Report of the Acting Clinical Director

National Advisory Environmental Health Sciences Council Meeting

February 2010

NIEHS Clinical Research Unit (CRU) Update

Since the last Council meeting, the CRU has continued to enroll patients under the IRB approved protocol titled *Innate Immunity Signal Transduction in Human Leukocytes* (Principal Investigators: Mike Fessler, Mike Resnick, Ron Mason, Doug Bell and John Cidlowski); 36 patients have been enrolled to date. There are currently two other IRB approved protocols that will utilize the CRU as a recruitment site in the near future: *Pathogenic Studies in Families with Twins or Siblings Discordant for Systemic Rheumatic Disorders* (Principal Investigator: Fred Miller) and *Genotypic and Phenotypic Data Analysis Requests from the Framingham Heart Study* (Principal Investigator: Stavros Garantziotis). Several additional protocols are being developed and are in various stages of review (see Appendix 1). The plan is to have 10-12 active CRU protocols by summer 2010.

Since the last Council meeting, five proposals for clinical-translational research based on the Environmental Polymorphism Registry (EPR) have been submitted for consideration and are in various stages of review. The EPR is a large DNA biobank available to investigators at NIEHS, UNC and other research institutions. EPR subjects are recruited from the greater NC Triangle region through volunteer study drives held at health fairs, corporate sites, community centers, universities and other locations, as well as through outpatient clinics within the UNC Healthcare System, and in the near future, through the NIEHS CRU. Blood samples are collected from these individuals and used for DNA isolation. The DNAs are then coded with unique personal identification numbers (PINs) that are linked back to the subjects' identities, contact information and basic demographic data. Investigators propose specific "genes of interest" and the DNA samples are genotyped for single nucleotide polymorphisms (SNPs) in those genes in the NIEHS Molecular Genetics Core (MGC) Facility. Once EPR subjects with specific genotypes of interest are identified through anonymous genetic screening, samples are decoded and personal information provided to investigators for follow-up studies. The ultimate objective of the EPR is to identify groups of individuals with polymorphisms of interest in environmentally responsive genes and to correlate genotype with biochemical, physiologic, pharmacologic and/or clinical phenotype. This process is known as "ascertainment by genotype." To date, over 16,000 subjects have been enrolled into the EPR and contact data is current for approximately 11,000 individuals. A race and ethnicity distribution of this cohort is provided in Appendix 2. To date, 24 intramural investigators have nominated 96 genes of interest for inclusion in the initial round of genotyping. The EPR will be the subject of a more detailed presentation at a future Council meeting.

Since the last Council meeting, NIEHS CRU Staff have worked closely with staff at the U.S. Environmental Protection Agency (EPA) to establish a collaborative arrangement to facilitate conduct of inhalation and bronchoscopy studies at the EPA Human Studies Facility located on the University of North Carolina campus in Chapel Hill, NC. The goal is to foster collaborations between two government institutions with an interest in environmental health issues, taking advantage of operational strengths of each institution. Two pilot projects have been proposed to test the feasibility of these

arrangements. One project will evaluate the role of transforming growth factor-β in the susceptibility to viral infections in asthmatic individuals; the other will identify genetic determinants of inflammatory cell response to lung injury (see Appendix 3). At this time an umbrella Memo of Understanding (MOU) which establishes the framework for the scientific collaborative arrangements between NIEHS and EPA has been signed by both parties. Documents establishing an interagency agreement (IAG) to initiate the specific pilot projects have been finalized and are awaiting approval by senior leadership at EPA and NIEHS.

Since the last Council meeting, the CRU purchased a FACSAriaII flow cytometer to support clinical studies at NIEHS. This flow cytometer is the most versatile unit available that would allow investigators to both analyze and sort specific populations of cells from a single biological sample. The FACSAriaII is a 3-laser, 13-parameter cytometer using digital technology that is capable of handling various clinical assays including immunophenotyping, apoptotic assessment, and DNA analysis of human cells. This instrument is equipped with the latest octagon and trigon fiber-coupled collection optics for increased quality and quantity of the fluorescent signal. Additionally, the FACSAriaII is a high-speed cell sorter which allows for the simultaneous recovery of up to 4 distinct populations of cells. The instrument is also equipped with an aerosol management system to protect cells being sorted from contamination and to protect the operator from potential blood-borne pathogen hazards associated with live human cells. The FACSAriaII will initially be used by a number of intramural investigators to isolate peripheral blood cells for *ex vivo* studies. Due to its advanced technology and high capabilities, the FACSAriaII has also attracted interest from academic investigators in the greater Triangle area.

Three IRA 2010 proposals with active CRU participation were submitted in response to a DIR Request for Applications. One project, which will take advantage of the EPR cohort, was selected for funding following a highly competitive review process. In addition one Bench-to-Bedside proposal with active CRU participation was submitted for consideration of funding to NIH.

Finally, CRU staff has actively sought collaborative projects with investigators in the National Toxicology Program (NTP). Tentative plans to investigate the effects of nutritional additives on human health are being explored. CRU staff has also been involved with the NIEHS nanotechnology initiative and has established a signature Nano-Healthy project which will investigate the effect of environmentally released nanoparticles on human health outcomes.

NIEHS Office of Human Research Compliance (OHRC) Update

Currently, the NIEHS has 14 IRB Members. There are 10 affiliated members and 4 non-affiliated or community members. The list of current members, their affiliations, and their areas of expertise is shown in Appendix 4.

Currently, NIEHS has 56 active clinical protocols that are handled by the OHRC. Forty-nine protocols are reviewed by the NIEHS Institutional Review Board (IRB) and eight protocols are reviewed by outside IRBs (3 at NIAID IRB, 4 at NIDDK IRB). Nineteen of the 56 protocols are reviewed by the full board and 28 protocols undergo expedited review. The total number of active Epidemiology Branch protocols is 39 whereas the total number of active Non-Epidemiology Branch protocols is 17 (see Appendix 5). Since the last Council meeting in September 2009, the NIEHS IRB conducted 4 initial reviews, 23 continuing reviews, reviewed 20 protocol amendments, handled 2 protocol deviations/violations, and facilitated a protocol close-out.

Since the last Council Meeting in September 2009, the OHRC has worked closely with the NIH Office of Human Subjects Research (OHSR) to establish reliance agreements for two NIEHS clinical protocols. A reliance agreement is between the NIH and another Federal Wide Assurance (FWA)-approved institution and acknowledges that review by a single IRB will suffice. Reliance agreements help to simplify the IRB review process by limiting the number of IRBs that review a particular clinical protocol.

Since the last Council meeting, the OHRC Quality Assurance/Quality Improvement Program completed a due-diligence audit of Copernicus Group. Due-diligence audits are an important safeguard to allow the IRB to have confidence that remote study sites understand and enforce human subjects' protections.

Since the last Council meeting, NIH decided to apply for the accreditation of its Human Research Protection Program (HRPP) through the Association for the Accreditation of Human Research Protection Programs (AAHRPP). AAHRPP is an independent, non-profit accrediting body that uses a voluntary, peer-driven, educational model to ensure that HRPPs meet rigorous standards for quality and protection. AAHRPP accreditation is the "gold standard" for HRPPs and offers assurances to research participants, researchers, sponsors, government regulators, and the general public that an *"HRPP is focused first and foremost on excellence."* AAHRPP will look at three separate components at NIEHS:

1. The NIEHS HRPP, which consists of all the components of NIEHS involved in the conduct and oversight of human research. At NIEHS the HRPP is managed by the Office of Human Research Compliance.
2. The NIEHS Institutional Review Board
3. All investigators with studies that involve human participants (including work with biological specimens)

AAHRPP accreditation is a collective effort for all who participate in the conduct, review, approval and facilitation of clinical research at NIEHS. NIEHS is preparing for AAHRPP accreditation by providing the necessary education and review sessions to all who participate in clinical research.

On December 2, 2009, the OHRC hosted a Town Hall meeting as the initial kick-off for the AAHRPP accreditation process. During this Town Hall meeting, Dr. Jeffrey Cohen (a contractor hired by NIH OHSR to prepare NIH for accreditation) and Ms. Leody Bojenowsky (Program Analyst, NIH OHSR) spoke about the accreditation process from

the NIH perspective. Dr. Joan Packenham spoke about the accreditation process from the NIEHS perspective. Investigators received information needed to successfully participate in the AAHRPP process. OHRC is in the process of developing mandatory review sessions for investigators, study staff, and IRB members so they will be prepared for the AAHRPP site visit that will occur in Fall 2010. OHRC is actively participating in the NIH AAHRPP process by participating on the Trans-NIH policies and procedures committee and the Trans-NIH Quality Assurance/Quality Improvement working group. Both committees were established by Dr. Gottesman, NIH Deputy Director of Intramural Research. In addition, the OHRC is working closely with NIH OHSR during this accreditation process.

Appendix 1 – NIEHS Active protocols and Protocols Under Development

PROTOCOL NAME (Principal Investigator)		PROTOCOL DRAFTED	REVIEW COMPLETED			PATIENT ACCRUAL
			Clinical Advisory Committee (CAC)	Scientific Review	IRB Review	
Active CRU Protocols						
Innate Immunity Signal Transduction in Human Leukocytes (PIs: Fessler, Garantziotis, Resnick, Mason)	Active	✓	N/A	✓	✓	43 (at CRU)
Genotypic and Phenotypic Data Analysis Requests from the Framingham Heart Study (PI: Garantziotis)	Active	✓	N/A	✓	✓	N/A
Pathogenic Studies in Families with Twins or Siblings Discordant for Systemic Rheumatic Disorders (PI: Miller)	Active (CRU serving as a recruitment site)	✓	N/A	N/A	✓	0
CRU Protocols Under Development / Review						
Sample Collection Registry for Quality Control of Biological and Environmental Specimens and Assay Development and Testing (PI: Garantziotis)	Final IRB stipulation addressed on Jan 18, 2010; awaiting IRB notice of approval	✓	✓	✓	Jan 14, 2010	
The Role of Glucocorticoid Receptor SNPs in Receptor Function and Metabolic Disease (PIs: Cidlowski, Garantziotis, Jewell)	Received provisional approval from Scientific Review Board – currently addressing comments	✓	✓	Provisionally approved	Mar 4, 2010	

PROTOCOL NAME (Principal Investigator)		PROTOCOL DRAFTED	REVIEW COMPLETED			PATIENT ACCRUAL
			Clinical Advisory Committee (CAC)	Scientific Review	IRB Review	
Study of the Effect of Innate Immunity on the Inflammatory Response to Endotoxin (PIs: Fessler, Garantziotis)	Received provisional approval from Scientific Review Board– currently addressing comments	✓	✓	Provisionally approved	Apr 1, 2010	
Study of the Effect of SNPs in p53 and p53 Response Elements on the Inflammatory Response to DNA Damage (PIs: Resnick, Menendez, Garantziotis)	Received provisional approval from Scientific Review Board– currently addressing comments	✓	✓	Provisionally approved	Mar 4, 2010	
Study of the Effect of MDC1 SNPs in the Repair of DNA Damage (PIs: Mason, Dronamraju, Garantziotis)	Protocol was tabled by the Scientific Review Board– currently addressing comments	✓	✓	Tabled	May 6, 2010	
TGF-beta Mediates Asthmatic Epithelial Susceptibility to RSV Infection (PIs: Imani, Garantziotis)	Protocol was tabled by the Scientific Review Board– currently addressing comments	✓	✓	Tabled	May 6, 2010	
Hyaluronan study (PI: Garantziotis)	Full NIEHS IRB review will be required for this study. CAC concerned over two protocols for this study (one at Duke, one at NIEHS).	✓	✓			
Short Term Cholesterol Kinetics in Humans after high fat meal (PIs: Garantziotis, Goodno)	Concept development					
Nanoparticle study (PIs: Garantziotis, Gutierrez)	Laboratory pilot study underway	✓	✓			

PROTOCOL NAME (Principal Investigator)		PROTOCOL DRAFTED	REVIEW COMPLETED			PATIENT ACCRUAL
			Clinical Advisory Committee (CAC)	Scientific Review	IRB Review	
Exempted Research						
Exemption request to use monocytes and epithelial cells obtained under UNC protocol #91-0679 (formerly IRB # 91-EPA-304) titled "Effects of in vitro pollutant exposure on functional and biochemical characteristics of human pulmonary cells in normal subjects" (PI: Garantziotis)	Active	N/A	N/A	N/A	N/A	10
Exemption request to measure physiological levels of IaI in patients with cystic fibrosis (PI: Garantziotis)	Active	N/A	N/A	N/A	N/A	10
Exemption request to receive sera from study at Duke titled "Gene Variant for NAG1 in inflammatory bowel disease study" (PI: Eling)	Active	N/A	N/A	N/A	N/A	34
Development of novel models of scleroderma (PI: Diaz)	Exemption request submitted to receive scleroderma samples from Duke; a second exemption will be submitted to receive comparator cells under the CRU Sample Collection Registry					

PROTOCOL NAME (Principal Investigator)		PROTOCOL DRAFTED	REVIEW COMPLETED			PATIENT ACCRUAL
			Clinical Advisory Committee (CAC)	Scientific Review	IRB Review	
Pilot Study of association of serum anti-flagellin antibodies with allergic asthma (PIs: Cook, Garantziotis)	Exemption request submitted to receive sera from asthmatics from Duke; a second exemption will be submitted to receive non-asthmatic sera under the CRU Sample Collection Registry		✓			

Appendix 2 – EPR Population by gender, age, race and ethnicity

Table 1. EPR Population by gender, age, race and ethnicity

Race	Age	Non-Hispanic Latino				Hispanic Latino				Unknown/NR				Totals
		Female	Male	NA	Subtotal	Female	Male	NA	Subtotal	Female	Male	NA	Subtotal	
White	18-40	2109	1346	13	3468	81	57		138	69	42	1	112	3718
	41-60	2533	1381	44	3958	78	33	1	112	115	72	2	189	4259
	61+	1255	919	84	2258	21	11		32	92	80	20	192	2482
	NA	53	34	13	100		1		1	1	2	1	4	105
		5950	3680	154	9784	180	102	1	283	277	196	24	497	10564
African American	18-40	1055	608	4	1667	21	9		30	101	85		186	1883
	41-60		478	12	1416	14	12		26	107	72	5	184	1626
	61+	280	117	13	410	3		1	4	35	20	8	63	477
	NA	926 32	19		51	1		1	2	2	1	1	4	57
		2293	1222	29	3544	39	21	2	62	245	178	14	437	4043
Asian	18-40	76	55	1	132	1			1	6	6		12	145
	41-60	51	39		90	1	1		2	4			4	96
	61+	20	19	2	41				0		3		3	44
	NA	1	3		4				0				0	4
		148	116	3	267	2	1	0	3	10	9	0	19	289
AIAN	18-40	67	34		101	4	6		10	3	1	1	5	116
	41-60	66	32	1	99	5	1		6	12	5		17	122
	61+	19	13	1	33	2	1		3	1		2	3	39
	NA	1			1				0				0	1
		153	79	2	234	11	8	0	19	16	6	3	25	278
NHOPI	18-40		4		11	1			1	1	2		3	15
	41-60		2		4				0				0	4
	61+	7			0		1		1	1			1	2
		2 9	6	0	15	1	1	0	2	2	2	0	4	21
Multiple	18-40	87	50		137	101	81		182	12	7	1	20	339
	41-60	82	33	2	117	42	30		72	3	2		5	194
	61+	30	16	4	50	7	8		15	2	3	2	7	72
	NA	3	2		5	1			1		1		1	7
		202	101	6	309	151	119	0	270	17	13	3	33	612
Unknown /NR	18-40	1	5		6	76	54		130	6	4	32	42	178
	41-60		2	1	7	40	32		72	12	8	72	92	171
	61+	2	3		5	5	3		8	14	7	63	84	97
	NA	4	1		1	4	1		5	3	2	17	22	28
		7	11	1	19	125	90	0	215	35	21	184	240	474

Totals	8762	5215	195	14172	509	342	3	854	602	425	228	1255	16281

Appendix 3 – NIEHS-EPA IAG Statement of Work

Background

As the guiding principle, this arrangement is of a collaborative nature on issues that are of mutual interest to the US Environmental Protection Agency (EPA) and the National Institute of Environmental Health Sciences (NIEHS). This IAG covers two specific projects that will be done in a collaborative fashion between investigators at the EPA Human Studies Facility (HSF) and NIEHS investigators. Both EPA and NIEHS investigators will be involved in the planning and execution of these studies.

The participants must understand that NIEHS is using the EPA Human Studies Facility for conducting bronchoscopy procedures. NIEHS is responsible for complying with applicable safety requirements and protocols, including human research protection protocols. Thus, all procedures will follow the EPA Standard Operating Procedures listed in the appendix.

Further, the participants should also understand that the EPA shall not be liable in contract or tort for any acts or omissions of employees or agents of the NIEHS.

Projects

The two projects outlined below are designed to collect bronchial cells by fiberoptic bronchoscopy with endobronchial brush biopsy and/or BAL. Bronchoscopy is proposed in normal and asthmatic subjects, and in subjects with specific genotypes of interest. All samples generated by bronchoscopy (bronchial and alveolar cells, BAL) will be shared between EPA and NIEHS investigators for in vitro studies. All information will be shared including correlative clinical information about phenotype of individuals from whom samples are obtained. In addition and in order to facilitate cooperation, BAL fluid and cells may be obtained from ongoing EPA studies and shared with NIEHS investigators.

