Supporting Statement B for:

**THE AGRICULTURAL HEALTH STUDY (AHS): A PROSPECTIVE COHORT STUDY**

**OF CANCER AND OTHER DISEASE AMONG MEN AND WOMEN**

**IN AGRICULTURE (NCI)**

**OMB Control Number: 0925-0406**

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Submitted by:

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**B. STATISTICAL METHODS**

**B.1 Respondent Universe and Sampling Methods**

Agricultural Health Study (AHS) cohort members considered eligible for contact for the phase III interview include all pesticide applicators who completed the Enrollment Questionnaire (and at least one other data collection form) and all spouses, who are alive, current residents of Iowa or North Carolina, cancer free at the time of the attempted phase III interview and have not yet been contacted during the initial phase III (2005-2011) time period. This is approximately 450 study subjects. There has been 80% response rates achieved for both phase I and phase II of the study. As of April 5, 2010 the response rate for phase III was 65 percent for the Computer Assisted Telephone Interviews (CATI) and a 69 percent response rate and completion of the buccal cell sampling. The respondent universe, interviews conducted to date, and remaining proposed data to be collected are outlined in Table B.1-1.

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| **TABLE B.1-1: Target Sample For Completion of Phase III** |
|  | Iowa | North Carolina | Total |
| **Private Applicators Enrolled** | **31,877** | **20,518** | **52,395** |
| Eligible for Phase III | 26,083 | 14,653 | 40,736 |
| Contacted during 2005-2010 | 26,083 | 14,521 | 39,501 |
| Phase III Private Applicator target population to complete (includes buccal cell sub-set) | 300 | 150 | 450 |
| **Spouses Enrolled** | **21,771** | **10,575** | **32,346** |
| Eligible for Phase III | 20,666 | 9,681 | 30,347 |
| Contacted during 2005-2010 | 20,666 | 9626 | 30,292 |
| Phase III Spouse target population to complete (includes buccal cell sub-set) | 0 | 0 | 0 |
| Total Enrolled | 53,648 | 31,093 | 89,658 |
| Total Phase III target population | 46,749 | 24,334 | 73,887 |
| Total Phase III Target to complete (includes buccal cell sub-set) | 300 | 150 | 450 |

For the new biomarker component of AHS, the Study of Biomarkers of Exposures and Effects in Agriculture (BEEA), cohort members will be eligible if they are: 1) male private pesticide applicators; 2) alive, cancer-free, currently residing in Iowa or North Carolina, and over 50 years of age at the time of initial contact for BEEA; and 3) have completed the phase I, II, and III interviews. Cohort members will be deemed ineligible for BEEA if they have a blood clotting disorder such as hemophilia, are unable to speak English, or are registered as a “no contact” with the AHS. We estimate that approximately 11,516 study subjects will be eligible to participate in BEEA. Over the five year study period we will enroll 1600 participants (1072 in Iowa, and 528 in North Carolina). Table B.1-2 represents the respondent universe and proposed data to be collected for BEEA for a three year period.

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| --- |
| **TABLE B.1-2: Target Sample For BEEA** |
|  | Iowa | North Carolina | Total |
| **Private Applicators Eligible for BEEA** | **7,229** | **4,287** | **11,516** |
| To be contacted for BEEA during Phase III | 1,930 | 950 | 2,880 |
| To be enrolled in BEEA during Phase III | 643 | 317 | 960 |

###### B.2 Procedures for the Collection of Information

Data collection for phase III began in the winter of 2003 and is anticipated to continue through until April 2013. To date, the interim response rate is approximately 65 percent but we anticipate this will increase once data collection is completed. This request is to complete the remaining phase III interviews and buccal collection and to include a new biomarker component (BEEA).

The cohort is divided into three groups; private applicators, spouses of private applicators, and commercial applicators. Each year, private applicators will be targeted for contact in the winter, spouses of private applicators in the summer, and the commercial applicators in the fall. While private applicators and spouses come from both states, commercial applicators were recruited only in Iowa.

