

Supporting Statement B for:

**THE AGRICULTURAL HEALTH STUDY (AHS): A PROSPECTIVE COHORT STUDY
OF CANCER AND OTHER DISEASE AMONG MEN AND WOMEN
IN AGRICULTURE (NIEHS)**

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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 IV interview include all pesticide applicators who completed the Enrollment Questionnaire (and at least one other data collection form), all spouses, and proxy for those cohort members unable to complete the follow up. This is approximately 74,000 study subjects. There has been 80% response rates achieved for both phases I and phase II of the study. The response rate for phase III was 63% for the Computer Assisted Telephone Interviews (CATI) and a 57% response rate and completion of the buccal cell sampling. The respondent universe for phase IV follow up and data to be collected is outlined in Table B.1-1. Cohort members or their proxy, who were eligible for Phase III follow up, are eligible for the Phase IV follow up.

	Total
Private Applicators Enrolled	52,395
Eligible for Phase IV	41,565
Spouses Enrolled	32,346
Eligible for Phase IV	30,603
Proxy eligible for Phase IV – known deceased	1,905
Total Phase IV target population	74,073

For the biomarker component of AHS, the Study of Biomarkers of Exposures and Effects in Agriculture (BEEA), cohort members are 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. It is estimated that approximately 11,516 study subjects will be eligible to participate in BEEA. Over the five year study period, a total of 1600 participants (1072 in Iowa, and 528 in

North Carolina) will be enrolled. Table B.1-2 represents the respondent universe and remaining data to be collected for BEEA.

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 IV	1700	1200	2900
To be enrolled in BEEA during Phase IV	743	507	1250

Table B.1-3 represents the respondent universe and remaining data to be collected for the buccal cell collection.

TABLE B.1-3: Target Sample For Buccal			
	Iowa	North Carolina	Total
Applicators Eligible for Buccal	3500	1750	5250
To be contacted for Buccal during Phase IV	3500	1750	5250
To be enrolled in Buccal during Phase IV	2100	1050	3150

B.2 Procedures for the Collection of Information

Data collection for phase IV will begin in the winter of 2013 and is anticipated to continue through until December 2015. This request is to complete the phase IV interviews, buccal collection and biomarker component (BEEA).

The cohort is divided into three groups; private applicators, spouses of private applicators, and commercial applicators. Commercial applicators will not be contacted for phase IV interview follow up.

Currently, the plan is to contact AHS cohort members to complete the phase IV follow-up interview allowing one of three modes of response: self-administered paper questionnaire, self-administered web survey (CAWI), and interviewer-administered telephone interview (CATI) (Attachment 25 and 26). The cohort will be sent an introductory letter describing the

purpose of the follow up and the volunteer nature of participation (**Attachment 24**). A second letter will be sent to those who do not respond encouraging their participation (**Attachment 24**). If neither mailing results in participation, then attempts will be made to complete the follow up by phone. Interviews are conducted by trained interviewers, and are scheduled for times that are convenient for the respondents, and callbacks will be made at the respondent's request. Proxy interviews for those cohort members unable to complete the follow up will be completed by using one of the three methods as well. For the questionnaires administered by telephone, informed consent will be documented verbally. Completion of the CAWI or return of the paper/pen questionnaire will document informed consent for those who choose to complete the follow up in either of these modes.

For buccal cell collection, each eligible cohort member who has not yet been asked for buccal cell specimen (N=5250) shall be sent an introductory letter describing the study's purpose and sponsors, and the volunteer nature of participation (**Attachment 14**). 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 were selected based on their cancer status. Those who agree are mailed a buccal cell collection kit which includes a cover letter (**Attachment 16**), informed consent form (**Attachment 15**), instructions for collecting the sample (**Attachment 16**), and questions and answers regarding the buccal cell collection (**Attachment 16**). 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 9**).

For the biomarker component of the study, BEEA, another subset of AHS participants will be asked to complete an in-person interview at their home (**Attachment 19**), and provide blood and urine specimens. **During Phase IV, 1250 participants (743 in Iowa and 507 in North Carolina) will be enrolled.** 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 10**) 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 17**) and study brochure or fact sheet (**Attachment 11**) 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 20 or 21**), 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, three questions will be asked regarding their history of cancer screening tests, including PSA testing, digital rectal exams, and colonoscopies and sigmoidoscopies. The follow-up call will take approximately 5 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 (**Attachment 18**), the

home visit confirmation letter (**Attachment 17**), a reminder card for key elements of the questionnaire (particularly recent pesticide use) (**Attachment 17**), and the urine collection kit (**Attachment 17**).

