than adults, thereby possessing a relatively greater surface for dermal contact, which we are focussing upon in this proposal. Younger children who have yet to develop good practices of hygiene and common sense are likely to become contaminated by surfaces which have pesticide residues. These dermal exposures could easily become oral exposures because of the propensity of young children to put their hands and other objects in their mouths. Therefore, it seems that children may well receive greater exposure to pesticides from contact with surfaces than from residues on foods. There is certainly substantial concern among scientists at present that the intermittent exposures of infants and children to pesticide residues from a variety of residential applications may be the greatest source of pesticide exposure to the immature, and may well bring the internal dose of pesticides into excursions above presumably safe levels into the realm of toxic outcomes. Scientists from a variety of perspectives agreed recently at the "Workshop to Develop a Framework for Cumulative Risk Assessment" organized by the International Life Sciences Institute (September, 1998), in which the Principal Investigator was a participant, that episodic residential exposures to pesticides were of greater concern than the low levels of pesticides occurring in the food supply. They also agreed that the data base to estimate these exposure levels for risk characterization was sorely deficient.

One type of surface which has been studied is carpeting which has been treated with flea control insecticides, such as chlorpyrifos. Researchers have found dislodgable residues exceeding the No Observed Effect Level (NOEL) by up to 5-fold which could be transferred from freshly-treated wet carpet to crawling infants (Fenske et al., 1990). Other studies on dried carpeting indicated that available residues were of a lesser magnitude with a relatively low transfer efficiency, yielding an 18-fold margin of safety to the single dose adult human NOEL (Vaccaro, 1993). Recent reports have indicated that soft toys have the potential to accumulate appreciable residues of insecticides and may serve as a reservoir (Gurunathan *et al.*, 1998).

B. Flea control

One area which does not seem to have been explored appreciably is the possibility that residues of flea control insecticides remaining on pet fur from flea control treatments could become a substantive source of pesticide exposure to children. Clearly, in order to be effective against fleas, the insecticide must have a residual effect on the animal for several days, weeks, or even longer. The insecticide residues, in many cases, are intended to remain on the surface of the animal in order to kill the pest. Any child who handles a pet treated for fleas could easily be exposed to insecticide residues which then could be absorbed through the child's skin or, as indicated above, could become an oral exposure from contaminated hands. There seems to be virtually no information available in the open literature on the magnitude of exposure which might be possible from contaminated pets. Our literature searches have identified food, water, occupational, and carpet exposures to insecticide (Fenske et al., 1990; Spear, 1991; Davis et al., 1992; NRC, 1993), but have yet to reveal accounts of exposure estimates from pets. It appears that the possibility of exposure to insecticides from pets

has been totally ignored until recently, even though an epidemiological study on households with children indicated substantial use of pesticides on pets when very young children are present (Davis et al., 1992). Part of the reason for this lack of information is probably the fact that animal subjects for such a study are not commonly available to most researchers.

We are currently exploring the possibility of contamination of children from pet dogs treated with flea control insecticides (EPA grant R825170). In this study we have investigated the dislodgable residues of two OP insecticide dips (active ingredients are chlorpyrifos and phosmet), and are currently investigating residues from a collar containing tetrachlorvinphos (TCVP) and will study a collar containing chlorpyrifos in the future. The protocol for all of these studies involves rubbing the back of treated dogs with cotton gloves and to quantitate by analytical chemistry the residues dislodged from the fur of dogs; the scope of the current project did not allow biomonitoring of the people in close contact with these pets. In this on-going study we chose to use a variety of dog sizes and fur types to yield a range of data useful in probabilistic predictions of exposure levels. These studies with the two dips have indicated that high levels of both insecticides are dislodgable shortly after the dogs have been treated with the dips and dried (4 hours after dipping), with average levels dislodged from a 10 inch long by 4 inch wide section along the back of the dog in a 5 min period of rubbing of 1 mg of chlorpyrifos (Boone *et al.*, 1998) and 3 mg of phosmet. These residues quickly dissipated to levels that were close to 0 by the time of the next prescribed dipping (according to label directions), 3 weeks for chlorpyrifos and 2 weeks for phosmet. The very earliest data coming from the recently initiated TCVP collar study indicates that at 4 hr after application about 15 mg is dislodged in rubbing for 5 minutes, and over 20 mg from a collar which has been on the dog for 3 days. While these numbers are from only about 12 samples and will be replicated further, they suggest a substantial source of concern. These data indicate a very likely source of intermittent exposures to pesticides to which children might be subject. Both the rapid dissipation of residues from high initial levels as well as the irregular levels of contact of the child with the pet contribute to the intermittent nature of the exposures. It is also very likely that a dog owner would use a flea control product on the dog and concurrently would treat the household with an insecticide (such as a carpet treatment) to try to eliminate all of the fleas at the same time. Therefore, multiple sources of exposure, as must be considered in cumulative risk assessments, are very likely to occur during flea remediation.

The possibility of pesticide exposure from contaminated pets is not a trivial concern. A recent demographic survey of companion animals by the American Veterinary Medical Association (Wise, 1992) indicated that approximately 34.6 million households in the United States owned a dog or dogs in 1991, a number essentially unchanged from 1987; this number represents 36.5% of all households. More households had dogs for pets than other types of animals. There was a mean of 1.52 dogs per dog-owning household, yielding a total estimate of 52.5 million dogs. Fifty percent of pet-owning households were parental households with children (compared to only 40% of the overall population). It was projected that there would be 53.6 million dogs in the USA in 1998 (Wise, 1994). Therefore, there seems to be an extremely large number of pet dogs living in households with children. These pets could be a source of exposure to children from flea control insecticides. If half of the 36.5% of the households which own dogs have children in them, then clearly almost one fifth of American households have children in contact with dogs, yielding a population of millions of children who could be in direct contact with flea control insecticides from dogs alone. There are also millions of cats and other pets or domestic animals, such as horses or cows, which are also be treated for insect pests, and which could serve as additional sources of

intermittent insecticide exposure to children. These pet-borne insecticide residues would then add to the residues already better documented, i.e., food, water, carpets, air, and lawns and might genuinely be among the highest sources of exposure, providing episodic exposure events whose magnitude are currently unknown.

Fleas are a constant and persistent problem for dogs throughout the country. Especially in the warmer regions, such as the South, West Coast and Southwest, fleas are present during most of the year, and pet owners must treat their animals continuously. Many flea control products, such as dips or spot treatments, are designed to leave insecticide residues on the fur so that they will continue to kill fleas for extended periods of time. Dips and spot treatments are applied to the animal's coat and are not rinsed off, thereby deliberately leaving an external residue. Collars are impregnated with insecticides; petting or hugging the dog's neck will yield direct contact with the insecticide-impregnated plastic. Fleas are a greater problem in warmer seasons when children will be wearing less clothing, thereby having less protection from contact with the insecticide residues. Thus, children are very likely to have direct dermal contact with the insecticides, a portion of which is likely to become an oral exposure.