Project 1. TGF-β mediates increased susceptibility of asthmatic epithelia to respiratory syncytial virus infections

Principal Investigators: Farhad Imani, PhD, Stavros Garantziotis, MD

Infection with common respiratory viruses can trigger severe asthma exacerbation and hospitalization in asthmatics, although these infections are generally well tolerated in normal subjects. The exact reason for this difference between the normal and asthmatic response to respiratory virus infections is not clear. Our recent data indicate that transforming growth factor-□ (TGF-□) may play a role in virus-induced asthma exacerbations. Our data showed that treatment of human epithelial cells *in vitro* with TGF-□ prior to infection with respiratory syncytial virus (RSV) causes a significant increase in RSV replication and RSV-induced TNF-□ expression. Since TGF-□ expression has been clearly associated with asthma phenotype and severity, we believe that our *in vitro* observations are relevant to *in vivo* events. We propose to isolate epithelial cells from asthmatic and normal subjects and use the cells *ex vivo* in RSV

infection studies. We hypothesize that asthmatic bronchial epithelia will express higher levels of TGF-□ and therefore have higher levels of infectivity and increased expression of pro-inflammatory cytokines after RSV exposure. We will include 32 subjects, age 18-60 years old, non-smokers, of any gender or race in this study (16 asthmatics and 16 non-asthmatics). Asthmatics will have to be free of acute exacerbations. We will screen patients for asthma and allergy during the initial visit, by performing a pulmonary function study, a methacholine challenge, skin allergy testing, and complete blood count with serum IgE testing. These tests will be performed at the NIEHS CRU. Suitable individuals will be called back for bronchoscopy with endobronchial brush biopsy at the EPA Facility in Chapel Hill. Primary cultures will be established at the NIEHS by seeding freshly brushed bronchial epithelial cells into hormonally supplemented appropriate bronchial epithelial growth medium. TGF-β release will be assessed at baseline. Cells will then be infected with RSV and viral replication, cell growth and cell cycle will be evaluated with or without TGF-β antagonism.

Project 2. Genetic determinants of inflammatory cell response to injury – Environmental Polymorphism Registry (EPR) project

Principal Investigators: Michael Resnick, PhD, Daniel Menendez, PhD, Stavros Garantziotis, MD

The cellular response to noxious agents and injury is strictly regulated. A number of proteins and cell organelles must act in coordination for optimal results. Genetic polymorphisms resulting in aberrant protein function can therefore result in pathological responses and lead to disease. Cell stress or DNA damage leads to activation of p53, which in turn activates a number of cellular response elements, such as cell cycle changes, DNA repair, apoptosis, inflammation etc. p53 binds on so-call response elements (REs) in the promoter region of its target genes, leading to gene expression. Failure of p53 activation leads to chromosomal instability, and failure of normal response by p53 target genes leads to abnormal cell repair and defective inflammatory response, and is associated with cancer pathogenesis. SNPs in the p53 promoter are known or predicted to have direct effects on p53 activation, and SNPs affecting p53 Res in p53 target genes affect these genes' activation by p53. We hypothesize that genetic polymorphisms in the p53 gene as well as p53 response elements in p53 target genes will affect the human primary cell response to damage *ex vivo*. To this end, we will utilize the Environmental Polymorphisms Registry (EPR), an NIEHS-established cohort of approximately 15,000 individuals who live in the greater Research Triangle Area, who have donated their DNA for genotyping and have agreed to be called back for phenotyping. We are following up these individuals annually and we have established a retention rate of over 80%. We will identify and recruit individuals carrying SNPs in the p53 promoter region or in p53 target gene response element regions, or age- and gender-matched "wild-type" controls, through genotyping done at the NIEHS. Screening will be performed at the NIEHS CRU. We will employ a questionnaire regarding past and family history of inflammatory disease, autoimmune disease, and cancer. We will exclude individuals with active cancer on chemotherapy/radiation therapy, or on immune-modifying drugs. Patients will undergo bronchoscopy at the

EPA Facility in Chapel Hill for isolation of bronchial epithelia (through brushing) and alveolar macrophages (through bronchoalveolar lavage). We will culture these cells at the NIEHS, and expose these cells to irradiation or doxorubicin (inducing DNA damage) and determine cell response depending on genotype. Primary endpoint is p53 target gene expression measured by RT-PCR. Secondary endpoints are p53 promoter occupancy (ChIP analysis), apoptosis (Annexin V-PI), cell cycle analysis (FACS), and DNA repair (TAFE gels). The primary endpoint is expected to yield the most conservative power calculations. Based on our power calculations, we will need 13 subjects per genotype group for a power of at least 80% for a 2-sided error of 0.05. We will examine 3 SNPs, therefore we need 4x13=52 patients for our study (3 SNP-carriers and one "wildtype" group).

Research Protocols

The research protocols that cover subject recruitment and bronchoscopy will undergo rigorous scientific and biostatistical review at NIEHS and will be prepared and submitted by the NIEHS investigators for NIEHS IRB approval. These protocols will then be reviewed with the EPA Medical Station nurses. Protocols may also require submission to the UNC IRB for review and approval. It is acknowledged that review by the UNC IRB may not be needed, and the UNC IRB may choose to defer to the NIEHS IRB.

All studies will require review and approval by the EPA HSRRO and the Director of the HSRO, The EPA may, at its sole discretion, also require review by the EPHD Division Director, the NHEERL Lab Director and his/her designee. The EPA agrees to conduct reviews in a timely manner.

Relevance to the Clean Air Act

The objective of these research projects is to establish host risk factors for environmentally induced airway disease. Host risk factors play a major role in the pathogenesis of pollution-related health effects. By identifying the mechanisms and factors that place humans at risk for environmentally induced cardiopulmonary disease, we can help assess, prevent and treat pollution-related adverse health effects.

Procedures

The subjects will be recruited and screened by NIEHS in the Clinical Research Unit in Research Triangle Park, NC. Screening procedures and criteria for medical suitability for bronchoscopy will be based on the current criteria used by EPA medical staff for research bronchoscopy (see appendix). The screening will include blood counts and chemistries, 12-lead EKG and spirometry. A pre-bronchoscopy training day will be scheduled at the EPA HSF to familiarize the subject with the staff, procedure and facility, and obtain consent for bronchoscopy. This will be performed by the NIEHS bronchoscopist or his designee. Screening medical charts will be made in duplicate at the NIEHS CRU and one copy will be distributed to EPA prior to the training day.

The bronchoscopy will be performed on a specific day/time and arranged in conjunction with nursing staff at the EPA HSF medical station in Chapel Hill. Scheduling of subjects

will be done by NIEHS. The procedure will be performed by one of three NIEHS physicians. All NIEHS physician will each observe three research bronchoscopies by an EPA physician to familiarize themselves with the protocol for research bronchoscopy at EPA HSF. Bronchoscopy will be done without sedation, using topical anesthesia of upper airway per EPA SOP (see appendix). Bronchoscopy with bronchoalveolar lavage and endobronchial brush biopsy will per performed per the EPA HSF SOP (see appendix). An EPA physician will be present for at least the first three bronchoscopies performed by a NIEHS physician at the EPA. If there is discussion regarding suitability of candidate for bronchoscopy, the final arbiter will be EPA bronchoscopy physicians. In some cases, EPA physicians may perform bronchoscopies and provide biological materials to NIEHS investigators. We anticipate that initially, one bronchoscopy per week will be done by NIEHS investigators. After a trial period of 2-4 months, if all parties agree, additional bronchoscopies may be added to the weekly schedule.

Appropriateness of the EPA as the performing site for above procedures

The NIEHS and the EPA serve complementary missions in the effort to understand, prevent, and treat environmentally-induced health problems. The EPA and the NIEHS have a long record of collaboration with regard to environmental health and disease. Scientifically, the questions addressed and methods employed in our projects are often very similar. Geographic proximity and ease of access are another relevant aspect. The EPA is therefore the clear choice in our decision to establish research partnerships in the area. Furthermore, the EPA brings substantial expertise in human research to the table, particularly with regard to environmental exposures and pollution effects on human health.

Logistics and Funding Needs

Current staffing of the EPA HSF medical station will not accommodate bronchoscopies in addition to what is already scheduled by EPA investigators. Thus, bronchoscopy of NIEHS subjects will require additional personnel and cannot commence until a new nurse has been identified and trained. As part of this IAG, NIEHS will agree to provide a Registered Nurse (RN) to recruit study subjects, interact with the subjects at the NIEHS CRU, and assist with bronchoscopies at the EPA. The RN will work closely with the EPA and NIEHS physicians and EPA nursing staff. The RN will undergo a period of training with EPA nursing staff and EPA physicians to assure optimal patient safety and consistency with EPA medical SOPs

There is currently one bronchoscopy suite at the EPA HSF. This will not accommodate the needs of this agreement. Prior to initiating activities covered in this agreement, one of the examination rooms at HSF medical station will be converted to a second bronchoscopy suite. This will require significant modifications and new bronchoscopy equipment which will interface with the current Olympus bronchoscope. Funds for purchase and installation of the bronchoscopic equipment will be provided by NIEHS as part of this IAG. EPA will use said funds to purchase this equipment which will become the sole property of the EPA. EPA will be responsible for maintaining the equipment in proper working order including payment of maintenance agreements, as applicable.

Reporting

An annual written report of activity on the two protocols will be made by NIEHS and EPA investigators to EPA EPHD Division Director, EPA Director of the Human Research Protocol Office, NIEHS Clinical Director and respective IRBs.

Estimated Costs

Equipment and Supplies (provided by NIEHS)

For compatibility with EPA bronchoscopy system already in place, an Olympus system would need to be purchased.

Olympus CV-180: EVIS EXERA II Bronchoscope, cart and monitor (attached)	$90,247
MSE Company General Transport Stretcher	$1,199
2 Berchtold ceiling mounted arms for bronch monitors ($8,093 ea)	$16,078
Olympus flat panel monitor for room 23	$3,400
Replace existing Spacelabs EKG monitors and support arms ($10,720 each)	$21,440
Supplies for 96 proposed bronchoscopes	$13,151
Total	**$145,515**

Construction (provided by NIEHS)

Suction stations ($100 each, 2 needed)	$200
Vacuum outlet stations ($150 each, 2 needed)	$300
Mounting hardware, pipe and fittings	$1,200
Labor for above installations	$2,000
Wall repair and paint	$1,200
Total	**$4,900**

Equipment Installation (provided by EPA)

Reconfigure cabinets and counters in room 25 to make comparable to room 23	$5,000
Total	**$5,000**

Personnel (provided by NIEHS)

1 - NIEHS RN (NIEHS contractor)
3 - NIEHS Pulmonary Physicians (bronchoscopists)

Personnel	Labor Cost	Fringe Benefits Cost
Zeldin	$32,038	$8,490
Garantziotis	$93,082	$24,666
Fessler	$34,505	$9,143
Yingling (Contractor)	$134,755	N/A
Total	**$294,381**	**$42,300**

Personnel (provided by EPA)
Access to EPA HSF RNs as needed
Access to EPA Pulmonary Physicians (bronchoscopists) as needed

Appendix 4: Current NIEHS IRB Members

Member name	Degree	Email address	Term end	Minority/Gender	Specialty	Affiliation
Baird, Donna	PhD	baird@niehs.nih.gov	09/30/2011	N / F	Epidemiology	NIEHS
Bishop, Jack	PhD	bishop@niehs.nih.gov	06/01/2010	N / M	Toxicology	NIEHS
Blackmon, Betty	--	bettyblackmon@csallc.com	01/30/2010	Y / F		Community Member
Fessler, Michael	MD	fesslerm@niehs.nih.gov	01/30/2010	N / M	Respiratory Biology	NIEHS
Hoppin, Jane	ScD	hoppin1@niehs.nih.gov	07/01/2010	N / F	Epidemiology	NIEHS
Lee, Craig R.	PharmD, PhD	craig_lee@unc.edu	04/22/2010	N / M	Pharmacology	NIEHS

Moore, Alicia	MS	moore5@niehs.nih.gov	03/31/2010	Y / F	Biology	NIEHS
Packenham, Joan (IRB Vice Chair)	PhD	packenhm@niehs.nih.gov	09/30/2010	Y / F	Pathology	NIEHS
Ramirez, Rey	BA	rey2@gte.net	01/30/2012	Y / M		Community Member
Resnik, David (IRB Chair)	JD, PhD	resnikd@niehs.nih.gov	09/30/2010	N / M	Research Ethics	NIEHS
Roberts, Michael	DDS	Mike_Roberts@dentistry.unc.edu	01/30/2010	N / M	Pediatric Dentistry	Community Member
Siegl, Adelaide	PhD, ThM	docs3boys@yahoo.com	7/23/2010	N / F		Community Member
Sills, Robert C.	PhD, DVM	sills@niehs.nih.gov	06/11/2010	Y / M	Pathology	NIEHS
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Appendix 5 - 2010 Active NIEHS Clinical Studies

NIEHS Epidemiology Branch Protocols

Title: The Study of Environment, Lifestyle & Fibroids (SELF)

PI: Donna D. Baird, Ph.D. (NIEHS/DIR/EB)

Precis:

Uterine leiomyomata (fibroids) are the leading indication for hysterectomy in the United States. The direct medical costs exceed $2 billion per year, not including indirect costs for managing the symptoms of bleeding, pain, urinary incontinence, and reproductive dysfunction. Fibroids are benign tumors of smooth muscle that develop during reproductive years and tend to regress after menopause. Estrogen and progesterone both increase proliferation of tumor tissue in vitro, and hormonal responsiveness is confirmed by in vivo data. Though hormonally mediated, the etiology is not understood. African Americans are at significantly higher risk with earlier onset than in whites and no decrease in tumor growth with age as seen in whites. An estimated 20% of African American women have a hysterectomy for fibroids before menopause, nearly three times the proportion in white women. The reasons for marked ethnic differences are unknown. No epidemiologic study has investigated disease incidence with ultrasound screening so that exposures can be assessed before disease onset. We propose to prospectively study incidence and growth of uterine fibroids in a cohort of 1600 African American women, ages 23-34, who have not been diagnosed with fibroids. Participants will be screened for fibroids with ultrasound at enrollment. There will be clinic visits with ultrasound examinations approximately every 15 months to identify incidence for those who do not have fibroids at enrollment. Fibroids that are detected at enrollment and during follow-up will be followed at subsequent visits for fibroid growth. We plan to collect risk factors and symptom data, physical measurements as well as specimens at each visit. We will test three primary hypotheses: 1. Vitamin D deficiency is a risk factor for fibroid incidence. 2. Reproductive tract infection is a risk factor for fibroid incidence. 3. A higher proportion of African ancestry is a risk factor for fibroid incidence. In addition to testing the hypotheses, we plan to collect a broad spectrum of information including data on recognized risk factors, data on common exposures with inconsistent risk estimates in the literature, exposures of interest for which there is very limited literature and detailed symptom data. In summary, this first prospective study of fibroid incidence will test important hypotheses regarding fibroid etiology which may lead to new strategies for prevention, and we will also store blood and urine for future analyses.

Title: Risk Factors for Uterine Fibroids: A Case Control Study and Follow-up Amendment to Study Disease Progression

PI: Donna D. Baird, Ph.D. (NIEHS/DIR/EB)

Precis:

Uterine leiomyomas are the leading cause of hysterectomy in the United States, accounting for over 200,000 procedures each year. Leiomyoma is a common condition with many tumors being asymptomatic. It is not known which women who have fibroids

will develop clinical symptoms. In 1996-1999 the NIEHS Uterine Fibroid Study enrolled 1245 randomly selected premenopausal women, aged 35 to 49, who had been randomly selected from the membership roles of George Washington University Health Plan, a large prepaid health plan in Washington, D.C. Slightly over half of the participants were African American. Participants were asked about prior diagnoses of uterine leiomyomas, and 87% were examined by abdominal/transvaginal ultrasound to screen for uterine leiomyoma. Sixty-two percent of the 1245 had either had a prior diagnosis of leiomyoma or had sonogram evidence of the condition.

Title: Hormonal Changes in Early Pregnancy: Pilot Study to Evaluate Stored Urine Specimens

PI: Donna D. Baird, Ph.D. (NIEHS/DIR/EB)

Precis:

Daily first morning urine specimens were collected throughout the first 8-9 weeks of 151 clinical pregnancies. These specimens are now approximately 20 years in storage, and the stability of markers of placental and corpus luteum function are not known. We will collect new urine specimens for 20 pregnancies to serve as a reference to evaluate the quality of those stored samples.

