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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LIST OF ATTACHMENTS
***Indicates a form/instrument/script/consent form**

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.

TABLE B.1-1 Target Sample for Phase IV

	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 eight year study period, a total of 2200 participants (1440 in Iowa, and 760

in North Carolina) will be enrolled. This includes increasing the overall AHS target sample for BEEA by 400 respondents, and adding the new control group. The controls will be identified from state voter registries and will be frequency-matched to BEEA participants on age, race, and state and county of residence; a total of 200 controls (100 in Iowa and 100 in North Carolina) will be enrolled. Table B.1-2 represents the AHS respondent universe and remaining data to be collected for BEEA in Phase IV.

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	**3300**	**1700**	**5000**
To be enrolled in BEEA during Phase IV	**1080**	**570**	**1650**

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 2018. This request is to complete the phase IV interviews, buccal collection and the biomarker component (BEEA), including the new BEEA control respondent group, buccal cell and air monitoring sample collection, and smartphone application (app) component.

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 (**Attachment 25.3**), self-administered web survey (CAWI)(**Attachment 25.1**), and interviewer-administered telephone interview (CATI) (**Attachment 25.2**). 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 **(Attachment 26.1, 26.2, or 26.3)**. For the questionnaires administered by telephone, informed consent will 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. To reiterate, participants or their proxy have the option to respond by web and receive the CAWI survey (Attachments 25.1 or 26.1, respectively); by phone and receive the CATI script (Attachments 25.2 or 26.2, respectively); or by mail and receive the paper and pen survey (Attachment 25.3 or 26.3, respectively). As needed, a standardized set of answers to frequently asked questions has been developed for the phase IV follow-up interview (Attachment 27).

Note that for many of the buccal and BEEA study materials described here, two versions exist: one for the Iowa cohort and one for the North Carolina cohort. The two versions provide different study center and IRB contact information, but otherwise are identical.

For buccal cell collection, each eligible cohort member who has not yet been asked for buccal cell specimen shall be sent an introductory letter describing the study's purpose and sponsors, and the volunteer nature of participation (**Attachment 14.1 or 14.2**). 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 16.1 or 16.4**), informed consent form (**Attachment 15.1 or 15.2**), instructions for collecting the sample (**Attachment 16.2 or 16.5**), and a list of standardized answers to frequently asked questions regarding the buccal cell collection (**Attachment 16.7**). 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. As needed, the subset of respondents who do not returned the buccal cell sample within 4 weeks of mailing the kit receives a reminder telephone call (**Attachment 9.2 or 9.3**); also as needed, respondents may be called to follow-up a missing consent form (**Attachment 9.4**) or damaged or missing sample (**Attachment 9.5**).

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 19.1**), and provide

blood, urine, buccal cell, and vacuum dust specimens. Together with this subset of AHS participants we now plan to enroll a small group of non-AHS controls, selected from lists of registered voters in Iowa and North Carolina and screened to ensure they have not worked in farming. Most BEEA participants, including the new control group will be asked to complete one home visit at a time that is convenient for them (**Attachment 20.1 or Attachment 20.4**). A smaller sample of subjects, identified during the telephone screener (A**ttachment 20.2, or Attachment 20.3**) according to their reported farming activities and potential exposure to selected pesticides or other agricultural exposures, will be asked to complete a total of three home visits, or two home visits and up to four separate air monitoring sample collection visits. Individuals will be mailed an introductory letter (**Attachment 17.1, 17.2, 17.14 or 17.15**) and study brochure (**Attachment 11.1, 11.2, or 11.3**) 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 20.1, 20.2, 20.3, or 20.4**), 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. Only controls who verbally consent to participate during the short questionnaire will be administered the cancer screening questions.

Prior to the scheduled home visit, subjects who verbally consent to participate will receive a mailing that includes copies of the consent forms (**Attachment 18.1, 18.2, 18.3, 18.4, 18.5, 18.6, 18.9, 18.10, or 18.11**), the home visit mailing cover letter (**Attachment 17.3, 17.4, 17.5, or 17.6**), a reminder card (show card) for key elements of the questionnaire (particularly recent pesticide use) (**Attachment 17.7, 17.8, 17.16, or 17.17**), a paper-and-pen Dust Specimen questionnaire (**Attachment 21**), and the urine collection kit and directions (**Attachment 17.9 or 17.10**). Subjects that agree to the air monitoring visits will receive consent forms for the air monitoring visits **(Attachment 18.7 or 18.8)** as well as the home visits. As needed, potential participants whose telephone numbers do not work can be sent a letter asking them to contact the study (**Attachment 17.12, 17.13, 17.18, or 17.19**).

The confirmation letters will include the scheduled visit date and phone numbers for the study team (toll-free number) and the phlebotomist (cell phone number). Subjects that agree to the air monitoring visits will receive a letter that confirms the first scheduled air monitoring visit as well as the home visit. 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 mailed dust questionnaire and the morning void urine collection (**Attachment 10.1**). Subjects that agree to the air monitoring visits will receive a call prior to the air monitoring visits **(Attachment 10.4)** as well as the home visits.