C. Flea control insecticides

A widely used group of insecticides which are utilized for flea control, along with numerous other agricultural and domestic applications, are the OP insecticides. The mechanism of toxicity of the OP insecticides or their active metabolites is the inhibition of nervous system enzyme acetylcholinesterase (AChE). AChE is responsible for rapidly inactivating the widely distributed neurotransmitter acetylcholine. When AChE is inhibited, acetylcholine accumulates leading to hypercholinergic activity, and a variety of signs and symptoms related to both central and peripheral pathways. Some of the OP's are highly toxic. Two of the more moderately toxic OP's which are used for flea control are TCVP [(Z)-isomer of 2-chloro-1(2,4,5-trichlorophenyl)-vinyl dimethyl phosphate; Rabon®] and chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate; Dursban®). As expected, both of these insecticides have a very low dermal toxicity level. Chlorpyrifos is a Class II insecticide with rat oral and rabbit dermal LD_{50}'s of 96-270 mg/kg and 2000 mg/kg, respectively; TCVP is a Class III insecticide with rat oral and rabbit dermal LD_{50}'s of 4000-5000 mg/kg and $>$2500 mg/kg, respectively (Farm Chemicals Handbook, 1996). OP insecticides act via a common mechanism of toxicity (Mileson et al., 1998), and therefore must be considered together in cumulative risk assessment when concurrent exposure is anticipated, as mandated by the Food Quality Protection Act (FQPA, 1996).

Synthetic pyrethroids are also in use as flea control insecticides. Synthetic pyrethroids act by holding open neuronal sodium channels and thereby induce spontaneous action potentials and nervous system hyperexcitability. Permethrin [(3-phenoxyphenyl)methyl(I)*cis,trans*-dichloroethenyl-2,2-dimethylcyclopropane-carboxylate, approximately 60-75% trans and 25-40% cis isomers], a Type I pyrethroid, has a large number of approved uses, including several formulations of flea control products. It is a Class II / III insecticide with rat oral and rabbit dermal LD_{50}'s of 430-4000 mg/kg and $>$2000 mg/kg, respectively. Synthetic pyrethroids may also operate under a common mechanism of toxicity, and therefore may be subject to future cumulative risk assessments under FQPA. Both the synthetic pyrethroids and the OP's being neurotoxicants may be particularly damaging to the developing nervous system.

We are not aware of an existing data base for the potential exposure of infants or children (or even adults) who handle a dog previously treated with one of these insecticides for flea control, except the data we are currently generating on chlorpyrifos, phosmet, and TCVP. We have estimated

from dislodgable residues measured for a 5 min rubbing over a limited surface area of the dog that the amount of chlorpyrifos which might be transferred to a child over the course of a day when peak residues were present could be about 10-20 mg. A dermal absorption rate of 3% has been measured for chlorpyrifos absorption through adult human skin (Berteau *et al.*, 1989; Nolan *et al.*, 1984); this may well be a very conservative estimate of the absorption of a highly lipophilic compound, and especially conservative when projecting to children who will probably have relatively greater dermal absorption than adults. Nevertheless, at 3%, the absorbed dose would be 0.3-0.6 mg; in a 10 kg child, this absorbed dosage would be 0.03-0.06 mg/kg. Since the 21-day repeated exposure NOEL (based on blood cholinesterase inhibition in adult humans) is 0.03 mg/kg/day and the R_fD is 0.003 mg/kg/day (FAO/WHO, 1973; USEPA, 1988), the R_fD projected for a 10 kg child would be 0.03 mg/day without consideration of the additional 10X safety factor (i.e., the FQPA safety factor) to account for the vulnerability of infants and children; this value is at the low end of the range we estimated. The exposure to phosmet would be approaching 3 times that of chlorpyrifos because it is applied in a more concentrated form. However, it should be borne in mind that children have greater absorption and less detoxication than adults, so that the current R_fD may yield inadequate protection for children. Thus, it is entirely possible that children could readily obtain residues of insecticides from a dog treated with an insecticidal application which could be a health threatening risk. Our initial results on the TCVP collar are even more suggestive of a potential intermittent exposure of concern.

One could easily question whether flea control insecticides are genuinely a concern today because of the effectiveness and presumably greater safety of the new generation of flea remedies such as the insect growth regulators [e.g., lufenuron (Program®) or pyriproxyfen (Nylar®)] or the highly insect selective neonicotinoids [e.g., imidacloprid (Advantage®)]. However, these superior products are expensive and require a visit to the veterinarian for a prescription which is also expensive. Poor people (and probably many affluent people as well) are not going to utilize these prescription products, and will routinely use inexpensive products which are readily available in such commercial establishments as discount department stores. While the concept of environmental justice is more typically applied to residential locations of poor populations near chemical waste sites or industrial outflows, the concept is also valid here where poor populations are more likely to encounter flea control insecticides because of their low price and ready availability.

D. Specific aims and hypothesis

We in the Center for Environmental Health Sciences of the College of Veterinary Medicine at Mississippi State University feel that we are uniquely positioned to determine what residues could be obtained from contacting dogs treated with flea control insecticides. We have a long standing research interest in insecticide toxicology, and we have access to a large number of dogs together with cooperative owners, along with a well established analytical chemistry laboratory, and experience in the quantitation of dislodgable residues from pet fur. Therefore, we will be able to set up a controlled study to expand our current efforts into the following:

1. The level of residues which could be transferred to a human from a dog which had received treatment with a spot treatment or a collar for flea control;
2. The time course of dissipation of these residues following the initial treatment;
3. The correlation of the residues dislodged from the fur of dogs (from gloves) to the residues occurring in a tee shirt worn by a child in the household of the treated dog, along with urinary metabolites of the insecticide in the child and a parent of the same household.

We propose the following hypothesis: *The residues of insecticides available for intermittent transfer*

to children from the fur of dogs treated by either a spot treatment or a collar for flea control will be appreciable and of a magnitude necessitating inclusion in cumulative risk assessments of pesticides to children; secondly, that the fur rubbing procedure developed to quantify dislodgable residues provides a useful estimate of insecticide residues which could be transferred from the fur of dogs to children.

This hypothesis will be tested by studying 3 specific aims, each of which will involve a correlation of residues from the rubbing procedure with cotton gloves (the technique we are using at present), residues from tee shirts worn by a child in the household, and urinary metabolites of this child and an adult in the household:

1. To determine the exposure of children to permethrin resulting from residues from the fur of dogs from a permethrin spot treatment;
2. To determine the exposure of children to TCVP resulting from residues from the fur of dogs treated with a TCVP flea collar; and
3. To determine the exposure of children to chlorpyrifos resulting from residues from the fur of dogs treated with a chlorpyrifos flea collar.