Title: Organochlorine Exposure in Relation to Timing of Natural Menopause

PI: Donna D. Baird, Ph.D. (NIEHS/DIR/EB)

Precis:

Smoking has been shown in many studies to be associated with a 1-2 year decrease in age at natural menopause. However, relatively little is known about the effect of other potential toxicants, including organochlorines such as polychlorinated biphenyls (PCBs), and 1, 1 dichloro-2,2-bis(p-chlorophenyl) ethylene (p,p'-DDE, referred to subsequently as DDE). We will assess timing of menopause among women who participated in the North Carolina Infant Feeding Study (total n = 865). Recruitment for this study was conducted between 1978 and 1982. PCB and DDE levels were analyzed in blood and breast milk samples around delivery and after pregnancy. The median age of the women as of March, 2002, is 50 years. Data will be collected in a telephone interview focusing on reproductive and menstrual history with additional information collected on demographic, social and behavioral factors that could affect timing of menopause. A blood sample will be collected from approximately 50% of participants based on sampling strata that involve criteria relating to age and menopausal status. Follicle stimulating hormone and luteinizing hormone will be measured in these samples in order to classify menopausal status of women who had undergone hysterectomy with retention of at least one ovary, women who are currently using hormone replacement therapy whose use began while still having periods, and women who report very short, very long, or irregular menstrual cycle lengths during the past 12 months. PCB and DDE levels will also be determined in these samples, allowing us to assess the correlation between current and baseline (1978-1982) PCB and DDE measures. The purpose of this study is to assess the association between the baseline organochlorine

measurements and timing of natural menopause. A secondary aim will be to conduct exploratory analyses of the association between specific factors (e.g., pregnancy history, weight change) and rate of change in organochlorine levels.

Title: Postpartum Uterine Regression

PI: Donna D. Baird, Ph.D. (NIEHS/DIR/EB)

Precis:

Background: Uterine leiomyomas are the leading cause of hysterectomy in the United States, accounting for over 200,000 procedures each year. Most epidemiologic studies of uterine leiomyoma show that parity has a protective association with leiomyoma, but the mechanism is not known. Both epidemiologic data and data from an animal model indicate that the protective association is not an artifact resulting from reduced fertility among women with fibroids. We hypothesize that the process of uterine regression following delivery results in loss of small fibroids due to selective apoptosis of transformed cells and the extensive remodeling of the entire uterus. Study Objectives: Monitor fibroids during pregnancy and after postpartum uterine regression to assess any loss of fibroids and change in size of fibroids. Methods: Add a postpartum ultrasound examination to an existing epidemiologic study of pregnant women. The parent study documents fibroid number, size, and location with a 7 week ultrasound examination. With the additional postpartum ultrasound proposed here, data on fibroid number, size, and location through pregnancy and postpartum uterine regression will be collected on approximately 400 women. A subsample of 30 women will also have an MRI after their postpartum ultrasound in order to evaluate the sensitivity of ultrasound imaging. Significance: This study will provide the first data on fibroid change with parturition/postpartum uterine regression for a large sample of women. If small fibroids disappear during this time, it will document a process that results in "natural regression" of these tumors in premenopausal women. Insights from the biology of this process may be useful in developing treatment that could be used by nonpregnant women with fibroids to induce tumor regression.

Title: Shanghai Parkinson's Study

PI: Honglei Chen, M.D., PhD.(NIEHS/DIR/EB)

Precis:

We propose to clinically examine self-reported Parkinson's disease (PD) patients and selected controls from the well established Shanghai Women's Health Study (SWHS) and thus initiate a long-term PD research study in this unique Chinese women cohort. The SWHS cohort was established 10 years ago by Dr. Wei Zheng from Vanderbilt University in collaboration with investigators from the Shanghai Cancer Institute (SCI) and the National Cancer Institute (NCI) of the US. Their primary aim was to examine several unique dietary hypotheses on cancer among Chinese women. From 1996 to 2000, the SWHS successfully recruited 74,942 Chinese women, aged 40 to 70, from selected communities in a single district in Shanghai with an overall consent rate of 92%. All participants completed a comprehensive baseline survey, 88% donated urine,

76% donated blood, and an additional 12% donated buccal cells.1 Follow-up surveys have since been conducted biennially with consistent participation rates of 95% or higher. Through the 3rd follow-up, the cohort has documented 220 self-reported PD cases and we expect to identify another 80 self-reports during the ongoing 4th follow-up survey (2007-2010). We hereby propose to clinically examine self-reported PD patients, and if the diagnosis is confirmed, examine matched controls. By doing so, we hope to achieve the following two major aims. Aim #1: To initiate a long-term prospective study on PD in this unique Chinese women cohort Aim #2: To examine the following specific hypotheses among women 1) Higher plasma levels of pro-inflammatory biomarkers predict higher PD risk. 2) Higher plasma uric acid is associated with a lower PD risk 3) Environmental tobacco smoke (ETS) is associated with lower PD risk a. Self-reported ETS exposure is associated with a lower PD risk b. Higher urine level of cotinine is associated with a lower PD risk We hereby propose a prospective study on PD in a unique women-only cohort. The infrastructure and the many desirable characteristics of this cohort offer us a rare opportunity for PD research in women, particularly on biomarkers. We expect to establish it as a long-term and excellent resource for PD research in women in the future. In the short term, we plan to examine several promising PD hypotheses that have not been adequately evaluated among women. These findings will apply directly to Chinese women and may also have implications for women in the West. PD etiological research is under-represented in women. Therefore, research in the SPS may not only corroborate findings on women in the west, but also lead to the identification of novel risk factors that could be generalizable to Western women.

Title: Confirmation of Self-Reported Incident ALS Cases in the AARP-Diet and Health (AARP-DH) Cohort

PI: Honglei Chen, M.D., PhD.(NIEHS/DIR/EB)

Precis:

Amyotrophic Lateral Sclerosis (ALS) is a rare but rapidly progressive neurological disease that often results in death within a few years after the diagnosis. The incidence of ALS in the US is approximately 2.0/100,000/year and is age dependent. Very few epidemiological studies have investigated the causes of ALS. Last year, Dr. Alberto Ascherio at Harvard School of Public Health successfully obtained a RO1 grant to investigate the risk of ALS by documenting ALS cases in five well-established large prospective cohorts: The Nurses's; Health Study (NHS), the Health Professionals Follow-up Study (HPFS), the Cancer Prevention Study II Nutrition Cohort (CPS IIN), the Multiethnic Cohort (MEC), and the NIH-American Association of Retired Persons Diet and Health Study (AARP-DH). The primary aims of this grant are to prospectively clarify the associations between diet and smoking and risk of ALS in this to date the largest epidemiological study on ALS. Incident ALS cases will be documented via biennial questionnaires in the first three cohorts. While mortality data will be obtained in the MEC and AARP-DH cohorts by searching the National Death Index (NDI) Plus, it is also desirable to identify surviving incident cases in these two cohorts. The objective of this specific proposal is to ascertain the self-reported incident ALS cases from the AARP-DH study and obtain consent for medical release following the procedures set up for

Parkinson's disease (PD) cases in the currently approved Parkinson's Genes and Environment (PAGE) Study IRB approval #06-E-N093. The confirmed ALS cases may be analyzed as part of the RO1 project. We expect to identify 300 self-reported ALS cases from the AARP cohort. Detailed analytic plans will be coordinated with Dr. Ascherio.

Title: The Parkinson's, Genes and Environment (PAGE) Study

PI: Honglei Chen, M.D., PhD.(NIEHS/DIR/EB)

Precis:

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease, affecting more than 1 million elderly Americans. The causes of PD are largely unknown, but may include both genetic and environmental factors. We thus propose a large study to investigate the roles of diet, lifestyle, genes and their potential interactions in PD etiology, using the NIH-AARP Diet and Health Study. The AARP cohort was established by investigators at NCI and recruited over half a million participants in 1995 and had prospectively collected detailed information on diet and lifestyle. At baseline, participants were 50 years or older and included 40% women. After more than 8 years of follow-up, we expect to confirm 1,208 incident PD cases with their neurologists. We will comprehensively examine the associations between diet and lifestyle and risk of PD, focusing on dietary antioxidants, fat, caffeine, dairy products, estrogen use, obesity, physical activity, and non steroidal anti-inflammatory drugs (NSAIDs). Further, we will collect saliva samples from PD patients and selected controls without PD for genetic analysis. These results will be used to explore the PD associations with several common genetic polymorphisms and, for the first time, their interactions with several promising diet or lifestyle exposures. Many of the specific aims are novel and important but have been rarely examined in previous investigations. The findings will improve our understanding of the complex relationships among diet, lifestyle, gene-environment interaction, and PD etiology and may potentially contribute to successful PD prevention strategies. Study aims: 1) Examine dietary factors that may increase or decrease PD risk. 2) Examine lifestyle factors that may increase or decrease PD risk. 3) Examine genetic polymorphisms in relation to PD risk and their interactions with diet and lifestyle on PD risk. Study population: The NIH-AARP Diet and Health Study cohort. Design 1) Longitudinal study for the first two study aims 2) Nested case-control study for the third study aim. Outcome parameters: Physician confirmed PD diagnoses.

Title: Genetic and Environmental Influences on Adult Asthma in the Agricultural Health Study: Lung Health in the Agricultural Health Study

PI: Jane A. Hoppin, D.Sc. (NIEHS/DIR/EB)

Precis:

Farmers have high rates of respiratory morbidity and mortality; the causes are unclear. Almost all farmers grow up on farms and thus possible early life exposure to endotoxin may modulate their later inflammatory response to farm and common

allergens. Endotoxins are biologically active lipopolysaccharides (LPS) that modulate innate immune responses via activation of inflammatory pathways. In addition to endotoxin, farmers are exposed to many other known respiratory toxicants that may activate inflammatory pathways to produce disease later in life. Such toxicants include other pathogen-associated-molecular patterns (PAMPs) (such as peptidoglycan and beta-sign-glucans), allergens, grain dust, diesel exhaust, and pesticides. While data on effects of endotoxin exposure are increasing, information on other PAMPs and these other farm co-exposures are limited. In cross-sectional analyses in the Agricultural Health Study, using questionnaire-based outcomes, exposures to animals, manure, pesticides, and diesel exhaust were associated with wheeze among farmers. To better understand the relationship among farm exposures, inflammatory pathways, and asthma in farm residents, this study will obtain objective measures of asthma, allergic status, and airway inflammation, to elicit more detailed farm exposure data, and to assess of the role of genetic variation in the inflammatory response pathways to these environmental exposures. This case-cohort study is nested within the Agricultural Health Study (AHS), a prospective cohort study of approximately 84,000 farmers and their spouses in North Carolina and Iowa. Participants in this cohort are well characterized for lifetime farming exposures and current asthma history. Participants in this study will include both the farmers and spouses in the AHS cohort. AHS expects by 2010, when the current wave of follow-up is complete, to have identified more than 2000 currently symptomatic asthma cases. This case-cohort study plans to enroll and conduct home visits for 1700 cases with symptomatic asthma and 1900 comparison subjects. Clinical assessments conducted at farm homes will include measurements of pulmonary function (spirometry conducted both before and after bronchodilator administration), and airway inflammation (exhaled nitric oxide), allergic sensitization, and collection of blood for DNA, allergic sensitization (total and specific IgE to common aeroallergens), and other analyses. Lifetime history of exposure to pesticides, animals, and grains will be assessed via questionnaire. Dust samples will be collected in the homes for analysis of endotoxin and other PAMPs. We anticipate that approximately 1000 selected AHS participants (470 cases and 530) controls might decline the home visit. On these subjects we will attempt to collect a minimal set of data, namely a saliva sample for DNA and the lifetime exposure questionnaire. All participants, whether or not they have the home visit, will also be asked to complete a telephone interview.

Title: Neurological Outcomes Among Pesticide Applicators

PI: Jane A. Hoppin, D.Sc. (NIEHS/DIR/EB)

Precis:

The investigators propose the collection of additional blood samples as part of an ongoing, approved epidemiological study of neurobehavioral function in 1000 farmers from the Agricultural Health Study, a prospective cohort study of approximately 60,000 farmers and their wives in North Carolina and Iowa conducted by NIEHS, NCI, and EPA. The parent study is designed to test the association between long-term organophosphate and carbamate pesticide exposure and neurological and neurobehavioral health outcomes among approximately 1000 pesticide applicators in Iowa and North Carolina (500 in each state). The parent study is oversampling those

individuals with the greatest lifetime organophosphate exposure; this provides a unique opportunity to explore the mechanisms of organophosphate toxicity. A blood sample is currently included to analyze for polymorphisms in the paraoxonase gene. This study is an extramurally funded study to Dr. Fred Gerr at the University of IA and has been approved by the University of IA IRB. NIEHS investigators are collaborators on this study and are proposing the collection of additional blood during the blood draw. Three additional tubes of blood are proposed for analysis of paraoxonase activity in serum, organophosphate adducts to cholinesterase, steroid hormones, and telomere length. Remaining samples will be stored for future analyses. A total of 40 mL of blood will be collected for this study; 10 mL for the parent study and 30 mL for the NIEHS add-on study. NIEHS Study Aims 1) Assess the role of serum PON1 activity on neurotoxicity and evaluate the relationship between serum PON1 activity and PON1 genotype; 2) Evaluate a biomarker of organophosphate (OP) exposure that measures OP adducts to acetylcholinesterase; and 3) Evaluate whether lifetime exposure to OP and carbamate insecticides affects steroid hormone metabolism and telomere length (a potential marker of long-term stress).

Title: Study of ALS in the Farming Environment (SAFE)

PI: Freya Kamel, Ph.D., MPH (NIEHS/DIR/EB)

Precis:

Objective: To evaluate the association of ALS with pesticide exposure and investigate gene-environment interaction. Background: ALS is a progressive neurodegenerative disease affecting motor neurons in the brain and spinal cord; symptoms include weakness and exaggerated reflexes. Patients generally die within two to five years of diagnosis. ALS likely has a multifactorial etiology, with both environmental exposures and genetic susceptibility playing a role. Exposures proposed to increase risk of ALS include pesticides, heavy metals, and electromagnetic fields. Design: The Agricultural Health Study (AHS) is a cohort of ~90,000 licensed pesticide applicators and their spouses in Iowa and North Carolina. Participants were first enrolled in 1993-97 by completing questionnaires, and two follow-up interviews have been conducted at five-year intervals. These contacts collected information on demographics, lifestyle, medical history, and lifetime pesticide exposure. Based on self-report or death certificates, there are ~60 AHS participants who may have ALS. We will contact these individuals or their proxies to request (1) permission to contact their physicians to obtain medical records and (2) a saliva sample from living cases to provide DNA. We will also attempt to retrieve buccal cell samples previously donated by deceased cases. Physicians of participants who consent will be requested to provide medical records. Outcome parameters: A study neurologist will make final ALS diagnoses based on available information. We will assess exposure using existing AHS data. Genotyping will focus on genes implicated in ALS or susceptibility to xenobiotic exposures. We will not collect DNA from controls for this study. Instead we will use data from 384 controls from the FAME study, another nested case-control study within the AHS with exposure and genotype data. Data analysis: Pesticide exposure analyses will compare the valid cases to the remaining AHS cohort using Poisson or Cox proportional hazards regression; genotype analyses will compare the valid cases to the FAME controls using logistic

regression. Assuming that we identify 40 actual ALS cases in the cohort, we will have 80% power (alpha=0.05) to detect relative risks of 2 to 5, depending on exposure and genotype prevalence. Thus this study has limited power. It is nevertheless worthwhile because it is the only study able to address the role of pesticides in ALS with detailed, prospectively collected exposure data. Further, additional cases will accrue as the AHS cohort ages, permitting future studies with greater power.

Title: Genes, Environment, and Age-Related Macular Degeneration (GENARM)

PI: Freya Kamel, Ph.D., MPH (NIEHS/DIR/EB)

Precis:

Age-related macular degeneration (AMD) affects an estimated 1.75 million people in the US, and in developed countries it is the leading cause of blindness among older adults. The overall goal of GENARM is to investigate the relationship of AMD to pesticide exposure. The study will be based in the Agricultural Health Study (AHS), a cohort study of licensed pesticide applicators and their spouses. Cohort members have provided information on physician diagnosis of AMD. GENARM has three objectives: (1) to confirm self-report of AMD using diagnostic information collected from cohort members' physicians; (2) to analyze the relationship of AMD to pesticide exposure, based on physician-confirmed cases; and (3) to evaluate the role of gene-environment interaction in AMD using case-only gene-environment analyses. The AHS cohort includes approximately 85,000 individuals, of whom approximately 57,000 completed follow-up telephone interviews in 1999-2003. Among the latter, 1,391 reported either a physician diagnosis of AMD or use of an Amsler grid, a diagnostic test used to monitor vision at home by individuals suspected by their physicians of having early AMD. We anticipate that 776 of 1,391 suspect cases will confirm a self-report of AMD and agree to participate in GENARM by providing contact information for their physicians. We will ask physicians to complete a diagnostic checklist based on medical records and to provide a retinal photograph if available. We anticipate that information provided by physicians will corroborate the self-reported diagnosis in 415 of 776 suspect cases. Using physician-confirmed cases, we will conduct a prospective analysis to classify the level of risk of AMD according to pesticide type and degree of exposure, comparing cases to the remaining AHS cohort. We will request that cohort members who participate in GENARM donate a saliva sample as a source of DNA; we anticipate that 621 of 776 individuals will provide saliva, including 332 of the physician-confirmed cases. We will analyze polymorphisms in several genes known to play a major role in AMD and evaluate interaction of these genes with pesticide exposure with case-only analyses. GENARM will have 80% power (alpha = 0.05) to detect an odds ratio of 1.5-2.0 for association of pesticide exposure with AMD, and similar power to detect gene-environment interactions.