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 17**). 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 10**).

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 (**Attachment 10**). 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. Questions regarding 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 will be asked.

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, participants are requested to refrigerate the sample and give 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 68% in Phase II 63% for the Computer Assisted Telephone Interviews (CATI) in Phase III and 57% rate for completion of the buccal cell sampling. At least six attempts shall be made to contact each eligible respondent for phase IV. 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 CAWI, paper/pen and CATI tools have been developed for ease of administration and efficiency. Certain information about each respondent will be pre-programmed into the CAWI and CATI systems. Invitation mailings to the cohort will be sent to the spouse and

applicator at the same time. This strategy is being used because spouse response tends to be higher, and it is anticipated the spouse to encourage the husband's participation if both are received at the same time. 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 is hoped to continue through to the end of data collection in phase IV.

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 an incentive 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 were selected to participate in the buccal cell collection. The participants targeted for the buccal cell collection are 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, a relatively larger number of samples need to be collected. 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 15**) and a script to re-contact those in which the buccal cells were either missing or arrived damaged (**Attachment 9**). The additional letters include a pre-telephone letter for those who have been chosen to participate in the buccal cell collection (**Attachment 14**), 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 9**), a re-mailed consent form cover letter (**Attachment 16**), and a re-mailed buccal cell kit cover letter (**Attachment 16**).

For the BEEA component, based on experience from prior substudies with the AHS cohort it is anticipated 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 17**). 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 17**). 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 10 and 17**).

Finally, participants will receive \$75 for completing each home visit as an incentive 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

The phase IV data will be collected by using one of three methods of the cohort member's choosing: self-administered computer assisted web survey (CAWI); self-administered paper-and-pen (Paper/pen); or an interviewer administered computer assisted telephone

interview (CATI). Proxy interviews for those cohort members unable to complete the follow up will be completed by using one of the three methods as well. For the BEEA Study, all procedures for contacting potentially eligible cohort members and conducting home visits were pilot tested and are currently being used. The BEEA CATI and CAPI both were validated and evaluated concerning time needed for and ease of administration, and data processing. The urine collection supplies and directions were 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 NIEHS has a staff of biostatisticians who are experts in this type of study. Dr. David Umbach, Staff Scientist, a member of this branch, is a study investigator.

The University of Iowa will be responsible for data collection activities for buccal cell collection and the BEEA study in IA and Westat (the AHS Coordinating Center) will be responsible for these activities in NC. Social & Scientific Systems, Inc. will be responsible for data collection activities for the phase IV follow up interview under this protocol:

University of Iowa
Department of Preventive Medicine
Iowa City, IA
(319/335-9633)
Charles Lynch, MD, Ph.D., Director

Westat, Inc.
Rockville, Maryland
(301/507-8087)
Marsha Dunn, AHS Coordinating Center Director

Social & Scientific Systems, Inc.
Durham, North Carolina
(919/287-4335)
Pamela Schwingl, Ph.D., Research Director

Currently, National Institute of Environmental Health Sciences (NIEHS) will be taking the lead role in collecting information for this phase of the study. NCI is responsible for the analysis of the cancer endpoint data and the NIEHS is responsible for analysis of the non-cancer outcomes. All data collected by the study are being shared jointly between NCI and NIEHS and this relationship will continue. Additionally, the co-investigators from each Institute share the Contracting Officer's Representatives (COR) responsibilities. NCI will continue to play a lead role through membership on the Agricultural Health Study Executive Committee and in actively evaluating the links between occupational and environmental exposures and cancer. Federal scientific investigators conducting data analysis include:

Michael Alavanja, Senior Investigator, National Cancer Institute, (301/435-4720)

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

Donna Baird, Senior Investigator, National Institute of Environmental Health Sciences, (919/541-2786)

Laura Beane Freeman, Investigator, National Cancer Institute, (301/496-9093)

Aaron Blair, Scientist Emeritus, National Cancer Institute, (301/496-9094)

Neil Caparaso, Senior Investigator, National Cancer Institute (301/496-4377)

Cynthia Hines, Industrial Hygienist, National Institute for Occupational Safety and Health, (513/841-4453)

Jonathan Hofmann, Staff Fellow, National Cancer Institute, (301/402-2605)

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