2. Approach

A. Objectives

As indicated above, this project is designed to test the following hypothesis: *The residues of insecticides available for intermittent transfer to children from the fur of dogs treated by either a spot treatment or a collar for flea control will be appreciable and of a magnitude necessitating inclusion in cumulative risk assessments of pesticides to children; secondly, that the fur rubbing procedure developed to quantify dislodgable residues provides a useful estimate of insecticide residues which could be transferred from the fur of dogs to children.*

The experiments will be designed to answer the following three questions:

1. Specific Aim 1: How much residue of permethrin can be transferred from a dog recently treated with a spot treatment to a child, how quickly does this dislodgable residue dissipate, does this dislodgable residue increase with subsequent treatments and does the residue correlate with urinary metabolite levels?
2. Specific Aim 2: How much residue of TCVP can be transferred from a dog treated with a flea collar to a child, how quickly does this dislodgable residue dissipate, and does the residue correlate with urinary metabolite levels?
3. Specific Aim 3: How much residue of chlorpyrifos can be transferred from a dog recently treated with a flea collar to a child, how quickly does this dislodgable residue dissipate, and does the residue correlate with urinary metabolite levels?

To answer these questions, dogs will be treated with an over-the-counter insecticide spot treatment or collar according to package directions, and they will be subsequently rubbed in a standardized protocol to transfer dislodgable residues to cotton gloves at set intervals after the initiation of the treatment; in the case of the spot treatment, the treatment will be repeated two times at the reapplication interval specified by the package label. Children will be provided with a cotton tee shirt to wear during the afternoon and evening at the same sampling times as the glove samples. These gloves and tee shirts will subsequently be extracted with solvents for quantitation of insecticide residues by gas chromatography with electron capture detection. Urine samples from the child and from an adult in the household will be analyzed by gas chromatography/mass spectrometry for urinary metabolites at the same sampling times as the residue samples. Residue and urine samples will also be taken before the insecticide treatments to assess baseline levels. The protocols

and specific methods proposed are described below in Section D.

B. Rationale

Dogs were selected because they represent the most numerous pets in households, are commonly plagued with fleas throughout the country necessitating frequent use of flea control insecticides, and are very often petted and hugged by young children. Additional data generated on dogs will correlate with the data being obtained in the current project. We have proposed the use of a variety of breeds of dogs to yield estimates from natural populations which will be useful in probabilistic methods of exposure assessment.

The insecticides permethrin, TCVP, and chlorpyrifos were selected because they are readily available in a variety of over-the-counter formulations at many consumer outlets. The TCVP and chlorpyrifos are subjects of our current study, which does not include biomonitoring; these two compounds will be studied again to gain additional data to correlate with the biomonitoring estimates and to determine whether the glove procedure accurately reflects dislodgable residues. The on-going study will provide information on the most appropriate sampling times, such as peak levels, to include in the proposed studies which will be more limited in time points so that the scope can be expanded into biomonitoring. Over-the-counter insecticides were selected because they are inexpensive and will be widely used by a representative cross-section of the population, especially poor people who cannot afford veterinary care and prescription products. We have chosen not to study flea control shampoos because these are specifically designed to be washed out so as not to leave residual insecticide; we feel that the shampoos are not as critical a problem in risk assessment as are the products designed to leave a residue.

The glove procedure was developed in our on-going project as a means to assess dislodgable insecticide residues from fur; it is felt to be more protective of the individual doing the sampling than direct hand contact. However, the glove residues alone are not an index of absorption, and we do not have data yet to indicate whether these glove residues will be an accurate surrogate for the levels of dislodgable residues during normal pet contact. Therefore, the proposed project will expand the on-going project into two new measures, a tee shirt for dislodgable residues to the child, and urinary metabolites to give an indication of internal dose. The tee shirts are felt to cover an area of the child's body which is likely to be in close contact with the pet dog, and is a piece of clothing which the child would readily wear without embarassment in front of his/her peers and siblings. The urinary metabolites will give an indication of the exposure of the child and an adult to the insecticide. Pre-treatment samples will give a baseline of exposure obtained from other residential uses of the insecticide, such as from crack and crevice, carpet or outdoor applications. One potential bias in the urinary metabolite levels comes from the possibility that the environmental hydrolysis products could be absorbed and would appear in the urine as though they originated from insecticide exposure. These hydrolysis products would be the same in most cases as the biologically-generated metabolites assayed in the urine. This possibility does not seem to have been considered very seriously in most of the residential exposure studies, but may have represented an important source of some of the inexplicably high levels of *p*-nitrophenol observed in the residents of houses illegally treated with methyl parathion in Mississippi during the last several years (Grissom *et al.*, 1998). However, it would be impossible to quantify this possibility without extensive additional tests.

Therefore, the proposed project is intended to expand our current studies into biomonitoring to gain a better estimate of the absorption of the insecticide. The studies will also generate data from both a child and an adult in the same household to yield as assessment of the difference in exposure levels that may result from differences in the time and degree of contact of the child and the adult

with the pet dog. Children aged 4-10 years will be selected as old enough to be out of diapers and to yield reliable urine samples, but young enough to still represent a population at risk for neurodevelopmental toxicity. First morning voids will be collected as representative of a significantly long period (8-10 hr in children and 6-8 hr in adults) of urine formation. We will not be including blood cholinesterase inhibition measurements from the dogs treated with OP insecticides as an index of internal dose to the dog, as we are currently doing, since we feel that the current study is giving us sufficient information. We are also not proposing to assess human blood cholinesterase inhibition because cholinesterase is not expected to be significantly inhibited from these approved insecticide usages, and collection would be an uncomfortable invasive procedure, especially to the children. We are also not proposing to study children's activity patterns or their behavior around their dogs, as this lies outside the scope of this analytical project, and is currently being investigated by researchers at other institutions.

C. Design

The overall design of this project involves: the treatment of dogs with one of three over-the-counter flea control insecticide formulations (a permethrin spot treatment, a TCVP collar, or a chlorpyrifos collar); at selected times after treatment the sampling and quantitation of dislodgable insecticide residues from fur samples by rubbing with white cotton gloves (the same as the protocol we are employing at present); the sampling and quantitation by analytical chemistry of insecticide residues from a tee shirt worn by a child (age 4-10) in the household on the same day as the glove samples are taken; the sampling of urine for quantitation by analytical chemistry of urinary insecticide metabolites from the child and from one adult in the household on the day after the glove and tee shirt samples are taken; and the correlation of the insecticide and metabolite data. Pretreatment samples will be obtained to determine a baseline in the participating individuals from other sources of residential exposure such as carpet or home garden applications. Estimates of potential human exposures from pets will be calculated.