Title: Veterans with Amyotrophic Lateral Sclerosis (ALS) and Lead Exposure (VALE)

PI: Freya Kamel, Ph.D., MPH (NIEHS/DIR/EB)

Precis:

The overall goal of the VALE study is to clarify the etiology of ALS. The study will address four hypotheses: (1) ALS risk is associated with blood lead levels; (2) genotype of ALAD, previously shown to affect lead toxicokinetics, affects the lead-ALS relationship; (3) bone turnover affects the lead-ALS relationship; and (4) ALS risk is associated with blood levels of mercury, manganese, cadmium, chromium, copper, or nickel. The study population will be drawn from the ongoing Duke University case-control study Genetic Epidemiology of ALS in Veterans, which is in turn based on the National Registry of Veterans with ALS conducted by the US Department of Veterans Affairs (VA). The VALE study will enroll 270 ALS cases and 200 matched controls. Cases will be selected among those enrolled in the VA ALS Registry after January 1, 2007. Controls will be selected among all those enrolled in the Duke ALS Study and frequency matched to cases on age, gender, race/ethnicity, and past VA health care use. The primary outcome measures will be biomarkers of metal exposure and bone turnover. For cases, blood samples already being collected in a metal-free tube for metal assays and a second will be used as a source of serum. For controls, a home visit will be conducted to collect two blood samples, one for metal assays and one for serum. Metals in whole blood will be measured using inductively coupled plasma mass spectrometry. Biomarkers of bone turnover in serum will be assessed using specific immunoassays to measure two markers of bone formation, bone-specific alkaline phosphatase and procollagen type I carboxyterminal peptide; and two markers of bone resorption, amino-terminal crosslinked telopeptide and tartrate-resistant alkaline phosphatase 5b. VALE will also have access to diagnostic information and other data from the medical record obtained by the VA ALS Registry for ALS cases and to genetic and risk factor information from the Duke ALS Study for cases and controls. Data will be analyzed using unconditional logistic regression. We will have greater than or equal to 80% power to detect odds ratios of 1.4 for hypotheses 1 and 4 and 1.8-2.0 for hypotheses 2 and 3.

Title: Exposure to Neurotoxins As Risk Factors For ALS: Measurement of Genes, Proteins, Neurotoxicants, and Other Factors Potentially Associated With ALS

PI: Freya Kamel, Ph.D., MPH (NIEHS/DIR/EB)

Precis:

Objectives: Stored blood and toenail samples are available from a case-control study of ALS conducted in 1993-1996. The purpose of the initial study was to examine the relationship to ALS of lead and other exposures, including mercury, pesticides, and solvents, as well as genetic susceptibility to these exposures. The protocol was closed in May 1997 (closed protocol #95-007) after recruitment of study subjects ended. The objective of the present proposal is to reopen the protocol to permit measurement in blood or toenails of genes, proteins, neurotoxicants, and other factors or agents potentially associated with ALS, as well as analyses of existing data. The short-term goal is to measure (i) polymorphisms in DNA repair genes; (ii) serum protein profiles; and (iii) serum organochlorine pesticide levels. Other factors may be of interest in the future, for example polymorphisms in other genes or levels of metals in blood or toenails. We also plan (iv) to continue analyses of existing data. Some data analysis will

involve combining data from the original ALS study with data from the Harvard Normative Aging Study, a cohort study of World War II verterans that has collected demographic, lifestyle, and medical data as well as information on bone and blood lead levels. Study population: No new subjects will be recruited for this protocol. The original study involved 110 ALS cases, 31 hospital controls with other neurologic diseases, and 256 population controls, recruited in New England between 1993 and 1996. The population controls were frequency matched to the cases on the basis of age, gender, race, and region within New England. Design: The original study was a case-control study. Data collection involved an interview collection of blood and toenail samples, and measurement of bone lead using x-ray fluorescence. Stored blood and/or toenail samples are available from 107 ALS cases, 31 hospital controls, and 39 population controls. The present study will use conventional techniques to measure genetic polymorphisms and serum organochlorine levels and newly developed SLDI-TOF technology to evaluate serum protein profiles. Outcome parameters: The outcome parameter is risk of ALS. Associations with ALS risk will be evaluated for polymorphisms in DNA repair genes; serum protein profiles; and organochlorine pesticide levels in serum.

Title: Farming and Movement Evaluation (FAME) Study

PI: Freya Kamel, Ph.D., MPH (NIEHS/DIR/EB)

Precis:

This study is a collaboration of investigators at the Parkinson's Institute in Sunnyvale, CA, with intramural investigators at NIEHS and NCI. The Principal Investigator is Dr. Caroline Tanner, Director of Clinical Research at the Parkinson's Institute. The study has been reviewed by Study Section and approved for funding by the Extramural Division of NIEHS. Investigators at the Parkinson's Institute will be responsible for enrolling participants and conducting interviews. Intramural investigators at NIEHS will be responsible for collecting, storing, and analyzing biological and environmental samples. An intramural investigator at NCI will serve as a consultant. The long-term goal of this research is to elucidate the cause(s) of Parkinson's disease (PD), with a focus on environmental determinants. We propose to investigate the relationship between PD and exposure to pesticides or other factors by conducting a nested case-control study in the Agricultural Health Study (AHS), using a 1:3 case-control ratio and employing classical methods for multivariate analysis. We will enroll 160 PD cases and 480 controls from the AHS cohort. Diagnosis of PD will be confirmed with a neurologic exam. We will supplement information on pesticide and other exposures already collected from the AHS cohort with additional interviews and measurements in blood, household dust, and soil. The study has five specific aims. Aim 1 will test the hypothesis that pesticide exposure increases PD risk using self-reported (life history) and direct (blood, dust) measurements. Aim 2 will test the hypothesis that non-pesticide chemical exposure increases PD risk, using a job-task-based occupational history, and blood testing. Aim 3 will test the hypothesis that exposure to the soil pathogen Nocardia asteroides is related to PD using a battery of assays in blood, soil, and dust. Aim 4 will assess the role of specific lifestyle and health factors previously reported to alter PD risk. Aim 5 will assess the effects of specific polymorphisms of xenobiotic metabolizing

enzymes previously linked to PD. The study will take full advantage of the AHS, a unique, prospectively studied cohort of licensed pesticide applicators and their spouses. We believe that this work could provide a critical and dramatic next step in furthering our knowledge of the environmental determinants of PD, and thereby take us closer to our goal of finding its cause.

Title: Genetic Susceptibility to Childhood Respiratory Illness in Mexico City

PI: Stephanie London, M.D., DrPH (NIEHS/DIR/EB)

Precis:

We propose to add a collection of genetic material to a clinical trial of anti-oxidant supplementation for the amelioration of asthma in 7-12 year olds being conducted at a public pediatric hospital in Mexico City. The anti-oxidant study has been approved by the Institutional Review Board of the National Institute of Public Health in Mexico City and is scheduled to begin in September 1998. The purpose of this add-on study is to examine genetic polymorphisms that may be related to asthma. Asthma cases will be compared with their parents as controls. In particular, we will save the buffy coat from the blood collection being done on the asthmatic child for measurements of plasma micronutrients in the anti-oxidant trial for extraction of DNA. This part of the sample would otherwise be discarded. In addition, we will enroll the parents of the asthmatic child as controls for the child. From the parents, either a 10 ml blood sample or if they prefer, a sample of buccal cells will be collected. Although our current plan is to compare the asthmatic child to the parents using statistical methods based on the "transmission disequilibrium test" because research into various family designs for candidate gene studies is rapidly evolving and various sibling control sample strategies have been discussed, we would also like to collect a genetic sample on as many of the child's siblings as possible with priority given to those closest in age. This will enhance the future usefulness of the samples. We anticipate enrolling approximately 200 families.

Title: A Cohort Study of Smoking Prevention and Health Promotion for Middle School Students in Wuhan, China

PI: Stephanie London, M.D., DrPH (NIEHS/DIR/EB)

Precis:

We propose to add a collection of buccal cells to a school-based cohort of 7th graders in Wuhan, a large industrial city in China. The cohort study is being conducted by the Wuhan Public Health and Anti-Epidemic Station (Li Yan MD, director and principal investigator). The cohort study is designed to look at several outcomes. One is initiation of smoking. The second is respiratory health in relation to active and passive smoking and other environmental exposures that are prevalent in Wuhan. The respiratory outcomes include changes in pulmonary function, asthma and other respiratory symptoms. Collection of buccal cells is a noninvasive method of obtaining DNA. The addition of a genetic sample will enable us to examine candidate gene associations for asthma and childhood respiratory illness within an interesting and well-

characterized Chinese population. In addition, it provides the capability to examine gene environment interaction with respect to common environmental exposures in Wuhan. The ability to examine gene-environment interaction can help to identify relatively subtle effects of pollutants such as environmental tobacco smoke which is becoming a very common exposure due to the major increase in smoking among Chinese men. Other exposures of interest in Wuhan are indoor coal burning and high ambient exposures to particules, ozone and nitrogen oxides. The proposed study has been approved by the human subjects committee of the Wuhan Public Health and Anti-Epidemic Station.

Title: Anonymous Sample Collection for Laboratory Assay Development and Testing (London)

PI: Stephanie London, M.D., DrPH (NIEHS/DIR/EB)

Precis:

In planning an epidemiologic study involving the analysis of laboratory specimens, it is necessary to evaluate the performance of available assays in the target population. It is essential to know if an assay is sufficiently reproducible such that differences in levels among individuals in the target population can be detected. In addition, if one is choosing between methods or between laboratories, it is important to have replicate samples from the target population to be able to choose the lab with greater precision. In deciding whether an assay is appropriate it is also necessary to know whether the samples handling the collection protocol used in a field study alters the measured levels. It may also be of interest to determine whether a non-invasive sample, such as urine or saliva, would be a good proxy for a blood sample. For all of these purposes, it is important to collect samples from the target population as levels of analytes will differ by gender, age and other characteristics. We propose a generic protocol for use in obtaining such blood or noninvasive samples anonymously from small numbers (up to 10 subjects completing a protocol) of healthy adult volunteers for use in laboratory assay evaluation. Volunteer subjects will be given informed consent. Assays could include evaluation of immunologic parameters, DNA, peptides, proteins, etc. Samples to be collected will include blood and/or urine and and/or saliva. The samples will be used to ascertain whether new tests are sufficiently valid and precise to be used in epidemiologic studies. Any other studies will be submitted for approval separately. For pregnant individuals, only a one time collection would be done and the maximum amount of blood would be 40 ml. For non-pregnant individuals, up to four collections could be done over a 16 week period and the amount of blood could be up to 100 ml of blood on any one occasion, although the typical amount would be 40 ml.

Title: Dietary and Genetic Factors in Asthma & Chronic Bronchitis in a Cohort of Chinese Singaporeans

PI: Stephanie London, M.D., DrPH (NIEHS/DIR/EB)

Precis:

There is suggestive evidence for a role of diet in the etiology of asthma and chronic bronchitis. However, there are few prospective data. We propose to expand our

collaboration with the Singapore Chinese Health Study to examine dietary, environmental, and genetic factors, along with their interactions, in relation to the risk of developing asthma and chronic bronchitis. The Singapore Chinese Health Study is a cohort of 63,257 men and women of Chinese ethnicity in Singapore who were aged 45-74 years at enrollment from 1993 to 1998. Telephone follow-up of the cohort to update and outcome information began in 1999 and is ongoing. We expect to identify 538 cases of incident asthma and 672 cases of incident chronic bronchitis when the current follow-up questionnaire cycle is complete in 2004. In this proposal, we would validate self-reports of incident asthma, obtain follow-up data from the entire cohort to perform analyses of diet and smoking in relation to these outcomes, and analyze genetic material on cases of incident asthma and chronic bronchitis and controls from the cohort. In this proposal we will examine the following hypotheses: 1. Higher intake of fruits and/or antioxidant micronutrients decreases the risk of developing asthma and chronic bronchitis. a. Effects of fruit and/or antioxidant micronutrients may differ by smoking history. 2. Common polymorphisms in genes involved in the response to oxidative stress influence the risk of asthma and chronic bronchitis. We initially propose to examine polymorphisms in three genes--glutathione S-tranferase M1, glutahione S-transferase P1, and matrix metalloproteinase-1. However, we plan to examine additional relevant polymorphisms in the future, especially taking advantage of high throughput screens of candidate genes for asthma and chronic bronchitis. It is possible that by 2004 when the sample set will be available that more compelling candidates and high throughput screens may be available to us at a low cost. Thus we will re-evaluate our choice when the samples are available. 3. Polymorphisms in these and other genes interact with fruit/antioxidant intake and/or smoking to influence the risk of asthma and chronic bronchitis.

Title: Perfluorinated Alkyls and Fecundability
PI: Matthew Longnecker, M.D., Sc.D. (NIEHS/DIR/EB)
Precis:
Perfluoroalkyl chemicals are used to make fluoropolymers and other materials that are heat resistant, impermeable, and stain resistant. They have been used as coatings for fabrics, leather, upholstery, carpets, paper, and in many other applications. Teflon®, Scotchgard®, and Stainmaster® are examples of perfluoroalkyl-based products. As a consequence of manufacturing these products two persistent organic pollutants are created: perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). These chemicals are ubiquitous in biota, including humans, worldwide. Humans are probably exposed via air and dust, but diet seems to be the major source. These compounds reside mostly in the liver and plasma, and bind to proteins, e.g., albumin. They are poorly metabolized. Excretion is not well understood but may be via bile and urine. Recently it has been demonstrated that these compounds can be accurately measured in plasma. Fei et al. has reported a strong association between the level of PFOS and PFOA and increased time-to-pregnancy (decreased fecundability). No biologic mechanism was obvious and corresponding findings in animals have not been reported, except for increased fetal loss at very high doses. Because of the strength of the association and its potential importance if it were causal, we propose to follow-up on

this finding using existing samples and data from the Norwegian Mother and Child (MoBa) Cohort Study. In addition to the hypothesis on fecundability, other recent studies suggest associations with size at birth, growth, and thyroid metabolism, and our study will also address these issues. We will analyze plasma for PFOS, PFOA, and albumin previously collected from 450 women who had a time-to-pregnancy of >12 months, and from 500 women who are selected at random from cohort members. The specific aims of the study are: 1. Test the hypothesis that higher blood levels of PFOS and PFOA are associated with a longer time-to-pregnancy in a nested case-control study of women in the Norwegian Mother and Child Cohort (MoBa) study. 2. Investigate several related matters such as whether levels of PFOS and PFOA are related to child growth, TSH levels on neonatal screening, and what the determinants of the plasma levels are (e.g., diet, etc.).

Title: Study of DDT and Loss of Clinically-Recognized Pregnancies in South Africa

PI: Matthew Longnecker, M.D., Sc.D. (NIEHS/DIR/EB)

Precis:

We will conduct a study of exposure to DDT in relation to loss of clinically-recognized pregnancies among women in South Africa. We will obtain a blood specimen from the women (n = 2,400) before pregnancy and then among those who become pregnant (n = 1,200), we will obtain a second blood sample at the time the pregnancy is verified. We will then determine the outcome of the pregnancy and collect a third sample of blood. The primary goal will be a nested case-control study among a subset of these 1,200 women that will examine the relation of pre-pregnancy DDT level with odds of loss of clinically-recognized pregnancies. Women who have a pregnancy loss that is less than six months gestation will have a fourth blood sample collected for HIV testing. A pilot study will first be conducted with 400 women (200 women each from sprayed and unsprayed villages). The goal of the pilot is to evaluate the field procedures. Additional goals are to carry out a reproducibility study among a subset of women (n = 200) to examine the reproducibility of levels of DDT, and a separate study among a subset of women (n = 200) to examine determinants of DDT levels.

Title: Temporal Variability of Prenatal Exposure to Organophosphate Pesticides and Bisphenol A in the MoBa Cohort

PI: Matthew Longnecker, M.D. Sc.D. (NIEHS/DIR/EB)

Precis:

We propose to measure the levels of metabolites of OP pesticides and BPA in the urine samples that were collected at 17, 23 and 29 weeks of gestation, in the “Reliability Substudy” of The Norway Mother and Child study (MoBa). The Reliability Substudy is ongoing and started in November of 2007, and by the end of 2008, ~650 women from four hospitals (St Olavs Hospital HF, Sykehuset Østfold HF in Fredrikstad, Sykehuset Sunnmøre HF in Ålesund, and Stavanger Universitetssykehus HF) will have been enrolled. For this study, urine samples from 160 subjects will be selected at random from the “Reliability Sub-study” of the MoBa Cohort.

Title: Anonymous Sample Collection for Laboratory Assay Development and Testing (Longnecker)

PI: Matthew Longnecker, M.D. Sc.D. (NIEHS/DIR/EB)

Precis:

In planning an epidemiologic study involving the analysis of laboratory specimens, it is necessary to evaluate the performance of available assays in the target population. It is essential to know if an assay is sufficiently reproducible such that differences in levels among individuals in the target population can be detected. In addition, if one is choosing between methods or between laboratories, it is important to have replicate samples from the target population to be able to choose the lab with greater precision. In deciding whether an assay is appropriate it is also necessary to know whether the sample handling and collection protocol used in a field study alters the measured levels. It may also be of interest to determine whether a non-invasive sample, such as urine or saliva, would be a good proxy for a blood sample. For all of these purposes, it is important to collect samples from the target population as levels of analytes will differ by gender, age and other characteristics. We propose a generic protocol for use in obtaining such blood or noninvasive samples anonymously from small numbers (up to 18 subjects completing a protocol) of healthy adult volunteers for use in laboratory assay evaluation. Volunteer subjects will be given informed consent. Assays could include evaluation of immunologic parameters, DNA, peptides, proteins, etc. Samples to be collected will include blood and/or urine and/or saliva. The samples will be used to ascertain whether new tests are sufficiently valid and precise to be used in epidemiological studies. Any other studies will be submitted for approval separately. For pregnant individuals only a one time collection would be done and the maximum amount of blood would be 50 ml. For non-pregnant individuals up to four collections could be done over a 16 week period and the amount of blood could be up to 450 ml of blood total in this period, although the typical amount would be 50 ml.