For subjects in the Recently Exposed group (i.e., those recently exposed to selected pesticides or other agricultural exposures), this call will also serve to verify that the subject has recently mixed, loaded, or applied a pesticide of interest or had another recent agricultural exposure of interest (**Attachment 10.2 or 10.3**). The purpose of this aspect of the study will be

to determine if recent exposure to selected pesticides or other agricultural exposures 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 before, during and after the exposure of interest.

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, 6) collect the urine sample, 7) collect and review the dust questionnaire, and 8) collect the vacuum dust specimen. 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 of the consent form and offer the subject a 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 for AHS participants 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. The CAPI for

controls will include many of the same general and medical questions, but will ask for other occupational exposures instead of farming exposures.

If the subject agrees to the blood sample, the phlebotomist will collect the blood sample by venipuncture using all standard procedures for safety. The following blood samples will be collected from participants the Random Select group: two 10.0-mL and one 6.0-mL serum tube, one 10.0-mL heparin tube, one 10.0-mL EDTA tube, one 8.5-mL acid citrate dextrose (ACD) tubes, and two 2.5-mL PAXgene tubes. For the subjects in the Recently Exposed and Control groups, the following tubes will be collected: two 10.0-mL serum tubes, two 10.0-mL heparin tubes, one 3.0-mL lithium heparin plasma separator tube, two 6.0-mL EDTA tubes, and two 2.5-mL PAXgene tubes. One of the ETDA tubes will be used for the hematologic assays (i.e., CBC with differential and lymphocyte subsets) and the 3.0-mL lithium heparin tube will be used for liver function tests. These tubes will be collected at each home visit. Consenting Recently Exposed and Control subjects are mailed a clinical test results letter (**Attachment 17.11**) – there are participant letters for Normal, Essentially Normal, and Abnormal results, as well as a physician letter for Abnormal results meriting physician contact (if consented).

For all participants, a 15-mL buccal cell sample will be collected by the same protocol used in Phase IV buccal collection, but will be collected in person instead of by mail. Participants are swish 10 ml of the mouthwash in their mouth for approximately one minute, and expectorate it back into the sample cup. The phlebotomist will track the time and, after the participant has finished, take the cup and cap it securely.

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 and Air Monitoring 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.

For all participants, a paper-and-pen Dust Specimen questionnaire **(Attachment 21)** will be sent to the home prior to the phlebotomy visit with instructions for selecting the vacuum cleaner of interest (the one used most often in the home), and completing the questionnaire. The questionnaire data will be collected to provide information about the vacuum machine from which the dust sample was collected, the time interval during which the vacuum was used, places in the home where the vacuum was used, and characteristics of the home related to the dust sample (e.g., number of carpets, age of home, type of air conditioning system). The phlebotomist will collect and review the dust questionnaire for completeness and clarity. If the subject agrees to the vacuum dust sample collection, the phlebotomist will ask to see the home's primary vacuum machine and, as applicable, will collect the disposable vacuum bag or empty the contents of the canister or reusable bag.

If a Recently Exposed group subject agrees to air monitoring visits, he will receive two home visits in different seasons (approximately 6 months apart) as well as separate air monitoring visits on the farm on the day prior to each of these two home visits. Up to two

additional air monitoring visits will be conducted on other days. For the air monitoring visits, an industrial hygienist will visit the farm and will collect airborne dust (bioaerosol) samples in the breathing zone of subjects while they perform their daily work tasks.

Finally, subjects who agree to complete the smartphone app component (**Attachment 22.2**), will sign a separate consent form (**Attachments 22.3 and Attachments 22.4**), after which the phlebotomist will assist them to set up the app on their smartphone. App participants will record their daily activity on 30 days during a 6-month timespan. Over the course of a selected day, this will involve periodically opening the app, choosing an activity from the list of pre-programmed options (or creating a new one), indicating when they start and stop the activity, and, as needed, making brief, related notations. This is expected to take no more than 10 minutes per selected day.

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. As noted in section B.2, 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 9.4**) and a script to re-contact those in which the buccal cells were either missing or arrived damaged (**Attachment 9.5**). The additional letters include a re-mailed consent form or buccal cell kit cover letter (**Attachment 16.3 or 16.6**).

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 17.3, 17.4, 17.5, or 17.6**). 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 10.1 or 10.4**). The participants in the Recently Exposed group will receive one additional contact by phone after their first visit that will help with scheduling their second home visit (**Attachments 10.2 or 10.3**).

Finally, participants will receive $100 for completing each home visit as an incentive for time spent in this study. Air monitoring participants will also receive $100 for each farm visit. Phone App participants will receive $100 for taking part in this component. 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. The

questions and methods for dust collection were based on previous NCI studies using this methodology. Likewise, the smartphone application is adapted from an app that was developed to assess physical activity for epidemiologic studies. The app uses procedures similar to those employed by other NCI studies (e.g., OMB #0925-0642), but will collect information to assess farming activities and agricultural exposures instead (**Attachment 22.1**).

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:

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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:

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