D. Experimental protocols and methods

1. Selection of canine test subjects

Dogs selected for this study will be owned by professional (DVM) or graduate students enrolled in the College of Veterinary Medicine, or staff/faculty members of Mississippi State University with a child aged 4-10 years in the household who routinely plays with this dog. Students or staff should be the most reliable group of owners (in contrast to the general public) in that they are accessible daily, their dogs can readily be treated and sampled when the students are in class or the staff members are at work, and as members of the academic community, the compliance and appreciation of the value of research should be high. Dogs participating in this study must be enrolled in the Small Animal Community Practice Health Maintenance Program, so that their health status and vaccination history are known (described also in Section 4,E, below).

Canine test subjects of mixed breeds must be healthy, not less than 10 lbs in weight or less than 4 months of age. Both sexes will be used, but pregnant or nursing females will not be included. Dogs will be characterized as having a thin coat (for example, pointers, beagles and labradors) or having a thick coat (for example, huskies, chows and collies). Subjects will not be used until at least 2 months have passed since the last treatment with the test insecticide.

While dogs are participating in this study, the owners will agree to not use any other products containing the test insecticide on this dog or other pets or in or around the household or products which could be metabolized to the same urinary metabolites. If the test insecticide is used for environmental insect control, such as in rental properties, the nature and dates of use of these will

be recorded. At the time of each sampling, the owner will be asked to supply any additional information about the dog's activities (such as swimming) or potential exposure to the test insecticide during the period since the last sampling which could affect residues. An informed consent form has been developed in the current project so that the owner agrees to the procedures proposed for the dog. Only one dog in any household will participate in this study at any given time.

As indicated below, dogs will have pretreatment samples for residues taken. Any dogs showing evidence of dermal residues of the test insecticide will be replaced in the study; this has not been necessary in the current study. Dogs will be observed during the study for signs of insecticide intoxication or any other health problem which would remove the dog from the study.

2. Selection of human test subjects

The human subjects will be residents in the same household as the canine test subject. The household must have a child (either sex) in the age range of 4-10 years who regularly plays with the dog, and an adult (probably a parent; either sex); both subjects must be willing to provide the samples required by the study protocol. The samples will be tee shirts from the child and first morning urine samples from the child and the adult. The age, sex, height and weight of both child and adult test subjects will be recorded, and the adult will be asked to give an estimate of the amount of time and degree of contact that the child and the adult had with the dog on the day of the tee shirt sample; these data will be available for later correlations with residue/metabolite data. A description of the protocol and an informed consent form will be developed, and the subjects will be assured of anonymity. These protocols and approval forms will be approved by the Institutional Review Board for Research on Human Subjects.

3. Glove sampling protocol

As has been used in our laboratories in the current project, white cotton gloves will be used for rubbing the dog in the standardized protocol. Samplers will be D.V.M. students. Samplers will not wear plastic gloves under the cotton gloves to prevent any possible contamination of the gloves with phthalate plasticizers. Samplers will wash their hands with detergent, rinse and dry them thoroughly before putting on the sampling gloves. The sampling time will be a continuous five minute period. For the dogs receiving the spot treatment (Specific Aim 1), rubbing will occur from the neck backwards along the midline toward the base of the tail in a region to include the spot treatment regions. For the dogs receiving the flea collar (Specific Aims 2 and 3), three samples will be taken in the following order to avoid cross contamination: 1) near the base of the tail; 2) around the neck with the collar removed; and 3) around the neck with the collar back in place. These three samples are designed to assess: 1) migration of the insecticide from the collar to distant areas of the body; 2) transfer of insecticide from the collar to the fur adjacent to the collar, and 3) from the collar itself, respectively. The weight and length of the dog (nose to base of tail) will be recorded for later estimation of surface area as will the breed, sex, age and thickness of coat. Dogs will be rubbed both with and against the hair coat. Firm pressure will be applied, but not so great as to cause discomfort to the dogs. (Depletion of insecticide by rubbing with the glove is negligible, as determined in the on-going experiment.) The samplers will be trained so that consistency in the sample collection is maintained among dogs and among samplers. Sample gloves will be placed into solvent-washed glass bottles for subsequent extraction. The particular schedule of obtaining glove samples is detailed below under the protocol description for each of the insecticides.

4. Tee shirt sampling protocol

The child subject will be supplied with a new clean (laundered but not solvent extracted) lightweight short sleeve white cotton tee shirt to wear at the times specified by the

protocols. The child will wear the tee shirt during the afternoon and evening of the sampling day for a 5 hour period. (While long sleeve shirts might give a better estimate of exposure, long sleeves would not be tolerable and could be a health risk because of the heat during 6- 8 months of the year in this region.) The child will not be instructed to alter his/her normal behavior with respect to the dog. At the end of the day, the tee shirt will be placed into a solvent-washed glass bottle for subsequent extraction. Prior to extraction, a square section, 6 inches on a side, of the chest area of the front of the shirt, will be cut out of the shirt and will be used for the extraction; this region is felt to be the most likely region of the shirt to contain residues from interacting with the dog.

5. Urine sampling protocol

Urine samples will be obtained from the child wearing the tee shirt and from one adult in the same household at the same times as the glove and tee shirt samples are obtained. First morning urine samples will be obtained, with the instructions to the test subjects to collect the entire void. These samples will be collected and brought to our laboratories on the day of collection, acidified with either hydrochloric acid or sulfuric acid (depending upon the extraction protocol for the individual metabolites to be assayed), and frozen. Samples will be thawed, pooled according to the schedule detailed below, mixed thoroughly, and a single sample will be drawn from the pooled sample for subsequent analysis.

6. Specific Aim 1 Protocol: Spot treatment with permethrin

On the day prior to treatment, dogs will be shampooed for 5 min with a non-insecticidal detergent shampoo, rinsed and allowed to dry overnight. On the day of treatment, the pretreatment fur sample will be taken. A permethrin spot treatment, Sergeant's X-Term PreTect®, which is 45.0% permethrin, will be used according to label instructions. A spot of insecticide formulation will be placed on the dorsal neck region of smaller dogs, or at the dorsal neck and tail regions of larger dogs, as specified by the label directions. Fur samples on gloves will be taken at 4 hours, and 3, 7, 14, and 21 days. On the 21st day, the dogs will be retreated and sampled in a similar manner. This will be repeated 1 more time for a total of 3 treatments over a 9 week period. No shampooing will be done during the experimental period. There will be 24 dogs. Dogs will be sampled 16 times: 1 pretreatment sample and 5 samples after each of the 3 treatments, for a total of 384 fur samples.