Title: The Norwegian Mother and Child Study - Environmental Specimen Collection

PI: Matthew Longnecker, M.D., Sc.D. (NIEHS/DIR/EB)

Precis:

The Norway Mother and Child study is a collaborative venture among health researchers in Norway funded by the Norwegian government. The study is being coordinated by the National Institute of Public Health (aka Folkehelsa) in Oslo and the Medical Birth Registry (MBR) in Bergen. NIEHS has the unique opportunity to participate through the collection of additional tubes of blood during the blood sample collection; these two tubes of blood and a urine sample will allow NIEHS and collaborators to explore environmental determinants for disease among women and their children. These additional samples will remain in the Biobank in Oslo, Norway, with other samples from cohort members and will be used under collaboration with Norwegian investigators. To achieve better health for mothers and children in the future, the Norway Mother and Child study is designed to test specific hypotheses about the

causes of a number of serious diseases by recruiting 110,000 pregnant women to a cohort study. As part of the primary aim of the study, women will be asked to provide a blood sample at 17 weeks gestation, at birth, and 4 days after birth. The NIEHS samples will be collected at the blood draw at 17 weeks gestation. Likely causal factors will be linked to information obtained from questionnaires, blood samples, and medical registers. The Norway Mother and Child study has multiple endpoints. Primarily those associated with adverse pregnancy outcomes will be studied, but also diseases affecting mother, father or child. Endpoints will be taken from questionnaires and medical registers. The study will be carried out nationally and any research groups with relevant questions will be able to participate. The Norway Mother and Child study has been approved by the Norwegian Parliament as well as their Data Inspectorate to ensure that the study and all protocols conform to Norwegian ethical standards as well as appropriate research ethical criteria. Further, the project has been evaluated by the Regional Ethics Committee for Medical Research which has approved all modifications to the project. Three new sub studies have been added since the start of the main protocol. 1. NIEHS will add an additional reliability sub-study. 900 woman who are currently enrolled in the main Norway Mother and Child study at four selected hospitals and have successfully completed the blood draw and urine collection at week 17 of gestation will be asked to donate two additional blood and urine collections at weeks 23 and 29 of gestation. The sub-study is being conducted to determine if biomarkers are stable during pregnancy. 2. An additional pilot study has been planned to add a follow-up questionnaire when children reach seven years of age. Although the goal is to follow all children with this questionnaire as they reach seven, the first four thousand children to receive the questionnaire will be part of a pilot test of which types of reminders will yield the best response rate, thus producing the most valid data. The comparison is between cell phone text message and repeat paper copy of the questionnaire. The purpose of the seven year questionnaire is to expand the capacity of the study to enable studies of early life exposures, genetics, and their interactions in relation to asthma and other common childhood diseases. 3. An additional new folate receptor sub-study, stored plasma samples from mothers of cases (children with birth defects) and randomly selected controls (children without birth defects) will be assayed to detect folate receptor antibodies. Plasma specimens collected at week 17 of pregnancy and at delivery will be used. Other researchers, nationally and internationally, will have access to the cohort on request and following approval from the project's executive group. NIEHS has the opportunity to add additional biological specimens for blood and urine to the base cohort. Two additional tubes of blood (total volume 9 ml) and a urine sample will be collected as part of the routine prenatal ultrasound visit and blood sample collections included in the overall study protocol. As part of a reliability sub-study, blood and urine samples will also be collected an additional two times, at weeks 23 and 29 of gestation. These samples are designed to allow investigators to explore environmental contributors to the health of women and their children. Low level exposure to environmental contaminants occurs in all industrialized countries, though the level of exposure may differ as the result of diet, cooking practices and pollution sources. However, the ability to explore the role of environmental exposure on health is often more limited by good population based information on health and disease then by exposure level. Thus, by creating a biological specimen repository in a country with

excellent disease registries, it will allow NIEHS to explore risk factors for disease relevant to US populations. All samples will be stored in Norway and will be used in collaboration with Norwegian and other investigators. NIEHS investigators will not have access to identifying information. NIEHS samples will not be used for genetic analyses.

Title: Effect of the Antiandrogen DDE on Anthropometric Measures at Birth

PI: Matthew Longnecker, M.D., Sc.D. (NIEHS/DIR/EB)

Precis:

We propose to follow the women and children enrolled in our original study (n= approximately 850 of each). In the original study, women were enrolled and interviewed while in the hospital for delivery, their blood was drawn, and anthropometric measurements were performed on their newborn male infants. The follow-up will be done primarily to determine the number of months that the mother breast feeds her child. Secondary endpoints will be infant infection as reported by the mother, and child growth as determined by measurement of height and weight and related measures (none in the genital region, as in the original study). Breast feeding duration, infections, and growth may be related to exposure to the DDT metabolite, DDE. The follow-up visits will be every three months from 6 to 18 months after birth, and study nurses will visit subjects in their home. For some subjects, there would be fewer follow-up visits, due to study scheduling or breastfeeding cessation. Mothers would be interviewed and mothers and children will undergo standard anthropometric assessments. This protocol does not call for collection of biologic specimens and poses minimal risk to subjects.

Title: Treatment of Lead-Exposed Children (TLC) Trial

PI: Walter Rogan, M.D. (NIEHS/ DIR/EB)

Precis:

At TLC enrollment, the children were between 12 and 33 months of age with baseline blood lead levels (PbB) between 20 and 44 microg/dl. Of 1,854 referred children who were screened for eligibility, 780 were randomized to the active drug (oral succimer) and placebo groups, stratified by clinical center, body surface area, blood lead level and language spoken at home; only the New Jersey Clinical Center enrolled Spanish-speaking participants. Up to three 26-day courses of succimer or placebo therapy were administered depending on response to treatment in those who were given the active drug. Eighty-nine percent of children had finished treatment by six months, with all children finishing by 13 months after randomization. Residential lead clean-up and nutritional supplementation with multivitamins and minerals were provided to all study children, irrespective of treatment group. Children were followed for three years, with regular physical exams, psychological and developmental testing, and measurement of lead concentration in venous blood. Treatment and follow up are closed for this trial; it is open for scientific analysis and report writing only. Although succimer lowered blood lead levels much more effectively than placebo, there was no difference between the two groups on any of the psychological tests at three years post randomization, when most children were about five years old. Follow up of TLC children continued into school

age. At age seven, 647 of 780 subjects remained in the study. Children were tested at age seven and again at seven and a half on standardized neuropsychological batteries that tap cognition, behavior, learning and memory, attention, and neuromotor skills. While chelation therapy with succimer had lowered average blood lead levels for approximately six months, it resulted in no benefit in cognitive, behavioral and neuromotor endpoints when measured at school ages in these children. These additional follow-up data confirm our previous finding that the TLC regimen of chelation therapy is not associated with neurodevelopmental benefits in children with blood lead levels between 20 and 44 microg/dL.

Title: Anonymous Sample Collection in Children for Collection, Processing and Laboratory Assay Evaluation

PI: Walter Rogan, M.D. (NIEHS/ DIR/EB)

Precis:

In planning an epidemiologic study involving the collection of laboratory specimens, it is necessary to evaluate collection and processing protocols as well as the performance of available assays in the target population. It is essential to know if a specimen collection and processing technique is adequate to obtain a satisfactory specimen sample for analysis and whether an assay is sufficiently reproducible such that differences in levels among individuals in the target population can be detected. In addition, if one is choosing between methods or between laboratories, it is important to have replicate samples from the target population to be able to choose the method or lab with greater precision. In deciding whether an assay is appropriate it is also necessary to know whether the sample handling and collection protocol used in a field study alters the measured levels. It may also be of interest to determine whether a non-invasive sample would be a good proxy for a blood sample. For all of these purposes, it is important to collect samples from the target population as levels of analytes will differ by gender, age and other characteristics. This is especially true for studies involving sample collections from children since sample volume limitation and levels of analytes differ between adults and children. Therefore, we propose a generic protocol for use in obtaining biological samples anonymously from healthy children volunteers (up to 100 subjects completing a single use of the protocol) for use in specimen collection, processing and laboratory assay evaluation. The parents/legal guardians of volunteer subjects will give informed consent. Assent will be obtained from all subjects who are between the age of 7 and 17 years. As with other anonymous sample collection protocols at the National Institute of Environmental Health Sciences (NIEHS) and the NIH Clinical Center, this protocol will serve as a generic protocol to obtain biological samples anonymously and IRB approval will not be required prior to each individual use of the protocol. This protocol may be used multiple times each year with the limitation that only 100 subjects can be enrolled for any given use. However, a detailed summary of each use of the protocol will be reported during the protocol's annual continuing review. Changes to the protocol (including the addition of investigators who play a role in the revision of the protocol), informed consent forms, other information to be completed by participants (e.g., survey instruments or questionnaires), and any proposed advertising/ recruitment materials) will receive IRB approval prior to

implementation. The investigator will notify the IRB of serious adverse events and protocol violations. A broad range of analysis may be performed on the sample including but not limited to immunologic parameters, DNA or RNA sequence, peptides and proteins. Histological evaluations may also occur. Cells may be immortalized. No assay information will be returned to the subject, since all samples are strictly anonymous. Samples to be collected can include blood, buccal cells, exhaled breath, hair, household dust, nail clippings, nasal mucosa cells, saliva, shed teeth, stool, urine and/or vaginal wall cells. The samples will be used to ascertain whether collection and processing techniques as well as new tests are sufficiently valid and precise to be used in epidemiologic studies. Up to five blood collections from a single subject could be done over a one year period. Depending of the weight of the child, the amount of blood could be up to 100 ml (7 tablespoons) total in this one year period, although the typical amount would be 3-20 ml in a single blood withdrawal. All blood draws will be within NIH Clinical Center guidelines for pediatric patients of no more than 3 ml/kg in a single blood withdrawal, and no more than 7 ml/kg over any six-week period.

Title: Study of Estrogen Activity & Development (SEAD) - SEAD 1 Sonography

PI: Walter Rogan, M.D. (NIEHS/ DIR/EB)

Precis:

Term newborns have anatomic and sonographic evidence of in utero estrogen exposure. Over the first six months to a year of life, as the influence of maternal hormone wanes, these findings recede. Soy infant formula contains large amounts of isoflavones (daidzein and genistein) that can occupy estrogen receptors and act as estrogens in the laboratory. A food substance with estrogen activity might prolong the effect of maternal hormones, or interfere with hormonal homeostasis in the child. This cross-sectional pilot study will be undertaken in order to establish methods for a future multi center study designed to assess the potential effects of soy formula on estrogen activity in infants. In this pilot study (SEAD 1), 156 examinations on full-term infants, ages 0-12 months and on one of three feeding regimens (soy formula, breast milk or cow's milk formula) will be completed to see if their exposure to soy estrogen compared to breast milk and non-soy formulas prolongs anatomical evidence of estrogen exposure and response. A schedule of visits for SEAD 1 is included in Appendix A. In addition to SEAD 1, two other cross-sectional studies (SEAD 2 and SEAD 3) will be undertaken to evaluate anatomical evidence by physical examination (SEAD 2) and assess the hormonal and biochemical status of full-term infants ages 0-12 months (SEAD 3). These studies will be conducted at Children's Hospital of Philadelphia (CHOP) in parallel with the SEAD 1 study. Further details of SEAD 2 and 3 will not be presented in this protocol. The purpose of the SEAD 1 pilot study is twofold. The first is to develop and finalize recruitment and collections procedures in preparation for a larger, comprehensive trial of biological response in children to soy formula with and without the estrogenic isoflavones. The second is to study the natural history of estrogen activity in infants on a variety of feeding regimens.

Title: Study of Estrogen Activity & Development (SEAD) SEAD2: Physical Exam and Ballard Markers; Sead 3: Biochemistry

PI: Walter Rogan, M.D. (NIEHS/ DIR/EB)

Precis:

Term newborns have anatomic and sonographic evidence of in utero estrogen exposure. Over the first six months to a year of life, as the influence of maternal hormone wanes, these findings recede. Soy infant formula contains large amounts of isoflavones (diadzein and genistein) that can occupy estrogen receptors and act as estrogens in the laboratory. A food substance with estrogen activity might prolong the effect of maternal hormones, or interfere with hormonal homeostasis the child. Two cross-sectional pilot study (SEAD 2 and SEAD 3) will be undertaken in order to establish the methods for a future, multi-center study designed to assess the potential effects of soy formula on estrogen activity in infants. Another cross-sectional pilot study (SEAD 1) will be undertaken to evaluate anatomical evidence detected by ultrasound and will be conducted at The Children's Hospital in Boston. Details of the SEAD 1 study will not be presented in this protocol. In the first pilot study (SEAD 2), 84 examinations of full-term infants, ages 0-6 months and on one of three feeding regimens (soy formula, breast milk or cow's milk formula) will be conducted. This study will establish a protocol for examining and sampling methods to be used for a later trial investigating whether exposure to soy estrogen compared to breast milk and non-soy formulas prolongs anatomical evidence of estrogen exposure and response. In addition to the physical examination, vaginal cells will be collected for cytology analysis. Breast nipple secretions, if present, will also be collected and analyzed for protein and sugar levels. In the second pilot study (SEAD 3), 372 evaluations on full-term infants will be conducted in parallel with the first study. SEAD 3 will assess the hormonal and biochemical status of full-term infants age 0-12 months. This study will establish a protocol for sampling methods to be used for collecting urine, saliva, serum, and hair samples. Endogenous hormone levels such as testosterone, estradiol, and isoflavone levels (genestein and diadzein) will be measured in serum and the results will be compared against the non-serum tests (urine, saliva and hair). The purpose of these two pilot studies is twofold. The first is to develop and finalize recruitment and collections procedures in preparation for a larger, comprehensive trial of biological response in children to soy formula with and without the estrogenic isoflavones. The second is to study the natural history of estrogen activity in infants on a variety of feeding regimens.

Title: Validation of Intrauterine and Early Life Exposures in the Sister Study

PI: Dale Sandler, Ph.D. (NIEHS/DIR/EB)

Precis:

Early life exposures may affect a woman's later risk of breast cancer and other health conditions. The Sister Study, a cohort study of breast cancer and other health conditions in 50,000 US and Puerto Rican women, aged 35 to 74, whose sister had breast cancer, asked participants information about their early life including during their mother's pregnancy and their family history of cancer and other diseases. Because this information may be difficult to recall, it is important for us to evaluate how well women were able to answer these questions. Therefore, we will conduct a study to determine

whether women's reporting of early life factors including those related to their mother's pregnancy is in agreement with information reported by their mother. We will also evaluate whether there are any study characteristics that predict differences in agreement between women and their mothers. We will invite a random sample of approximately 1,400 women with living mothers from the participants who were ages 35 to 59 when fully enrolled in the Sister Study as of December 2009. If our random sample of 1,400 does not have sufficient numbers with positive responses to rare exposures, we will select additional women reporting rare exposures resulting in up to 2,200 women in our total sample. We expect that approximately 1,500 mothers will complete our study based on an assumption of a 70% response rate. We will mail the selected participants a family history questionnaire that is modified to reflect the maternal point of view with a letter for participants to send to their mother to invite her to participate in our study. Participants will also be asked whether they contacted their mother or other relatives when they filled out the original questionnaire and to send us a copy of their birth certificate or birth announcement. We will not contact mothers directly. Mothers will complete the questionnaire and will be provided with a postage-paid envelope to return to us. We will allow mothers to contact us if they prefer to answer questions by phone. We will estimate measures of accuracy (sensitivity, specificity, and positive predictive value) for self-reported information in which we consider the maternal report or birth certificate as the gold standard. We will also estimate Cohen's kappa coefficients, a measure of agreement between the maternal and self-report of information.

Title: Pesticide Exposure and Health Status in NC African American Male Farmers and Farm Workers

PI: Dale Sandler, Ph.D. (NIEHS/DIR/EB)

Precis:

We propose to conduct a study to increase our understanding of farming practices and of the potential for previous exposure to DDT in North Carolina African American male agricultural workers, a group especially likely to have had high DDT exposure. Because of potentially widespread DDT exposure from agricultural activities, African American farmers and farm workers may be uniquely at risk for any associated health effects. However, little is known about levels of pesticide exposure in African American farming populations. Three hundred and eighty-nine African American men who are current or former farmers or farm workers will be asked to participate in the study. These men are members of the Supplemental Minority Cohort of the NCI/NIEHS/EPA Agricultural Health Study (AHS). The AHS is a prospective study of the potential health effects associated with agricultural exposures. Men in the supplemental cohort were recruited in 1996. At that time, they completed an enrollment questionnaire that focused on lifetime agricultural exposures, demographics, lifestyle factors and health. In this new study, a follow-up questionnaire will be administered by telephone by a trained interviewer to all men. Following the interview, the participant will be asked to donate a blood specimen, to have anthropometric indices (e.g., height, weight, waist and hip circumference, etc.) measured, and to have a top- and side view Polaroid photograph of their head taken during one visit to a central community location, such as the local

church or health clinic. We will quantitatively document serum p,p'-DDE levels, correlate these with self-reported farming activities and DDT exposure, and describe the health status of these men. We will also correlate serum p,p'-DDE levels and androgen concentrations, since p,p'-DDE is thought to be a potent androgen receptor antagonist. This exploration of the potential link between levels is important because the demonstration of any subclinical physiological effects of p,p'DDE would increase the plausibility of a wide range of health effects that have been postulated to be associated with the endogenous endocrine environment and the so-called endocrine disrupters. In addition, we will evaluate the relationship between several important physiological parameters which can be easily and accurately measured in serum, and physical characteristics including hair patterning and distribution of body fat in these African American men.