A few weeks prior to initiating telephone contact, for phase III each eligible cohort member who has not yet been interviewed and/or asked for buccal cell specimen (N=450) shall be sent an introductory letter describing the study’s purpose and sponsors, and the volunteer nature of participation (Attachment 10A, 10B, or 10C). The letter reminds participants of the on-going nature of the AHS, the importance of their continued participation, and describes the planned follow-up call. Interviews are conducted by trained interviewers, and are scheduled for times that are convenient for the respondents. Up to six attempts are made to contact each respondent, and callbacks will be made at the respondent’s request.

Since the interview is conducted over the telephone, a prepared script is used to introduce the interview and gain verbal consent (Attachment 3A or 3B). At the Iowa field station, quality control is performed by tape recording the telephone interviews, a percentage of which are then reviewed by the interviewer supervisors. The North Carolina field station does not have the capability to record interviews. Instead, supervisors directly listen to a portion of the interviews in order to monitor interviewer performance. These activities (and differences) are acknowledged in the introductory telephone script. The interview is conducted by a trained interviewer at a time that is convenient for the respondent.

After administration of the CATI, selected participants (N=450) are asked if they would be willing to provide a saliva (e.g. buccal cell) sample (Attachment 4A, 4B, or 4C). The purpose of the buccal cell collection is to collect samples from highly exposed study subjects in order to have enough statistical power to evaluate gene-environment interactions. Participants will be selected based on their cancer status. Those who agree are mailed a buccal cell collection kit which includes a cover letter (Attachment 15A or 15E), informed consent form (Attachment 14A or 14B), instructions for collecting the sample (Attachment 15B or 15F), and questions and answers regarding the buccal cell collection (Attachments 15C,15D or 15G). The collection protocol involves the use of a simple mouth rinse: a kit containing a small bottle of mouthwash and a 15 ml container. Participants are asked to pour 10 ml of the mouthwash into a cup, rinse the mouth for approximately one minute, expectorate back into the cup, seal it with the enclosed cap, and return the cup using an enclosed shipping mailer that contains an absorbent pad. The shipping mailer is pre-stamped and addressed for convenience. Each respondent who has not returned the buccal cell sample within 4 weeks of mailing the kit receives a reminder telephone call (Attachment 16A or 16B).

Finally, some phase III participants receive a letter (Attachments 13A and 13C), and telephone contact (Attachment 4B or 4C) to request the buccal sample separately from their phase III interview contact.

For the proposed biomarker component of the study, BEEA, we will ask another subset of AHS participants to complete an in-person interview at their home (Attachment 19), and provide blood and urine specimens. Over the five year study period we will enroll 1600 participants (1072 in Iowa, and 528 in North Carolina); however, the numbers referenced here apply to the respondent universe for a three-year period. Most BEEA participants (N=935) will be asked to complete one home visit at a time that is convenient for them. A smaller sample of 30 subjects, identified during the telephone screener (Attachment 20B) according to their reported plans to use diazinon in the coming year, will be asked to complete a total of three home visits.

A sample of approximately 2,880 individuals enrolled in the AHS over the age of 50 will be mailed an introductory letter (Attachments 21A and 21B) and study brochure or fact sheet (Attachments 21C-21F) explaining the study and indicating that a follow-up call will occur later. During the follow-up call, the interviewer will verify that the subject received the introductory letter, address subjects’ questions, determine eligibility via a short questionnaire (including eligibility for a blood draw) (Attachment 20A or 20 B), assess interest in participation, obtain verbal consent, provide additional instructions, and schedule the home visit. Additionally, all of the AHS participants who are contacted by phone (including those who decline to participate in the entire BEEA Study or are ineligible) will be asked for permission to collect some information about their cancer screening practices. If they verbally consent, we will ask three questions regarding their history of cancer screening tests, including PSA testing, digital rectal exams, and colonoscopies and sigmoidoscopies. The follow-up call will take approximately 10 minutes to administer.

Four weeks prior to the scheduled home visit, subjects who verbally consent to participate will receive a mailing that includes copies of the consent forms (Attachments 22A- 22D), the home visit confirmation letter (Attachments 23A or 23B), a reminder card for key elements of the questionnaire (particularly recent pesticide use) (Attachments 23C or 23D), and the urine collection kit (Attachments 23E or 23F).

Letters with the scheduled visit date and phone numbers for the study team (toll-free number) and the phlebotomist (cell phone number) will be mailed to all subjects (Attachment 24A and 24B). Finally, one to two days before the scheduled home visit, all scheduled subjects will receive a reminder call from the phlebotomist to confirm the date and time, reiterate that questionnaire item responses will be needed, and remind subjects about the morning void urine collection (Attachment 24C).