Tee shirt residues will be collected on the day after the above glove samples, except for the last sampling time before permethrin re-treatment; in these cases, the samples will be taken on the day before the retreatment. Therefore, samples will be taken at day -1 (pretreatment), and 1, 4, 8, 15, and 20 days. There will be 24 children. Therefore, 16 tee shirts will be worn by each child: 1 pretreatment sample and 5 samples after each of the 3 treatments, for a total of 384 tee shirt samples.

Urine samples will be collected by the test child and one adult in the household on the morning following the wearing of the tee shirt. A first morning void sample will be requested, and will be acidified and frozen on the day of collection. Because of the high cost of the urinary metabolite analysis, it was decided to pool samples from the same relative collection time so that the overall number of replications could be maximized. Therefore, samples will taken for pretreatment, and at 2, 5, 9, 16, and 21 days post-treatment. All 2 day samples will be pooled, all 5 day, etc. There will be 48 test subjects (24 children and 24 adults). Therefore, 16 urine samples will be taken by each individual: 1 pretreatment and 5 samples after each of the 3 treatments which will be pooled by comparable day to yield a total of 288 samples analyzed.

7. Specific Aims 2 and 3 Protocols: Flea collar treatments

The protocol will be the same for both of the flea collar treatments. Animals will be

shampooed prior to treatment. Hartz Control Ultimate Flea Collar® containing 14.55% TCVP (Specific Aim 2) or Lassie Flea and Tick Collar for Dogs® containing 8.0% chlorpyrifos will be attached around the neck of the dog loosely in accordance with label directions. Residue samples from rubbing the fur will be obtained on the day prior to treatment and at 3 days (or whatever peak time is identified by our current experiments), 2 months and 4 months. Because of the 4 month duration of this project, it is assumed that owners will desire to have the dogs bathed during this period. The dogs will be shampooed once per month with a non-insecticidal shampoo, with the 2 month shampooing occurring within 1 day following the 2 month fur sampling. This will allow two months for the insecticide to distribute through the fur again before the next sampling. The flea collar will be removed during the bath. Three fur samples will be taken on each sampling occasion: a) at the caudal end of the dog; b) with the collar removed, the fur will be sampled around the neck; and c) with the collar back in place, the neck fur will be sampled. Therefore, each dog will be sampled for residues on 4 occasions: pretreatment and 3 days (or peak day) , 2 months and 4 months post-treatment. One pretreatment residue sample will be taken along the back of the dog, and 3 samples on each of the 3 post-treatment samplings (caudal region, neck without collar, neck with collar), yielding 10 residue samples per dog. There will be 24 dogs, and therefore a total of 240 fur samples. (A less extensive set of samples will be taken with the collars because we are currently performing a more detailed time course study with these collars assaying glove residues, and are proposing here to spot check several important sampling times for correlation of glove residues, tee shirt residues and urinary metabolites.)

To gain a better idea of the range of residues which could be obtained from interacting with a pet dog, tee shirt residues will be collected on 7 consecutive days around the time of the above post-treatment fur samples. Therefore, samples will be taken once prior to treatment, and for 7 days at 1 week, 2 months and 4 months. There will be 24 children. Therefore, 22 tee shirts will be worn by each child: 1 pretreatment, and a set of 7 samples for each of the 3 sampling occasions, for a total of 528 tee shirt samples.

Urine samples will be collected by the test child and one adult in the household for 7 consecutive days near the occasion of the above samples. A first morning void sample will be requested, and will be acidified and frozen on the day of collection. Because of the high cost of the urinary metabolite analysis, it was decided to pool samples from each collection week so that the overall number of replications could be maximized. Therefore, samples will be taken once prior to treatment, and for 7 days at 1 week, 2 months and 4 months, and each week's samples will be pooled. There will be 48 test subjects (24 children and 24 adults). Therefore, 22 urine samples will be taken by each individual: 1 week of pretreatment samples and 3 weeks of post-treatment samples which will be pooled by week to yield a total of 192 samples analyzed for each insecticide.

8. Insecticide analysis

Cotton gloves will be used to sample for dislodgable residues of *cis*- and *trans*-permethrin, TCVP, or chlorpyrifos from the fur of the dogs. The gloves will be washed in soap and water, rinsed in water 3 times, and then undergo a soxhlet extraction using methylene chloride to remove any potential interfering compounds. The gloves will be stored in solvent-rinsed glass jars with Teflon lids to prevent contamination. The dogs will be rubbed as described earlier. After sampling, the gloves will be placed back into the glass jars. Each glove will be extracted with the solvent yielding the best recovery (hexane, acetone, or petroleum ether) in an Accelerated Solvent Extractor (ASE). An electron capture detector (for halides) will be used to quantify all three insecticides.

The percent recovery of the compounds from the gloves will be assessed by the addition of a known amount of *cis*- and *trans*-permethrin, TCVP, or chlorpyrifos to the gloves and by taking the gloves through the extraction and analysis procedure. Standards will be run for each extraction and will be from the high and low end of detection limits. The lower detection limits for TCVP and chlorpyrifos is 5 pg, and for *cis*- and *trans*-permethrin is 10 pg. We have found in our current experiments that the residues are well within the detection limits of our instruments even several weeks after treatment. All of these procedures are in place in our laboratories, and we are currently analyzing for TCVP and chlorpyrifos from gloves. Shirts will be processed the same as gloves but no pre-extraction will be performed; additional clean-up may be required by Florisil or C_{18} clean-up columns. These extractions and analyses are modifications of the methods of Zweig and Sherma (1977), Luke and Dahl (1976), and EPA Test Methods 8141A, 8081 and 3540 (EPA, 1993).

9. Urinary metabolite analysis

Urine samples will be analyzed for insecticide metabolites using standard methods by PTRL East, Inc., a contract analytical chemistry laboratory in Richmond, KY. PTRL has years of experience in analytical chemistry and has methods in place for quantitation of metabolites of all three of the selected insecticides. The metabolites of permethrin which will be quantified are *cis*- and *trans*-dichloroethenyl-2,2-dimethylcyclopropane-1-carboxylate (*cis*-Cl_2CA and *trans*-Cl_2CA) and 3-phenoxybenzoic acid (3-PBA). The conjugated permethrin metabolites will be released from urine by hydrolysis with sulfuric acid, and analytes will be isolated by solid phase extraction on a C_{18} column, and derivatized to their methyl esters. The analyte esters will be quantified by gas chromatography (GC) on a CP-SIL-8CB capillary column with detection by selected-ion monitoring mass spectrometry (SIMS) (Angerer and Ritter, 1997). The metabolites of TCVP will be 2-(2,4,5)-tetrachloroacetophenone and 1-(2,4,5-trichlorophenyl)ethanol whose conjugates will be released by HCl-hydrolysis. Analytes will be isolated on a C_{18} column, and will be derivatized with *N,O*-bis(trimethylsilyl)acetamide. These derivatives will be analyzed by GC on a DB-17 capillary column with detection by electron capture or SIMS. This method was developed by PTRL. The chlorpyrifos metabolite to be monitored will be 3,5,6-trichloropyridinol. The urine will be acidified and the analytes derivatized with *N*-(*t*-butyldimethylsilyl)-*N*-methyltrifluoro-acetamide. The analyte will be quantified by GC on a DB-1 capillary column with detection by SIMS (Bartels and Kastl, 1992).