Title: The Growth and Puberty Study: Agricultural Exposures and Puberty Onset in the Agricultural Health Study

PI: Dale Sandler, Ph.D. (NIEHS/DIR/EB)

Precis:

Children of pesticide applicators are exposed to a variety of chemicals with the potential to disrupt endocrine function. Recent studies have determined that in utero and early childhood exposure to endocrine disrupting chemicals can result in altered pubertal development that may have long term health consequences for affected children. Longitudinal investigations of puberty in healthy children have relied largely on Tanner staging, a qualitative estimate based on physical appearance and external signs of pubertal development. While Tanner staging has many advantages, it can be unreliable, subjective and uncomfortable for participants. More objective measures such as alterations in serum hormone concentrations require invasive procedures, which limit their utility for longitudinal studies of healthy children. However, hormone levels can now be measured in the urine and saliva of children, making longitudinal assessment more acceptable. Little is known about the normal levels of these hormones in children and the relationship of urinary and salivary hormone levels to the external signs of puberty. We propose to conduct a pilot study among children whose parents enrolled in the Agricultural Health Study (AHS) cohort to support the development of a larger prospective study of pubertal development in this population using a suite of non-invasive measures. The study will provide preliminary information on urinary and salivary hormone concentrations, and timing and magnitude of diurnal variation in urinary hormone concentrations and supply information vital to the refinement of the data collection proposed for the larger study. For example, we will determine the feasibility of using parent and child-assessed Tanner stages to replace clinical assessment and explore the optimal timing of data collection among participants. This study will add to our knowledge of the inflection points of anthropometric measures (e.g. points at which, for example, changes in the rate of height velocity occur), and of the relationships between changes in anthropometric measures and non-invasive pubertal biomarkers. This study also will provide preliminary data on potential links between farm exposures, and the timing of puberty and development among the children of pesticide applicators. A total of 102 Iowa families with at least one child between the ages of 7

and 15 (girls 7-14, boys 7-15) will be enrolled. Data will be collected at three time points over a six-month period. In addition to urine and saliva samples, children and parents will be asked to provide questionnaire data on farm activities, medical history, and growth.

Title: Anonymous Sample Collection for Quality Control of Biological and Environmental Specimens and Assay Development and Testing

PI: Dale Sandler, Ph.D. (NIEHS/DIR/EB)

Precis:

We propose a generic protocol for use in obtaining blood, urine, saliva and dust samples anonymously from adult volunteers for use in laboratory assay evaluation. The samples will be used to determine if new tests are sufficiently valid and precise to be used in epidemiologic studies and for quality control purposes. In epidemiologic studies involving the storage and analysis of laboratory specimens, it is essential to evaluate the performance of available assays in the target population and to actively assess the impact of storage and handling on the quality of samples and laboratory analyses. Specifically, it is essential to 1) demonstrate that an assay is sufficiently reproducible and stable over time such that differences in levels among individuals in the target population can be detected; 2) evaluate assay reliability by sending blinded anonymous samples along with study samples to testing labs for quality control purposes; 3) test proposed laboratories or methods by using replicate samples from the target population in order to choose the laboratory or method with the greater precision; and 4) assess how sample collection, handling, and storage procedures affect measured levels of specific analytes to allow for informed decisions about whether to proceed with a specific analysis. Under this protocol, assays could include evaluation of immunologic parameters, genes and gene products, peptides, proteins, hormones, lipids, viability of cells, evaporation and dilution effects, chemical toxins and their metabolites, micronutrients, metals, and more. For some samples, lymphocytes will be extracted for use in studies of DNA damage and for creation of cell lines. Assays will be performed on lymphocytes prior to freezing and the remaining sample will be cryopreserved to facilitate validation of studies involving frozen lymphocytes and/or immortalized cells. Samples to be collected will include blood and/or urine and/or household dust and/or saliva samples. Although this protocol may be used to evaluate assays for other studies, the primary purpose for this proposal is to provide quality control samples for the Sister Study. Since it is important to collect samples from the target population, this protocol covers collection of samples from non-pregnant women age 35-74 years who have not been diagnosed with breast cancer. The samples will be collected and stored using protocols developed for the Sister Study. This protocol covers two types of collection from non-pregnant adult women. Up to 200 volunteer women will be asked to provide a one-time collection of one blood sample (up to 45 ml) and one urine sample. A subset may be asked to provide a dust sample and/or saliva sample for assay evaluation or develoment purposes. A second group of up to 100 non-pregnant volunteer women will be asked to provide blood (45 ml), urine, household dust, and possible saliva- on as many as seven occasions over a period of approximately one year.

Title: The Sister Study: Environmental and Genetic Risk Factors for Breast Cancer

PI: Dale Sandler, Ph.D. (NIEHS/DIR/EB)

Precis:

We will study environmental and genetic risk factors for breast cancer in a cohort of 50,000 sisters of women who have had breast cancer. In the United States, there were approximately 192,000 new cases of breast cancer in 2001, with more than 200,000 cases expected in 2002. Breast Cancer accounts for over 30% of all new cancer cases among women and 15% of cancer deaths. The etiology of breast cancer is complex, with both genetic and environmental factors playing a role. By focusing on a genetically susceptible group, more precise estimates of the contribution of environmental and other non-genetic factors to disease risk may be possible. The cohort will be followed actively for the development of breast cancer and other diseases. We expect, on average, 300 new cases of breast cancer to be diagnosed each year in a cohort of 50,000 sisters aged 35-74 years. Thus, after five years of follow-up, we will have sufficient power, with about 1,500 new breast cancer cases, to address many key hypotheses regarding gene-environment interactions. Baseline questionnaires, banked blood, urine, and toenail samples, as well as banked environmental samples will provide a rich resource for testing current and future hypotheses regarding breast cancer risk. Follow-up questionnaires will update exposure and medical histories as well as provide an opportunity to collect new data and environmental samples to evaluate emerging hypotheses. Nested case-control or case-cohort analyses will be carried out among sisters who develop cancer and a sample of those who do not, to assess specific gene-environment interactions. Once assembled, the cohort also will provide the structure for assessing gene-environment interactions in risk for other diseases and will provide opportunities for add-on studies. Because sisters of women with breast cancer have about twice the risk of developing breast cancer themselves and because they may share many relevant genes and exposures, the Sister Study will have greater efficiency than a similar size general population cohort. An advantage of the prospective design over population-based case-control studies is the collection of blood samples and risk factor information prior to diagnosis of disease. Another advantage to the proposed design is that sisters of women with breast cancer are likely to be highly motivated to participate over time. While sampling may be prone to self-selection, the sampling for those who develop breast cancer will be identical to that for those who do not. Finally, we plan to collect detailed information on environmental and educational exposures as well as environmental samples such as household dust and plan to enroll only those women who are willing to provide the biological and environmental samples. Most of the existing cohort studies have only limited information on environmental exposures, focusing largely on lifestyle factors and diet, and many include biological samples for only a portion of the cohort. A nationwide publicity campaign coupled with an outreach program that takes advantage of the large networks of breast cancer advocates will be used to recruit a cohort of sisters that is ethnically, geographically, and socio-economically diverse. Focus groups and preliminary study contacts suggest a high level of interest in the study among these advocacy groups an women with family histories of

breast cancer. Ongoing pilot work has demonstrated the feasibility of recruiting sufficient numbers of women for the study.

Title: Anonymous Sample Collection for Laboratory Assay Development and Testing (Taylor)

PI: Jack Taylor, M.D., Ph.D. (NIEHS/DIR/EB)

Precis:

Development, validation and fine tuning of specific laboratory assays for new study protocols on human subjects require the testing of human blood samples collected from various age/gender groups. The purpose of the testing is to check the accuracy and quality of these newly developed laboratory and genetic tests. We propose the need for a generic protocol for use in obtaining such blood samples from small numbers of healthy volunteers for use in laboratory assay development. Volunteer subjects will be given informed consent. Assays will include evaluation of DNA and levels of growth factors and hormones in human serum. Once these tests are validated, they will be used as part of other, independent studies on health investigating genetic susceptibility to disease.

Title: Fluorescence Bronchoscopy and Molecular Characterization of Abnormal Bronchial Lesions: Novel Approaches for Early Detection of Lung Cancer in High Risk Patients

PI: Jack Taylor, M.D., Ph.D. (NIEHS/DIR/EB)

Precis:

Despite intensive research efforts, there are still no simple and effective screening tools to detect early lung cancer. The majority of newly diagnosed patients have higher stage, often disseminated, non-resectable disease. A better understanding of the natural biology and molecular abnormalities in early lung lesions may aid in the development of more effective screening tools. The Lung Imaging Fluorescence Endoscopy (LIFE) is FDA approved as an adjunct to WL bronchoscopy for the screening of lung cancer. Using the LIFE unit, this study will set the stage for the collection of a unique set of biopsy specimens that will be used to learn more about the natural biology and molecular changes in early lung lesions. We will study abnormalities in p53 by immunohistochemistry and by molecular analyses. The p53 results will be compared with histological grade and with genomic instability. Measures for genomic instability will be the loss of chromosomal information and cellular aneuploidy. Recent advances in molecular pathology, such as the development of Laser Capture Microdissection (LCM), have made the molecular profiling of these extremely small lesions feasible. The information obtained by these techniques will be used for comparison with clinical and exposure information. Future plans include the culturing of bronchial epithelial cells to study genomic instability in the multistep process of cancer progression. It is our hope that the application of these new technologies will improve the early detection of human lung cancer and provide insight into the natural biology and molecular changes of early lung lesions which may progress towards overt cancers.

Title: Svangerskap, Arv, Og Miljo (Pregnancy, Heredity and Environment)

PI: Jack Taylor, M.D., Ph.D. (NIEHS/DIR/EB)

Precis:

We propose to conduct a population-based case-control study of facial clefts (cleft lip or palate) in Norwegian newborns. Cleft lip and palate are one of the most common type of birth defect, and both genetic and environmental causes have been suspected. This project will combine tests of recently-discovered genes associated with facial clefting and personal interviews of the mothers soon after birth. With this information we expect to be able to assess the combined role of genetic susceptibility and environmental exposures in causation of facial clefts. We plan to enroll 750 cases and 1100 controls over five years. This will provide a 2:1 ratio of controls to cases for the largest sub-category of facial clefts (cleft lip with or without cleft palate). This study is being conducted as a collaboration between the US National Institute of Environmental Health Sciences, and the Norwegian Institute of Public Health/Medical Birth Registry. Norway offers a unique opportunity for carrying out such a study. First, all Norwegian babies with facial clefts are treated at one of two medical centers at the expense of the national government. Our collaboration with these two clinics will provide access to virtually every case born in the country. Second, the Medical Birth Registry maintains a file of all newborn babies, from which control infants can be randomly drawn from the whole population. Random population controls are the gold standard for such studies, but are seldom feasible. Third, blood samples from all Norwegian newborns are sent to one laboratory for analysis of PKU. Our collaboration with the PKU laboratory will permit mothers of control infants to consent to the study of their baby's blood without having to submit their baby to an additional needle stick. Mothers of cases and controls will be initially contacted through their physicians, and will be asked to participate in a telephone interview. With their consent, the mothers will also be asked to provide biological samples for analysis of genes that predispose to facial clefts. We will collect cheek swabs from control mothers and their infants. Case mothers will be asked to provide additional blood samples and cheek swabs for more intensive study of genetic factors within their family. Protection of privacy will be paramount. Mothers of case infants will be given the option of receiving individual results, but only if a consulting panel of ethicists and other experts agree that such information will be beneficial. Analysis and presentation of data will be carried out jointly by US and Norwegian investigators.

Title: Inhibition of Fried Meat-Induced DNA Damage: A Dietary Intervention Study

PI: Jack Taylor, M.D., Ph.D. (NIEHS/DIR/EB)

Precis:

Dietary exposures have been implicated as risk factors in colorectal cancer. Such agents may act by causing DNA damage or may be protective against DNA damage. The effect of dietary exposures in either causing or preventing damage has not been directly assessed in colon tissues. We are proposing a pilot study of dietary factors and

DNA damage, involving 16 healthy volunteers in a four-week controlled feeding study. The primary focus of this study is to assess genetic damage to colonic epithelium and blood lymphocytes induced by pyrolysis products formed in cooked meat, as well as the putative protective effects of cruciferous vegetables, yogurt, and chlorophyllin against that damage. In the first phase of this pilot study, eight subjects will be fed either a baseline diet or a diet high in fried meat in two-week intervals. In the second phase, the remaining eight subjects will be fed either the fried meat diet or a diet containing fried meat along with putative inhibitors. In both phases of the study, blood will be drawn and rectal biopsies will be obtained from subjects each week during the four-week study periods. Damage in the lymphocytes and colon epithelium from the different dietary regimens will be evaluated using the single cell gel electrophoresis (comet) assay. Rectal biopsies used in this study are painless and generally without risk. In previous studies conducted by Dr. Robert Sandler, at UNC, over 2,000 rectal biopsies have been obtained without any adverse events. The goal of this study will be to determine the feasibility of conducting a larger study to examine the interaction of genotoxic components in fried meat with "protective" dietary factors on a molecular level.

Title: Venous or Arterial Ligation and Intraoperative Dissemination (VALID) of Cancer Cells: A Randomized Clinical Trial For Patients With Resectable Non-Small Cell Lung Cancer

PI: Jack Taylor, M.D., Ph.D. (NIEHS/DIR/EB)

Precis:

The VALID study is designed to obtain information regarding factors associated with the risk of recurrence after resection of early stage Non-Small Cell Lung Cancer (NSCLC). Until recently, the only clearly identified prognostic factor was the stage of the disease. Recent development of sensitive molecular assays has provided a way to study the effect of circulating tumor cells on clinical outcome. Preliminary studies have shown that tumor cells detected by such means in lymph nodes or in bone marrow are associated with an increased risk of recurrence. Preliminary studies have also indicated that the level of circulating tumor cells in the blood stream is effected by intraoperative factors, i.e. the sequence of vessel ligation. The main objective for this study is to investigate the influence of intraoperative sequence of vessel ligation and how this affects tumor recurrence and survival. In addition, we will also investigate the use of molecular assays to detect circulating tumor cells as a surrogate endpoint for the occurrence of distant metastases and/or death after surgery for NSCL. Several of these molecular markers have proven their value in case series but have not been rigorously tested for association with the clinical endpoints of interest, tumor recurrence and survival. We believe that this study may lead to important answers about how the spread of tumor cells occurs and if novel detection methods can be used to predict patient outcome.

Title: PCOS Twin Study - Environmental Factors in the Development of Polycystic Ovary Syndrome, Phase 2

PI: Patricia C. Chulada, Ph.D., MHS (NIEHS/DIR/OSD)

Precis:

Polycystic Ovary Syndrome, or PCOS, is the most common endocrine disorder in women. Depending on the strictness of the diagnostic criteria used, it is thought to occur in about 6-10% of all women, many of whom do not know they have the syndrome. Women with PCOS produce abnormally high levels of male hormones (hyperandrogenism); this counteracts their ovaries' ability to make enough of the female hormones estrogen and progesterone needed for normal menstruation. PCOS is the number one cause of hormonally related infertility and also increases women's risks for diabetes, high blood pressure, hypercholesteremia, cardiovascular disease and certain cancers. It is currently unclear to what extent PCOS and PCOS-associated traits (hyperandrogenism, hyperinsulinemia, insulin resistance, type 2 diabetes, dyslipidemia, hypertension, obesity, and coronary artery disease) are the results of environmental factors or genetic predisposition. Therefore, the NIEHS Program in Clinical Research is conducting a multi-phase twin study to measure the extent of PCOS heritability and to identify environmental and genetic factors involved in the development of PCOS. The proposal described here is for Phase 2 of this study. The goals of Phase 2 are to: 1) establish more reliable concordance rates and baseline heritability estimates for PCOS in MZ and DZ twins; and 2) establish a cohort of intact MZ and DZ female twin pairs with PCOS as a resource for future studies. In Phase 1, about 1500 individual female twins were identified from the Mid-Atlantic Twin Registry (MATR) based on self report of a history of irregular periods and/or cystic ovaries in the MATR General Health Screening Questionnaire. Those twins were surveyed by phone for other traits associated with PCOS. In Phase 2, the twins most likely to have PCOS based on their answers to the Phase 1 phone survey will be recontacted for further PCOS screening. One or both twins in a pair will be screened for elevated levels of testosterone (total and free testosterone, bioavailable testosterone or BaT; free androgen index or FAI). Hyperandrogenism is one of the hallmark traits of PCOS and can be exhibited either biochemically (elevated testosterone) or clinically (hirsutism, acne, hair loss, alopecia, other). If one twin in a pair has an elevated BaT level, then both twins in the pair will be asked to undergo a medical evaluation for PCOS confirmation. This includes a physical exam, medical history, ultrasound, 2-hour glucose tolerance and other biochemical blood tests, and a Ferriman-Gallwey evaluation for abnormal hirsutism (another characteristic of PCOS). The women will also be tested for pregnancy and zygosity. Their female co-twins will be invited to undergo a similar medical evaluation. Depending on their PCOS traits, twin pairs in which neither member has elevated testosterone levels might be asked to undergo the medical evaluation as well. In clinical practice, PCOS diagnoses are often made on women with normal testosterone levels if they have other certain PCOS traits. The determination to include pairs in which both members have normal testosterone levels will be made depending on their collective PCOS traits that they reported on their Phase 1 survey.