For 50 subjects in the Recently Exposed group (i.e., those recently exposed to diazinon), this call will also serve to verify that the subject has recently mixed, loaded, or applied diazinon (Attachments 25A-25D). The purpose of this aspect of the study will be to determine if recent exposure to diazinon (previously associated with leukemia in the AHS cohort) produces hematologic changes [i.e., alterations in peripheral blood cell counts measured in a complete blood count (CBC) and CD4+ T-cells or other lymphocyte subsets]. Serial measurements will be obtained before and 1-day after exposure and 21 days after exposure to diazinon.

For each home visit, the phlebotomist will travel to the subject’s home to: 1) recheck eligibility, 2) review the informed consent form obtain signed consents for the interview and biospecimen collection, 3) administer a questionnaire via a computer-assisted personal interview (CAPI), 4) recheck to be sure the subject does not have an inherited blood clotting problem, 5) collect the blood samples, and 6) collect the urine sample. In order to validate residential information in the AHS cohort and facilitate future environmental studies through linkage to existing data sources, the phlebotomist will use a handheld Global Positioning System (GPS) receiver to record the exact location of the home of each study participant at the time of the home visit. Each home visit will take approximately 90 minutes of the participant’s time.

The phlebotomist will collect one signed copy and ask the subject to retain the other signed copy for his records. If the subject agrees to the interview, the phlebotomist will administer the CAPI, which will be audio recorded with the subject’s consent. The CAPI will elicit information about recent medication use, medical conditions, smoking status, alcohol consumption, and pesticide use during the current/previous growing season. We will ask about the duration of pesticide use (number of days and hours per day), dates of recent applications, the product formulation (liquid or other), handling activities and method of application, and use of personal protective equipment.

If the subject agrees to the blood sample, the phlebotomist will collect a 44.0-mL blood sample by venipuncture using all standard procedures for safety. The following blood samples will be collected from all participants: one 10.0-mL serum tube, one 6.0-mL heparin tube, one 6.0-mL EDTA tube, two 8.5-mL acid citrate dextrose (ACD) tubes, and two 2.5-mL PAXgene tubes. For the subjects in the Recently Exposed group, these tubes will be collected at the pre-season home visit; an additional 6.0-mL EDTA tube will also be collected from Recently Exposed subjects for the hematologic assays (i.e., CBC, lymphocyte subset measures). This aspect of the study will take approximately 20 minutes of the respondent’s time.

For the subsequent post-exposure home visits among subjects in the Recently Exposed group, the following blood samples will be collected: two 6.0-mL EDTA tubes (one for the repository and one for the hematologic assays), two 8.5-mL ACD tubes, and one 2.5-mL PAXgene tube.

For all participants, a 1.0-L urine collection kit will be sent to the home prior to the phlebotomy visit with instructions for collecting the first morning void on the day of the phlebotomy visit. The urine sample will be collected to quantify and validate pesticide exposures in a separately funded effort and to provide biospecimens for emerging analytical technologies. The subject will be asked to collect the entire void volume, and to record the time of sample collection and the previous void time. Also, we will request that the subject refrigerates the sample and gives it to the phlebotomist at the scheduled visit. Participants in the Recently Exposed group will be asked to provide a urine sample for each scheduled visit.

If the subject forgot to collect the urine sample, the phlebotomist will make sure that the subject has the necessary supplies, request that he collect the urine sample the following morning, and make sure that he understands all that is required of him to collect and ship the sample with the shipping materials provided.

###### B.3 Methods to Maximize Response Rates and Deal with Nonresponse

The overall response rate for eligible cohort members using the CATI techniques was 80.1 % in Phase II, and to date the response rate for phase III was 65 percent for the Computer Assisted Telephone Interviews (CATI) and a 69 percent response rate and completion of the buccal cell sampling. At least six attempts shall be made to contact each eligible respondent. Changes in telephone numbers will be tracked and an additional six attempts will be made for each new number. Interviewers are trained in refusal conversion techniques. Refusals, no contacts, deceased, etc., will also be tracked.