All urine samples will be analyzed for creatinine concentration (Angerer and Ritter, 1997). The amount of metabolite will be adjusted to creatinine concentration as a means of standardization to account for variability in degree of concentration of the urine.

10. Data calculations and statistics

Residue data will be obtained for the surface area sampled and will be calculated projected to the estimated surface area of the dog (Bonagura, 1995) to obtain a total dislodgable residue present on an individual dog. Size, coat thickness, and sex of dog will also be recorded.

Data for each specific aim will be analyzed using general linear model analysis of variance (ANOVA) for a repeated measures design. The household will constitute the experimental unit; multiple observations at each time point will be summarized as the average concentration over the data collection interval and as the maximum concentration during the interval. The residuals from each ANOVA will be examined using frequency histograms and normal probability plots; the data will be transformed and reanalyzed if the normality assumption appears to be substantially violated. ANOVA will be performed using the SAS® procedure GLM (SAS Institute Inc., 1996). If significant effects are found, means will be separated using the Least Significant Difference Test. Time trends will be examined using orthogonal polynomial contrasts. If substantial and sustained

levels of pesticide are found, the orthogonal polynomials will be used to develop predictive equations. The biological importance of statistically significant differences will be assessed using confidence intervals (Braitman, 1991). Correlations among various measures of residue exposure will be calculated using the SAS® procedure CORR (SAS Institute Inc., 1996). All calculations will be performed using SAS® Version 6.11 (SAS Institute Inc.); all statistical tests will use the 0.05 level of significance.

In addition, the residue data will be used to estimate human exposure levels as done above in Section 1,C, and as described in Fenske *et al.* (1990). These exposure estimates will be based on the dislodgable residues projected to the surface area of the dog for several exposure periods, i.e., 5 min (which will be the experimental sampling period but which is considered too conservative an estimate of the time a child would be in contact with his/her pet dog) and also 30 min and 60 min (which are considered to be more realistic estimates of times of daily close contact with pet dogs). These will be compared to the levels of residues obtained on the section of tee shirt analyzed (extrapolated to the surface area of the child which would be in contact with the dog), and a correlation analysis will be performed to determine whether the glove can serve as a suitable surrogate for the clothing. The projected exposure data, calculated from the residues and an estimated absorption factor, will be compared to estimates of internal dose based on the urinary metabolite concentrations. These residue and metabolite dose calculations will be compared to the R_fD to determine whether the expected exposure levels are of potential risk and therefore should be placed into risk assessment calculations.

3. Expected Results or Benefits

The study proposed here, i.e., a determination of the residues of flea control insecticides on the fur of dogs which could be transferred to humans, will yield unique information to the field of exposure assessment which does not currently exist. No estimates are currently available for the likely amount of contamination which humans, either adults or children, would obtain from handling treated dogs. These residues are likely to be relatively high, more akin to an occupational exposure to a treated crop in an agricultural field than to the general consumer exposures of food residues. These exposures will also be episodic, occurring when the flea control insecticides are high from fresh products and when the contact of the child with the pet is intense. Thus, this information will be very useful in adapting the current risk assessments to greater protection of children (and adults as well) who live in households with pets. Since dogs are so frequently contacted by children, the opportunity for these exposures is extremely high. Without information about the potential for transfer of some of these residues to humans, risk assessments ignore what may well be a major, if not **the major**, source of pesticide exposure in children. Since fleas are such a ubiquitous problem, the frequency of application of these insecticides suggests that exposures will be chronic over the course of several months at least and possibly continuous all year, depending upon climate, yet intermittent in nature. The information generated here will give an indication of the extent to which these residues dissipate with time following treatment, and how much variability in the exposure levels occurs because of different patterns of contact between the child and the pet. The scope of this project includes absorption estimates for humans which could be correlated to other data on urinary metabolites in people exposed to insecticides in occupational and residential settings. We predict that the rubbing procedure we have been using in our current project will be a useful, and more easily obtained, surrogate for the actual exposure of a child to insecticide residues from treated pets. We predict that the data on the tee shirts will correlate with the urinary metabolites of the child to provide an estimate of exposure, and that the level of urinary metabolites will be generally higher

in the child than the adult in the household if the child has had closer contact with the dog than the adult does. New risk assessments including exposures from pet residues along with the food and water exposures and environmental exposures (such as carpeting) will assist in better protecting vulnerable children from potentially damaging levels of neurotoxic insecticides during the formative years of their nervous systems. These data on a range of types and sizes of dogs will yield a range of exposure estimates useful in probabilistic techniques for estimating insecticide levels in exposure assessment.

4. General Project Information

A. Incentives for dog owners

In order to provide incentives for the cooperative participation of the dog owners in this study, we propose to offer $100 equivalent of veterinary care provided by the Animal Health Center of the College of Veterinary Medicine for each dog participating in the study. In addition, the spot treatments, shampoos, and collars constituting the study will be provided to the owners without additional charge. An additional $150 cash incentive will also be given to each participating household because of the shirt and urine samples. We predict that these incentives will lead to more than an adequate number of households to participate in this study. We currently estimate that there will be at least 500 dogs owned by veterinary and graduate students and faculty and staff at the College of Veterinary Medicine at any given time, and many of these households would have a child of the appropriate age. In the unlikely event that insufficient numbers of households can be enrolled for the study, we will open the participation to students, faculty, and staff members of Mississippi State University outside the College of Veterinary Medicine; these individuals would be offered the same incentives.

B. Schedule

Year 1: Initial recruitment of subjects/owners, training of student help, verification of recoveries, and detection limits of the chemical analysis, initiation of Specific Aim 1.

Year 2: Completion of Specific Aim 1, data analysis, initiation, and completion of Specific Aim 2, data analysis.

Year 3: Initiation and completion of Specific Aim 3, data analysis, and exposure estimate calculations.