Title: Environmental Polymorphism Registry (EPR)

PI: Patricia C. Chulada, Ph.D., MHS (NIEHS/DIR/OSD)

Precis:

The Environmental Genome Project (EGP) has completely or partially resequenced the protein coding and regulatory regions of 53 environmentally sensitive genes from 72 anonymous individuals of varying ethnic backgrounds to date. Some of the same genes have been resequenced in an additional set of 20 samples, and, in a subset of these, the introns and promoter regions have been sequenced as well. Within this population, 523 allelic variants (genetic polymorphisms), mostly single nucleotide polymorphisms (SNPs), have been found to date. If the polymorphism alters the behavior or expression of the encoded protein, it might be of clinical significance. The Program in Clinical Research is planning to establish a large resource bank of frozen DNA samples (n=20,000) and make this available to NIEHS intramural investigators, their collaborators at the University of North Carolina (UNC), Duke University and other research institutions to screen for the presence of these SNPs and other mutations by standard genotyping methods. To investigate the feasibility of such a large DNA sample collection, we recently completed a small pilot study consisting of approximately 481 samples from patients at two UNC outpatient clinics, the Family Practice Clinic (FPC) and the Ambulatory Care Center (ACC). The major goal of this pilot study was to assess the willingness of general outpatients to participate in a genetic study of this sort and identify potential problems that might arise when conducting the larger, 20,000 sample effort. In the pilot study, recruitment procedures worked well and accrual rates were high at both sites. Of all the patients asked to participate, 75.6% and 78.1% of the patients from the FPC and ACC, respectively, agreed to participate as evidenced by signing an informed consent form. Only two patients from the FPC withdrew from the study at a later date. Based on the excellent results of the pilot study, we have therefore decided to proceed with the larger, 20,000 sample collection, which is the focus of this protocol. Similar to the pilot study, for the larger study blood left over from patients already having their blood drawn for specific tests requiring EDTA-anticoagulated blood or purple top tube (complete blood count or CBC, sedimentation rate and hemoglobin A1c or HbA1c) as part of their routine clinical management will be asked to donate their left over blood from these tests. We will also ask other patients already having their blood drawn for other tests that do not require a purple top tube, if they will have an extra tube of blood drawn (3.0 ml purple top) just for this registry. This will eliminate having a needle stick done just for this study in the UNC Healthcare participants. In addition we plan to recruit EPR participants from other sources such as volunteer drives at various corporations throughout the RTP and other areas in NC, at the UNC and Duke campuses and the Medical Centers at both campuses, and from our EPR website. Once the samples have been obtained, the blood will be transferred into storage tubes that are identifiable only with a unique identification number and shipped to an NIEHS contractor for DNA isolation. During recruitment, interviewers will explain the study to potential participants, obtain their signatures on the informed consent documents, and

answer any questions they might have concerning this study. At this time, potential participants will be informed that, depending on the results of the genetic analyses of their blood samples, they may be recontacted at a later date and asked to participate in follow-up genotype/phenotype studies. These follow-up studies will be separate from this protocol and require their own IRB approval. The ultimate objective of these sample collections, combined with the follow-up genotype/phenotype studies, is to identify groups of individuals with genetic polymorphisms in environmentally sensitive genes, and to correlate their genotype with their clinical phenotype, a process known as "ascertainment by genotype".

Title: Environmental Polymorphism Study (EPS)

PI: Patricia C. Chulada, Ph.D., MHS (NIEHS/DIR/OSD)

Precis:

The Environmental Genome Project (EGP) has completely or partially resequenced the protein coding and regulatory regions of 53 environmentally sensitive genes from 72 anonymous individuals of varying ethnic backgrounds to date. Some of the same genes have been resequenced in an additional set of 20 samples, and, in a subset of these, the introns and promoter regions have been sequenced as well. Within this population, 523 allelic variants (genetic polymorphisms), mostly single nucleotide polymorphisms (SNPs), have been found to date. If the polymorphism alters the behavior or expression of the encoded protein, it might be of clinical significance. The Program in Clinical Research is planning to establish a large resource bank of frozen DNA samples (20,000) and make it available to NIEHS intramural investigators involved in the EGP to screen for the presence of these SNPs and other mutations by standard genotyping methods. To investigate the feasibility of such a large collection of samples, we plan to first conduct a pilot study to estimate the accrual rate and uncover potential problems that may be encountered in the larger effort. This IRB proposal is for the pilot study in which we will collect whole blood samples (EDTA-anticoagulated) from 481 patients at UNC Medical Center. Once the pilot study is complete, we will decide whether to proceed with the larger, 20,000 sample collection and if so, develop and submit for review a new IRB protocol for its implementation taking data from the pilot study into account. For both the pilot study and larger, 20,000 sample collection, only blood left over from patients already having their blood drawn for hematology (complete blood count or CBC) and hemoglobin A1c (HbA1c) assays as part of their routine clinical management will be used, thus eliminating the need to collect extra blood. Once the samples have been obtained from the clinical laboratory and processed, they will be identifiable only with a unique identification number and sent to an NIEHS contractor (BioServe Biotechnlolgies, Laurel, MD) for DNA isolation. During recruitment, interviewers will explain the study to potential participants, obtain their signatures on the informed consent documents, and answer any questions they have concerning this study. At this time, potential participants will be informed that, depending on the results of the genetic analyses of their blood samples, they may be recontacted at a later date and asked to participate in follow-up genotype/phenotype studies. These follow-up studies will be separate from this protocol and the subjects of future IRB proposals. The ultimate objective of these sample collections, combined with the follow-up

genotype/phenotype studies, is to identify groups of individuals with genetic polymorphisms in environmentally sensitive genes, and to correlate their genotype with their clinical phenotype, a process known as "ascertainment by genotype."

Title: Environmental Factors in the Development of Polycystic Ovary Syndrome

PI: Patricia C. Chulada, Ph.D., MHS (NIEHS/DIR/OSD)

Precis:

Polycystic Ovary Syndrome (PCOS) is manifested as a heterogeneous mixture of clinical and bichemical characteristics that complicate study of its etiology. It is currently unclear to what extent PCOS-associated traits (hyperandrogenism, hyperinsulinemia, insulin resistance, type 2 diabetes, dyslipidemia, hypertension, obesity, and coronary artery disease) are the result of environmental factors or genetic predisposition. We propose to conduct a twin study to investigate the possibility that environmental factors are important in the development of the PCOS phenotype. Twin studies are considered to be the gold standard for determining the extent of heritability of a trait. The proposal described here is only for Step 1 of a larger, multi-step study. The major goal of step 1 is to identify a large cohort of twin pairs, in which at least one member of each pair is likely to have PCOS. Participants for this study will come from the Mid-Atlantic Twin Registry (MATR). Many (3283) potential participants have already been identified based on their answers to a preliminary MATR screening questionnaire. Out of the approximately 7145 twin women of reproductive age who completed these MATR screening questionnaires, 1803 women reported irregular periods, 954 reported ovarian cysts, and 526 reported both irregular periods and ovarian cysts. Many of the women in this last group are likely to have PCOS. They represent 7.4% of the total sample, matching current estimates of PCOS prevalence (4-7%) in reproductive age women. We will also add new twin pairs who meet the criteria (irregular periods and evidence of PCOS or cystic ovaries) as they are recruited into the MATR and take the preliminary surveys. According to MATR statistics, about 33% of twin pairs are monozygotic (MZ, identical). Therefore, approximately 174 of the 526 women likely to have PCOS are members of a MZ pair. Step 1 of the proposed study consists of a telephone survey of the 3282 women with irregular periods and/or ovarian cysts. The survey will be conducted by the MATR. The instrument to be used contains a series of simple and direct questions and will take about 10 minutes to complete. The questions were designed to identify PCOS and their content deals with the frequency of menstrual periods (six or fewer per year being a major diagnostic criterion), a previous diagnosis of PCOS, obesity, excess facial hair and other evidence of hyperandrogenism. The women will also be asked if they have a living twin sister. On the basis of this survey, women will be identified who are likely to have PCOS and have a living female twin.

Title: Innate Immunity Signal Transduction in Human Leukocytes

PI: Michael B. Fessler, M.D. (NIEHS/DIR/LRB)

Precis:

The objective is to define the signaling pathways activated by lipopolysaccharide

(LPS) and other selected innate immunity stimuli, and the downstream inflammatory functional consequences, in human leukocytes in vitro. Adult (greater than or equal to 18 years old), nonpregnant, healthy volunteers will have 320 ml of whole blood collected by venipuncture in a monitored setting no more frequently than once every 8 weeks. No further interventions will be exercised upon the subjects. The whole blood will be fractionated into neutrophil, red blood cell, mononuclear cell, and plasma fractions using plasma-Percoll discontinuous centrifugation. Leukocytes will be subjected in vitro to inflammatory stimuli (eg, LPS), and selected signaling outcomes (eg, mitogen-activated protein kinase activation, Rho GTPase activation, protein-protein interactions) and functional measures (eg, chemotaxis, superoxide anion and cytokine production) quantified in the absence and presence of relevant chemical inhibitors (eg, SB203580, a p38 MAPK inhibitor). In each such experiment, cells from the daily donor will be used as paired controls to the in vitro experimental intervention (eg, SB203580 inhibitor vs. DMSO vehicle). Three or more repetitions (on different donors) of each specific experimental outcome, as necessary, will be performed to establish statistical significance of findings. A specific focus of the studies planned will be to define the role of lipid raft membrane microdomains in transduction of the LPS signal in human leukocytes. Lipid rafts are cholesterol-rich microdomains in the plasma membrane, within which the LPS receptor, Toll-like Receptor 4 (TLR4), has been described to reside. LPS signaling has been reported to be sensitive to raft cholesterol content, presumably because the specific repertoire of proteins in rafts is sensitive to raft cholesterol content. Rafts are thought to act as dynamic signaling platforms for co-segregation of proximal adaptor proteins, kinases, and other signaling proteins. Of interest, while LPS has been described to modulate the activity of proteins that determine raft cholesterol content (eg, Liver X Receptor, ABCA1), virtually no work has been done to clarify: 1) the mechanisms underlying LPS-induced intracellular cholesterol redistribution, and, more importantly, 2) whether such intracellular redistribution of cholesterol is causal to the signaling events triggered by LPS, or 3) whether innate immunity signaling is dependent upon inter-subject variations in raft cholesterol content. Furthermore, we will investigate the role of the tumor suppressor gene p53 in the regulation of inflammation. It is now widely accepted that inflammation and cancer development are interconnected. Dr. Resnick is one of the international leaders in the study of the tumor suppressor gene p53. His group has discovered that activation of p53 through exposure to carcinogenic stimuli leads to differential expression of genes that have a direct effect on the inflammatory response, such as several toll-like-receptor genes. Dr. Resnick will use human leukocytes that will be isolated from whole blood. He will expose these cells to stimuli that activate p53, such as doxorubicin (a chemotherapy agent) or radiation, and examine the expression of toll-like-receptor genes as well as the response to LPS and other inflammatory agents in vitro. In addition to cell signaling experiments, we plan to test a novel detection system for the presence of oxidized lipoprotein (LDL) in the blood. Inflammation in the body (like sepsis, radiation injury, cancer) can induce the generation of reactive oxygen radicals (ROS) which can react with proteins, DNA and other cell structures and alter their structure, therefore causing cell damage. No reliable minimally invasive tests exist to detect biomarkers for oxidative stress in humans. One such biomarker is N-formyl kynurenine (NFK) which can be found on lipoproteins like LDL. We are developing

polyclonal antiserum to NKF with the goal of producing a simplified and high throughput method of detecting NFK via ELISA and Western analyses. We propose to purify LDL from human serum by standard methods and use ELISA analysis to determine if the samples contain KFK as detected using our anti-NFK polyclonal serum. These experiments could lead to the development of a simple and reliable non-invasive assay that detects a biomarker for oxidative stress in humans.

Title: Role of Oxidant Susceptibility Genes in Severity of Neonatal Diseases Associated with Hyperoxic Injury

PI: Steven R. Kleeberger, M.D. (NIEHS/DIR/LRB)

Precis:

Hyperoxia treatment-induced oxidative stress leads to the severe, debilitating diseases bronchopulmonary dysplasia (BPD) and retinopathy of prematurity (ROP) in pediatric populations. BPD is the most frequent cause of chronic pulmonary illness in infants, affecting 25 percent of premature neonates weighing less than 1,500g in Latin America. ROP is a vasoproliferative disorder associated with hyperoxic injury that can progress to retinal detachment and blindness. Each year, approximately 3000 infants are blinded by ROP in the United States. In Argentina, ROP is the number one cause of blindness in infancy. Many antioxidant genes modulate oxidative stress and nuclear factor, erythroid 2 related factor 2 (NRF2) activates cellular rescue pathways against oxidative injury, inflammation and apoptosis. In mice, deficiency of NRF2 is associated with inflammation and death of capillary endothelial and alveolar epithelial cells, severe impairment of pulmonary function, and reduced expression of multiple antioxidant enzymes. In humans, the Kleeberger laboratory identified a C/A single nucleotide polymorphism (SNP) in the NRF2 gene promoter, associated with an increased incidence of adult respiratory distress syndrome (ARDS) in trauma patients under intensive medical care. Evidence supports a role for other genes in the NRF2 pathway in modulation of hyperoxic stress in lung diseases of infants, but no studies have examined this in very low birth weight (VLBW) infants.

Title: Head-Off Environmental Asthma in Louisiana (HEAL) Study

PI: William J. Martin II, M.D. (NIEHS/DIR/LRB)

Precis:

This document presents the protocol for conducting the Head-off Environmental Asthma in Louisiana (HEAL) study in New Orleans, Louisiana. The goal of the HEAL study is to implement and test an Asthma Counselor (AC) intervention program that addresses the multidimensional impact of hurricane Katrina on children with asthma in New Orleans. HEAL is a prospective, controlled trial in which children will be randomly assigned to one of two treatment groups. Group 1 children will receive an AC intervention focused on case management, adherence to medications and education on allergen avoidance. The responsibilities of the AC in this study are enhanced relative to previous initiatives such as the National Cooperative Inner City Asthma Study (NCICAS) AC intervention to also provide families with education aimed at reducing mold,

allergens, and moisture in their homes, and materials to aid this effort (dehumidifiers and HEPA air filters). Since 90% of children with asthma in New Orleans are sensitive to dust allergens, established allergen control measures will be incorporated into the AC intervention as well. Group 2 children will receive a high quality standard of care based on the National Asthma Education and Prevention Program (NAEPP)-NIH guidelines. An abbreviated AC intervention will be provided to children in Group 2 at the conclusion of the study. Both treatments will be administered over a 12-month period during which the children in both groups will receive an extensive clinical evaluation (at baseline and 12-months) and three environmental home evaluations (focused on moisture, mold and other allergens). In addition to monitoring the effectiveness of the AC at environmental remediation education, the environmental home evaluations will also allow some characterization of the relationships between allergens, post-Katrina environmental exposures and asthma morbidity. In addition to the enhanced AC intervention, a separate school based survey will be administered to a sample population of children to examine asthma prevalence and assess living conditions and stress levels that may have been influenced by hurricane Katrina. The survey population will consist of a sample of the families of Orleans Parish school children stratified by age, schools and geographic area of the Parish. The results of the survey, in combination with the results of the AC intervention, will give us an overview of the extent and severity of the asthma problem in post hurricane Katrina New Orleans. The HEAL project is a collaborative multi-institutional research project conducted by the Tulane University School of Public Health and Tropical Medicine and the New Orleans Department of Health. To support those efforts, Rho, Inc. will provide for coordination of data and study activities.

Title: Rituximab in the Treatment of Refractory Adult and Juvenile Dermatomyositis (DM) and Adult Polymyositis (PM)

PI: Frederick W. Miller, M.D. (NIEHS/DIR/PCR)

Precis:

The idiopathic inflammatory myopathies (IIM) are orphan diseases defined by inflammation in muscle, skin and other tissues and are associated with substantial morbidity and mortality. The treatment of IIM is unsatisfactory, and no agents are currently FDA-approved for this indication. Rituximab, a chimeric murine-human monoclonal antibody directed against the CD20 antigen on B cells, induces a targeted B cell depletion with the aim of eradicating B cell clones. Although rituximab inhibits mitogen-induced B-cell proliferation and differentiation and reduces circulating B cell numbers, exactly how it results in clinical improvement in a wide array of autoimmune disorders, some of which are thought to be T cell-mediated, remains unclear. Pathologic studies imply a role for B cells in the pathogenesis of myositis, and clinical anecdotes suggested rituximab can induce prolonged responses in refractory patients. The core Rituximab in Myositis (RIM) trial is a multicenter, double-blind, placebo-controlled study that will assess the efficacy and safety of rituximab in 202 treatment-resistant dermatomyositis (DM, N=76), polymyositis (PM, N=76), and juvenile dermatomyositis (JDM, N=50) patients. The RIM study provides the EAG and other participating centers a unique opportunity to study a number of IIM features, including gene expression patterns and imaging profiles of refractory disease and how they respond to a specific

immune intervention. Identifying such molecular and imaging features may not only allow early recognition of patients requiring more aggressive treatment but could enhance our understanding of the pathogenesis of myositis and related autoimmune syndromes. The goals of this proposal are to define molecular and imaging characteristics of disease responsiveness to rituximab therapy and to better understand the role of B cells and their subsets in the pathogenesis of myositis. We propose to take advantage of this trial to identify changes in gene expression patterns in muscle, skin and peripheral blood and the imaging features and immunopathology of muscle, skin and peripheral cells before (week 0) and after (week 16) therapy. These will also be correlated with the large number of clinical, laboratory and research variables already planned to be collected in the core RIM study. Furthermore, knowing specifically which gene expression patterns are altered in resistant patients before rituximab, and which are changed after rituximab therapy - in conjunction with flow cytometry of peripheral cells and immunopathology of the tissues - will help in understanding more about the pathogenesis of myositis and the possible contribution of B lymphocytes and their subsets.