The CATI tool itself has been programmed for ease of administration and efficiency. Certain information about each respondent will be pre-programmed into the CATI system. So it will be available to the interviewers at the appropriate sections of the interview. The rate of participation by the cohort has been excellent to date and the ongoing AHS has achieved a high level of acceptance in the agricultural community, which we hope to continue through to the end of data collection in phase III.

To date, approximately 35,978 participants have supplied buccal cells. As had been done previously each eligible respondent who returns a buccal cell sample shall receive $5.00 as reimbursement for the time spent providing the sample. This provides an incentive and maximizes the response rates. An additional 1,000 participants during the extension of phase III of the study have been selected to participate in the buccal cell collection. The participants targeted for the buccal cell collection will be those found to have selected cancers such as prostate cancer and non-Hodgkin lymphoma. In order for us to adequately examine gene-environment interactions in relationship to these selected cancers, we need to collect a relatively larger number of samples. To ensure compliance and take into account mishaps with the buccal cell collection, there are a number of additional scripts and letters that have been developed by the Iowa Field Station. The scripts include a script in which the buccal cell kit arrived without a signed consent form (Attachment 17A) and a script to re-contact those in which the buccal cells were either missing or arrived damaged (Attachment 18A). The additional letters include a pre-telephone letter for those who have been chosen to participate in the buccal cell collection (Attachment 13A for respondents with prostate cancer, and Attachment 13C for respondents with NHL), a cover letter and buccal cell kit for respondents who have been chosen but who have language difficulties and will not be contacted initially by telephone (Attachment 13B for respondents with prostate cancer, and Attachment 13D for respondents with NHL), a re-mailed consent form cover letter (Attachment 17B), and a re-mailed buccal cell kit cover letter (Attachment 18B).

For the BEEA component, based on experience from prior substudies with the AHS cohort we anticipate that approximately one-third of the potentially eligible participants who are contacted by phone will participate in the home visits. To ensure compliance with urine collection and other pre-visit preparations, a number of contacts using scripts and letters are built into the schedule prior to each home visit. As noted in section B.2, letters with the scheduled visit date and phone numbers for the study team (toll-free number) will be mailed to all subjects (Attachment 24A and 24B). Additionally, one or two days before the scheduled home visit, all scheduled subjects will receive a reminder call from the phlebotomist to confirm the date and time, reiterate that questionnaire item responses will be needed, and remind subjects about the morning void urine collection (Attachment 24C). The participants in the Recently Exposed group will receive multiple additional contacts by letter and phone after their first visit that will serve to pinpoint the timing of planned diazinon application and help with scheduling their second and third home visits (Attachments 25A-25D).

Finally, participants will receive $75 for completing each home visit as reimbursement for time spent in this study. Personal checks will be cut prior to the scheduled visits, so that reimbursement can be immediately provided as the phlebotomist completes the home visit. The number of cohort members who refuse to participate in BEEA or are determined not to be eligible will also be tracked.

###### B.4 Test of Procedures or Methods to be Undertaken

Phase II CATI methods, which proved to be very successful, are being employed again to continue and complete data collection of phase III in the AHS. For the BEEA Study, all procedures for contacting potentially eligible cohort members and conducting home visits are being pilot tested. Nine pilot home visits in both Iowa and North Carolina will be conducted. The BEEA CATI and CAPI both will be validated and evaluated concerning time needed for and ease of administration, and data processing. The urine collection supplies and directions likewise will be evaluated for participant acceptance and ease of use.

**B.5 Individuals Consulted on Statistical Aspects and Individuals Collecting and/or**

 **Analyzing Data**

The Biostatistics Branch of the NCI Division of Cancer Epidemiology and Genetics has a staff of biostatisticians who are experts in this type of study. Dr. Jay Lubin, Senior Investigator, (301/496-3356), a member of this branch, is a study investigator.

The Field Stations in Iowa and North Carolina will be responsible for data collection activities under this protocol:

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Department of Preventive Medicine

Iowa City, IA

(319/335-9633)

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Charles Knott, MPA, Director

The National Cancer Institute is responsible for overall study management and coordination and the analysis of the cancer endpoint data and the National Institute for Environmental Health Sciences is responsible for analysis of the non-cancer outcomes. Federal scientific investigators conducting data analysis include:

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Gabriella Andreotti, Staff Fellow, National Cancer Institute (301/496-9093)

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