C. Role of investigators

Dr. Janice Chambers has extensive experience in the toxicology of organophosphorus insecticides, including neurotoxicology and metabolism, and is the Principal Investigator of the current project monitoring dislodgable fur residues. She will be the Principal Investigator, will coordinate all of the studies and data analysis, and will be responsible for project management and report and manuscript writing; she will devote a 20% effort. Dr. J. Scott Boone has experience in biochemical and chemical analyses, and is a Co-Investigator on the current project, coordinating sample collection and analytical chemistry. He is the Assistant Director of the Analytical Support and Food Safety Laboratory, which will do the analytical chemistry on the parent insecticides. He will coordinate the sample collection and manage the analytical chemistry samples. He will devote a 40% effort. Dr. John Tyler is a practicing veterinarian at the College of Veterinary Medicine, has expertise in dermatology, and extensive experience in the treatment of flea infestation with insecticides. He is a Co-Investigator on the current grant. He will be responsible for recruiting the households for participation, training the students for fur sampling, will oversee the treatment of dogs with insecticides and the fur sampling and the survey information, and will monitor the health of the dogs included in the study, especially for factors such as signs of insecticide toxicity which

would require removal of a dog from the study. He will devote a 10% effort.

D. Facilities

The College of Veterinary Medicine of Mississippi State University has all of the facilities required for the proposed study except for the quantitation of urinary metabolites. The Animal Health Center has facilities for treatment, holding the dogs when needed, and the fur sampling. The Analytical Support and Food Safety Laboratory (ASFSL) is well equipped for sample preparation and extraction, and for gas and high pressure liquid chromatography. The specific instruments to be used on this project are two Hewlett Packard gas chromatographs, model 5890 Series II, with electron capture detectors. Additionally, there are several analytical chemists with experience in pesticide residue analysis in the Mississippi State Chemical Laboratory on our campus who can serve as resource persons, if needed.

The urinary metabolites will be quantitated by PTRL, East, of Richmond, KY, a contract analytical chemistry laboratory with specific experience in isolation, clean-up, derivatization, and quantitation of the urinary metabolites of all three of the test insecticides. PTRL has 4 Hewlett Packard 5890 gas chromatography systems with electron capture detectors and autosamplers and a Hewlett Packard 5970 MSD with GC capillary interface and autosampler, which would specifically be used on this project.

E. Animals

Dogs participating in this study will be required to be enrolled in MSU's Small Animal Community Practice Health Maintenance Program whereby the dog's health will be known by physical examination and vaccination history. If the dog was not previously enrolled in the program, the cost of the initial physical will be borne within the $100 incentive offered to each participant. These protocols will be approved by MSU's Institutional Animal Care and Use Committee (IACUC) prior to initiation of the project. We do not anticipate that there will be any reservations by the IACUC since the insecticides will be over-the-counter and no procedures will cause distress to the animals. Our current protocol is approved under protocol number 96-062. Veterinary observation by Dr. Tyler will occur regularly on all participating dogs. It is felt that 24 replications will be required because of the diversity of animals in the study and the resultant predicted high variability.

F. Human subjects

The human subjects for this study will be one child aged 4-10 years (either sex) who routinely interacts with the test dog plus one adult (either sex) in the same household. An informed consent form will be developed which will explain the protocol and sampling requirements to the individuals. The adult will give consent for himself/herself. A parent or the legal guardian of the child will give consent for the child. The protocols do not require any invasive procedures and are not expected to cause any physical or mental distress to the individuals involved. The test subjects will be asked to continue their routine activities and to not modify their activities in any way. They will be asked to provide urine samples on a set schedule; we will give them written assurance that the urine sample will be used only for quantitation of insecticide urinary metabolites and will not be used for any other purposes, and that all data will remain anonymous. The child will be asked to wear a tee shirt for 5 hours of the days on the protocol's schedule. All procedures will be approved by MSU's Institutional Research Board for Research on Human Subjects before initiation of the project.

assent from the child

References

Angerer, J., and Ritter, A. (1997) Determination of metabolites of pyrethroids in human urine using solid-phase extraction and gas chromatography-mass spectrometry. *J. Chromatog.* 695:217-226.

Atterberry, T.T., Burnett, W.T., and Chambers, J.E. (1997) Age-related differences in parathion and chlorpyrifos toxicity in male rats: Target and nontarget esterase sensitivity and cytochrome P450-mediated metabolism. *Toxicol. Appl. Pharmacol.* 147:411-418.

Bartels, M.J., and Kastl, P.J. (1992) Analysis of 3,5,6-trichloropyridinol in human urine using negative-ion chemical ionization gas chromatography-mass spectrometry. *J Chromatog.* 575:69-74.

Benke, G.M., and Murphy, S.D. (1975) The influence of age on the toxicity and metabolism of methyl parathion and parathion in male and female rats. *Toxicol. Appl. Pharmacol.* 31:254-269.

Berteau, P.E., Knaak, J.B., and Mengle, D.C. (1989) Insecticide absorption from indoor surfaces: hazard assessment and regulatory requirements. In: Wang, R.G.M., Franklin, C.A., Honeycutt, R.C., Reinert, J.C. (eds.) *Biological Monitoring for Pesticide Exposure*, ACS Symposium Series 382. American Chemical Society, Washington, D.C.

Bonagura, J.D. (1995) *Kirks Current Veterinary Therapy*; XII: Small Animal Practice. W.B. Saunders Company, Philadelphia, PA.

Boone, J.S., Tyler, J.W., and Chambers, J.E. (1998) Dislodgable fur residues and plasma cholinesterase inhibition in dogs treated with flea control dip containing chlorpyrifos. *Toxicol. Sci.* 42:783.

Braitman, L.E. (1991) Confidence intervals assess both clinical significance and statistical significance. *Annals of Internal Medicine*. 114:515-517.

Brodeur, J., and DuBois, K.P. (1963) Comparison of acute toxicity of anticholinesterase insecticides to weanling and adult male rats. *Proc. Soc. Exp. Biol. Med.* 114:509-511.

Burnett, W.T. (1994) Age-related differences in esterase activities and organophosphate sensitivity in neonate and juvenile rats, M.S. thesis, Mississippi State University, Mississippi State, MS, 41 pp.

Davis, J.R., Brownson, R.C., and Garcia, R. (1992) Family pesticide use in the home, garden, orchard, and yard. *Arch. Environ. Contam. Toxicol.* 22:260-266.

Diggory, H.J.P., Landrigan, P.J., Latimer, K.P., Ellington, A.C., Kimbrough, R.D., Liddle, J.A., Cline, R.E., and Smrek, A.L. (1977) Fatal parathion poisoning caused by contamination of flour in international commerce. *Am. J. Epidemiology* 106:145-153.

Dorough, G.D. (1992) The effects of specific cytochrome P450 inhibitors on the metabolism of the organophosphorus insecticide parathion: An investigation into the age related toxicity exhibited by the organophosphorus insecticide parathion, Ph.D. dissertation, Mississippi State Univeristy,

Mississippi State, MS, 57 pp.

Eitzman, D.V., and Wolfson, S.L. (1967) Acute parathion poisonings in children. *Amer. Journ. Dietary Health* 114:397-400.

Environmental Protection Agency. (1993) *Test Methods for Evaluating Solid Waste, Physicial/Chemical Methods.* EPA 530/SW-846.

FAO/WHO: *1972 evaluations of some pesticide residues in foods.* (1973) WHO Pesticide Residue Series No. 2. World Health Organization, Geneva.

Farm Chemicals Handbook. (1996) *Pesticide Dictionary Farm Chemicals Handbook '96.* Meister Publishing Company, Willoughby, OH.

Fenske, R.A., Black, K.G., Elkner, K.P., Lee, C.L., Methner, M.M., and Soto, R. (1990) Potential exposure and health risks of infants following indoor residential pesticide applications. *Am. J. Public Health* 80:689-693.

Food Quality Protection Act (FQPA). (1996) Public Law No. 104-107.

Gagné, J., and Brodeur, J. (1972) Metabolic studies on the mechanisms of increased susceptibility of weanling rats to parathion. *Can. J. Physiol. Pharmacol.* 50:902-915.

Goldenthal, E.I. (1971) A complilation of LD_{50} values for newborn and adult animals. *Toxicol. Appl. Pharmacol.* 18:185-207.

Grissom, R.E., Akin, E., Susten, A.S., Brackin, B., and Stilman, T. (1998) Residential exposure to methyl parathion (MP). *Toxicol. Sci.* 42:44.

Gurunathan, S., Robson, M., Freeman, N., Buckley, B., Roy, A., Meyer, R., Bukowski, J., and Lioy, P.J. (1998) Accumulation of chlorpyrifos on residential surfaces and toys accessible to children. *Environ. Health Perspect.* 106:9-16.

Knaak, J.B., Yee, K., Ackerman, C.R., Zweig, G., and Wilson, B.W. (1984) Percutaneous absorption of triadimefon in the adult and young male and female rat. *Toxicol. Appl. Pharmacol.* 72:406-416.

Kupferberg, H.J., and Way, E.L. (1963) Pharmacologic basis for the increased sensitivity of the newborn rat to morphine. *J. Pharmacol. Exp. Ther.* 141:105-112.

Lee, C.C. (1966) Comparative pharmacologic responses to antihistamines in newborn and young rats. *Toxicol. Appl. Pharmacol.* 8:210-217.

Long, G.G., Scheidt, A.B., Everson, R.J., and Oehme, F.W. (1986) Age related susceptibility of newborn pigs to the cutaneous application of chlorpyrifos. *Vet. Hum. Toxicol.* 28:297-299.

Luke, B.G., and Dahl, C.J. (1976) Detection of *O,O*-diethyl-*O*-(2,5-dichlorophenyl) phosphorothioate and *O,O*-diethyl-*O*-(3,6-dichloro-2-pyridyl) phosphorothioate in meat fat. *J. Assoc. Off. Anal. Chem.* 59:1081-1087.

Mileson, B.E., J.E. Chambers, W.L. Chen, W. Dettbarn, M. Ehrich, A.T. Eldefrawi, D.W. Gaylor, K. Hamernik, E. Hodgson, A.G. Karczmar, S. Padilla, C.N. Pope, R.J. Richardson, D.R. Saunders, L.P. Sheets, L.G. Sultatos, and K.B. Wallace. (1998) Common mechanism of toxicity: A case study of organophosphorus pesticides. *Toxicol. Sci.* 41:8-20.

Mortenson, S.R., Cahanda, S.M., Hooper, M.J., and Padilla, S. (1996) Maturational differences in chlorpyrifos-oxonase activity may contribute to age-related sensitivity to chlorpyrifos. *J. Biochem. Toxicol.* 11:279-287.

Nolan, R.J., Rick, D.L., Freshour, N.L., and Saunders, J.H. (1984) Chlorpyrifos: pharmacokinetics in human volunteers. *Toxicol. Appl. Pharmacol.* 73:8-15.

NRC. (1993) *Pesticides in the diets of infants and children.* National Research Council, National Academy Press. Washington, D.C. 386 pp.

Pope, C.N., Chakraborti, T.K., Chapman, M.L., Farrar, J.D., and Arthun, D. (1991) Comparison or *in vivo* cholinesterase inhibition in neonatal and adult rats by three organophosphorothionate insecticides. *Toxicol.* 68:51-61.

SAS Institute. 1996. *SAS/STAT® Software: Changes and Enhancements through Release 6.11.* SAS Institute, Inc., Cary, NC, USA.

Shah, P.V., Fisher, H.L., Sumler, M.R., Monroe, R.J., Chernoff, N., and Hall, L.L. (1987) Comparison of the penetration of 14 pesticides through the skin of young and adult rats. *J. Toxicol. Environ. Health* 21:353-366.

Spear, R. (1991) *Handbook of Pesticide Toxicology*: General Principles, Chap. 6, "Recognized and Possible Exposure to Pesticides," Academic Press, Inc., New York, NY, pp. 245-274.

U.S. EPA. (1988) *Integrated risk information system data base (IRIS).* U.S. Environmental Protection Agency, Washington, DC.

Vaccaro, J.R. (1993) Risks associated with exposure to chlorpyrifos and chlorpyrifos formulation components. In: K.D. Racke and A.R. Leslie, eds. *Pesticides in urban environments: fate and significance.* American Chemical Society, Washington, D.C., ACS symposium series, 522.

Warren, M.C., Conrad, J.P., Jr., Bocian, J.J., and Hayes, M. (1963) Clothing borne epidemic: Organic phosphate poisonings in children. *J. Amer. Med. Assoc.* 184:226-268.

Wise, J.K. (1992) The information exchange, veterinary service market for companion animals, 1992; Part I: Companion animal ownership and demographics. *J. Amer. Vet. Med. Assoc.*

201(7):990-992.

Wise, J.K. (1994) The information exchange, dog and cat ownership, 1991-1998, *J. Amer. Vet. Med. Assoc.* 204(8):1166-1167.

Woody, R.C. (1984) The clinical spectrum of pediatric organophosphate intoxications. *Neurotoxicology* 5:75.

Zweig, G., and Sherma, J. (1977) *Analytical methods for pesticides and plant growth regualtors.* Volume VI, gas chromatographuc analysis. G. Zweig, ed. Academic Press, New York, NY. pp. 408-413.

Zwiener, R.J., and Ginsburg, C.M. (1988) Organophosphate and carbamate poisoning in infants and children. *Pediatrics* 81:121-126.