Title: Studies of the Natural History and Pathogenesis of Autoimmune/Connective Tissue Diseases

PI: Frederick W. Miller, M.D. (NIEHS/DIR/PCR)

Precis:

Individuals who develop chronic harmful inflammation in association with self-reactive autoantibodies or T cells are said to have autoimmune diseases. The causes of these diseases are unknown but they are thought to occur in genetically susceptible individuals after exposure to selected environmental agents. There are many forms of these diseases, but we have been focusing on one of the rarest and most poorly-studied group of autoimmune disorders, known as the Idiopathic Inflammatory Myopathies (IIM). This heterogeneous group of diseases includes polymyositis, dermatomyositis and related disorders. This is a natural history protocol designed to continue our study of these diseases and begin the evaluation of related connective tissue disorders associated with environmental exposures. We plan to further delineate important groups of patients and familial cases, and obtain useful material for further investigations of the clinical presentations, etiology, pathogenesis, and immunologic abnormalities of autoimmune/connective tissue diseases. Clinical data and patient blood, urine and tissue specimens have been collected by referring physicians and sent to us. The blood samples have been separated into cells and plasma, frozen and then placed into cell and plasma banks. Often the diagnosis of an IIM can be confused with other illness (such as adult-onset dystrophies), and therefore, we have also included patients with other illnesses (who are referred with a preliminary diagnosis of an IIM or an unknown myopathy), including patients with other autoimmune diseases. In order to understand more fully the genetic risk factors for these diseases, family members of selected patients who have several blood relatives with autoimmune or connective tissue diseases will also be studied. In summary, this natural history protocol will attempt, through a series of hypothesis-testing and hypothesis-generating studies, to

obtain new information regarding the clinical presentation, risk and protective factors, pathogeneses and prognostic features for myositis and related conditions.

Title: Pathogenic Studies In Families With Twins Or Siblings Discordant For Systemic Rheumatic Disorders

PI: Frederick W. Miller, M.D. (NIEHS/DIR/PCR)

Precis:

Most autoimmune diseases are thought to develop as a result of chronic immune activation and dysregulation after selected environmental exposures in genetically susceptible individuals. Current evidence suggests that the adult and juvenile forms of systemic rheumatic disorders – defined here as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and idiopathic inflammatory myopathies (IIM) – share many common clinical manifestations, immune responses, genetic, hormonal and environmental risk factors, and possible pathogeneses. Conversely, other studies imply that each rheumatic disease, as currently defined, may be composed more of homogeneous subgroups, known as elemental disorders, with different pathogeneses. This protocol will explore pathogenic mechanisms for systemic rheumatic disorders and possible elemental disorders through the evaluation of families with monozygotic or dizygotic twins or other siblings discordant for systemic rheumatic disorders (twin-sib pairs). Parents, normal volunteers and offspring of microchimeric female twin-sibs will also be evaluated as needed for the experimental designs of each portion of the protocol. A clinical evaluation, using standardized physician and patient clinical and environmental exposure questionnaires, and specimen collections from 400 twin-sib pairs discordant for systemic rheumatic disorders will be performed to confirm diagnoses, document medical histories and assess possible risk factors implicated in the development of autoimmunity. This study will evaluate children, who will make up 25-50% of the twin-sib pairs, and adults in similar ways to attempt to understand possible similarities and differences in pathogeneses of systemic rheumatic disorders based upon age of onset. Hypothesis-testing studies will assess differences in peripheral blood cell gene activation/suppression, levels and types of microchimerism between affected and unaffected individuals, selected genetic risk factors for these disorders and occupational and hormonal exposures hypothesized to be potential risk factors for these diseases. Exploratory studies will be conducted to begin to assess other environmental risk factors for systemic rheumatic disorders and to better understand associations among phenotypes and genotypes. Biologic specimens--including blood, urine, and other clinical specimens or biopsies no longer necessary for clinical care--will be collected for directed biomarker assays and the development of repositories for future research. Yearly follow-up of all twin-sib pairs for five years will be performed to assess clinical changes and a final comprehensive assessment will repeat the initial studies at five years after enrollment.

Title: Fibroid Growth Study

PI: Shyamal Peddada, Ph.D. (NIEHS/DIR/BB

Precis:

Uterine leiomyomas, commonly called fibroids, are a major health concern for women of reproductive age. The objectives of the study described herein are to investigate the growth dynamics of uterine leiomyomas in a clinically relevant population of women. We will test the hypotheses that uterine leiomyomas are heterogeneous in terms of their growth characteristics and in their clinical symptoms or outcomes, and that differences in leiomyoma growth dynamics can be discriminated by molecular markers and cellular phenotypes. Participants will include 300 premenopausal women (greater than 18 years old) with at least one uterine leiomyoma. The inclusion criteria for patient enrollment is confirmed diagnosis of leiomyoma by ultrasound. At least one leiomyoma must be equal to or greater than 2 cm in diameter and the uterus must be enlarged to the size typical during the eigth week of pregnancy. After enrollment and informed consent, T1- and T2-weighted magnetic resonance image (MRI) scans will be conducted beginning at the first visit and then at 3, 6, and 12 months. Each patient will have a physical exam, provide urine and blood samples at each MRI visit, and respond to an initial extensive telephone-administered questionnaire followed by abbreviated monthly questionnaire updates. A number of the enrolled women will require surgical intervention (hysterectomy/myomectomy) as standard care. If surgery is an outcome for women enrolled in the study, MRI will be conducted before surgery and the surgical pathologist will map uterine leiomyomas for comparison to MRI. Leiomyoma samples will be analyzed for histopathological and molecular changes correlated with growth. Because hysterectomy and myomectomy are common outcomes in women with leiomyomas, we anticipate tissue will be available from at least 100 of the 300 women in the study. For those women who opt for surgery, we will also administer a brief (less than 5 minute) questionnaire clarifying their reason for electing surgery. Upon completion of data collection, we will be able to compare leiomyoma growth as a function of multiplicity and location; examine the relationship between leiomyoma growth and clinical symptoms or outcome; identify molecular, cellular, and pathological characteristics of leiomyomas with differing growth dynamics; and examine endocrinological parameters and lifestyle factors related to differential growth dynamics of uterine leiomyomas. The data may be used to establish a clinical severity scale and establish diagnostic markers currently not available for uterine leiomyomas.

Title: Studies in the Natural History and Pathogenesis of Childhood-Onset and Adult-Onset Idiopathic Inflammatory Myopathies

PI: Lisa G. Rider, M.D. (NIEHS/DIR/EA)

Precis:

This protocol is designed to extend our description of the idiopathic inflammatory myopathies (IIM) to children with juvenile dermatomyositis, polymyositis and other IIM. This study will attempt to delineate groups of patients with similar prognoses who share common clinical features, immunogenetics and autoantibodies; to develop an objective, comprehensive assessment tool of disease activity and chronicity which could be used to assess efficacy in future therapeutic trials; and to obtain specimens for study of the immunologic abnormalities, pathogenesis, and etiology of childhood IIM. Often the diagnosis of an IIM can be confused with other illness such as dystrophies or metabolic

myopathies. Therefore, we will evaluate children who are referred with a preliminary diagnosis of an IIM or who have muscle weakness, myalgias, or an elevated creatine phosphokinase (CK) without an established diagnosis. Children and adult patients with IIM undergo a comprehensive evaluation at NIH to collect epidemiologic and clinical data and laboratory specimens; these materials may be used for research purposes as well as to confirm a diagnosis of IIM. Some patients may elect to participate in serial clinical evaluations at the NIH to assess the clinical course of the disease, including the extent and severity of their illness. The data collected in this study will be used to develop a comprehensive clinical disease activity assessment tool that may benefit the patient by providing a thorough medical evaluation of their condition and treatment recommendations to their referring physicians. No treatment is provided as part of this protocol.

Title: Oral Bacteria and History of Allergic Disease in Children: A Pilot Study

PI: Michelle Sever (NIEHS/DIR/LRB)

Precis:

The purpose of this study is to examine the feasibility of and to establish methods for a future study that will investigate associations between oral bacteria and allergic diseases in children. Rates of allergic diseases such as asthma, hay fever, and eczema have increased in the U.S. over the past several decades. One explanation for those increases is the Hygiene Hypothesis, which contends that decreases in microbial exposures have made the population more reactive to environmental allergens. Reports of protective associations for various infections and exposures to farms, pets, siblings, and day care have provided support for the Hypothesis. Preliminary work by NIEHS researchers suggests that some oral bacterial exposures may be beneficial. NIEHS researchers recently reported that elevated serum antibody concentrations to two common oral pathogens were associated with lower prevalences of asthma, wheeze, and hay fever in the U.S. population. Using a mouse model, NIEHS researchers found that immune responses involved in allergic airway inflammation could be modulated by infection with an oral pathogen. To further investigate these associations in humans, NIEHS researchers, in collaboration with UNC researchers, are planning an observational study that will collect saliva samples from and allergy information on child patients at the UNC-Chapel Hill School of Dentistry. Because we anticipate that several hundred children might have to be enrolled, we are proposing to test methods in a pilot study of 60 children. Twenty children will be recruited from each of three pediatric clinics. A research assistant will obtain the parent's consent and the child's assent and administer a questionnaire to the parent. The child's dentist will collect one teaspoon of saliva by having the child chew a piece of inert wax and spit into a sterile collection cup. Samples will be transported to the NIEHS and analyzed for bacterial species and for allergy-related cytokines. The specific aims for this pilot study are: 1) to identify the most efficient recruitment strategies, 2) to estimate response rates, 3) to estimate the distribution of allergic diseases among the children, 4) to identify any problematic consent form and questionnaire items, 5) to optimize saliva collection and laboratory protocols, and 6) to estimate statistical parameters required for more precise sample size calculations. Information gained from this pilot study will allow us to decide whether

a larger study among this clinic population is feasible and to design a more efficient study if we decide to proceed.

Title: The Two Sister Study

PI: Clarice Weinberg, Ph.D. (NIEHS/DIR/BB)

Precis:

Although the disease is rare below age 50, women with young-onset breast cancer may be a particularly informative group for elucidating genetic and environmental causes, and they may experience a particularly aggressive form of the disease. The ongoing Sister Study enrolls unaffected sisters, but makes no direct use of their more than 50,000 affected sisters. We propose to enroll 1,600 recently (within 4 years) diagnosed sisters, among those who were under 50 when diagnosed. We will collect DNA from the affected sister and from their parents, creating a nuclear family foursome or "tetrad." This novel design will enable powerful analyses of joint effects of genes and environmental factors. The proposed study, now funded by the Susan G. Komen for the Cure Foundation will rapidly identify and access many motivated women with breast cancer and their parents, and can effectively use the DNA and environmental data now being collected from their unaffected sisters. Based on the first 32,000 Sister Study enrollees, more than 1,600 have an eligible sister and about 86 percent of those have one or both parents living. Mail-back saliva kits will provide DNA from cases and parents. We will collect clinical data and validate the diagnoses for all 1,600 cases. Follow-up of these cases (through the Sister Study, at no cost to the proposed study) will also allow us to identify environmental, clinical, and genetic factors that influence health after treatment. Case-parent analyses of gene variants are protected against bias due to confounding by genetic heritage, and also permit detection of both maternally-mediated genetic effects and parent-of-origin (imprinting) effects. Environmental effects will be identifiable through a paired comparison of affected and unaffected sisters. Gene-by-exposure interactions will be assessed with novel statistical methods. We plan to genotype 1,536 markers on some 150 candidate genes, including some expected to be related to risk and others expected to be related to prognosis. In addition, archived DNA will provide a resource for future tests of not-yet-known candidates to be found in ongoing whole genome scans. In the proposed study, affected sisters will complete many of the same activities done by the unaffected sister on the Sister Study. The affected sister will complete a 2-hour CATI providing information about breast cancer diagnosis and treatment as well as risk factors related to breast cancer. Self-administered questionnaires on diet, personal care products, and some limited questions about family history will be collected. A household dust sample and toenail clippings for environmental exposures and saliva for genetic analysis will be collected using mail-back kits. Participating parents will be asked to provide saliva, using the same mail-back kit. Patient permission for release of medical records and tumor tissue blocks will be requested. Once the initial Two Sister baseline information is collected, the affected sisters will be rolled into the annual follow-up of the Sister Study. In summary, the proposed study leverages off the ongoing Sister Study to build a cost-effective, powerful, and statistically independent study of young-onset breast cancer.

Title: National Survey of Lead Hazards and Allergens in Housing

PI: Darryl C. Zeldin, M.D. (NIEHS/DIR/LRB)

Precis:

We propose to conduct a scientifically valid, descriptive survey to measure the prevalence and levels of lead in dust, soil, and paint, and the prevalence and levels of various indoor allergens in floor and bedding dust in the nation's housing stock. The survey strategy is a population-based, multi-stage area probability sample designed to represent all 50 states. The survey will include approximately 1000 homes in at least 100 primary sampling units (PSU, a metropolitan area or cluster of counties). Residents of candidate participant housing units (HUs) will initially be contacted by a letter to introduce and provide a brief explanation of the study. A field interviewer will then visit each candidate HU to screen and recruit eligible units into the study. A short Screening Questionnaire will be administered to an adult HU resident and an invitation will be extended to those HUs that are eligible to participate in the study. A field data collection visit will be scheduled for the following week, at the resident's convenience. The collection visit will be conducted by a two member team (including the same field interviewer that will conduct the screening/recruiting visit) and will consist of administration of an informed consent form and Data Collection Questionnaire, completion of home observation forms, collection of interior dust and exterior soil samples, and conduct of nondestructive paint lead analyses on both interior and exterior walls. Soil and dust samples will be shipped to analytical laboratories for lead and allergen analysis. Extensive survey design, procedure, and reporting details are provided in the National Survey Lead Hazards and Allergens in Housing: Protocol and Sample Design Report. It is anticipated that this study will provide allergen-specific data regarding: 1) housing conditions, demographic factors, and climate to facilitate evaluation of regional, ethnic, socioeconomic, and housing characteristic differences in the indoor allergen burden; 2) an estimate of indoor allergen exposure in the U.S. population; 3) baseline data that can be used as a reference point for future allergen surveys; and 4) a database that can be used to stimulate future studies which attempt to correlate allergen exposure to disease outcome. The study will yield lead hazard data to: 1) estimate the number and percent of homes with dust and soil lead levels above selected thresholds; 2) identify sources of lead in dust in housing; 3) permit future analysis of lead hazard control strategies and costs, including associated policy and regulatory guidelines.

Title: Dust Mite Allergen Reduction Study

PI: Darryl C. Zeldin, M.D. (NIEHS/DIR/LRB)

Precis:

Sensitization to dust mite allergens is a major risk factor for the development and exacerbation of asthma. Asthmatics and others with dust mite allergies often implement strategies to avoid dust mite exposure, but lack objective evidence that their efforts are successful in reducing dust mite populations. Recently developed in-home test kits have introduced the capability to monitor the effectiveness of allergen reduction strategies by

providing an affordable, simple way to measure dust mite allergens on a regular basis. The primary objective of this study is to determine if use of an in-home test kit result in decreased dust mite allergen levels in homes of children sensitive or allergic to dust mites. A secondary objective is to determine if use of an in-home test kit result in attitudinal and/or behavioral changes related to implementing and maintaining dust mite reduction strategies. This study is a randomized controlled intervention trial designed to test the efficacy of an in-home test kit in influencing behaviors to reduce dust mite allergen levels. Households will be recruited through flyers and screened for eligibility through a recruitment call line. Study participants will be randomly assigned to a treatment or control group. The treatment group will receive educational materials and an in-home test kit at set intervals, while the control group will receive educational materials alone. Vacuumed dust samples will be collected and delivered to the NIEHS laboratory for ELISA-based measurements of the dust mite allergens Der f 2 and Der p 2. A questionnaire will be used to collect information on home characteristics and on dust mite reduction attitudes and behaviors. The Precaution Adoption Process Model (PAPM) will be used to assess changes in attitudes and behaviors. Data will be collected at baseline, 6-months, and 12-months. The primary statistical analysis will measure the change in allergen concentrations from baseline to month 12. This change will be compared between the two study arms using a generalized linear model to test the null hypothesis of no difference between groups. In secondary analyses, data from each sampling location will be analyzed separately and changes from 0-to-6 and 6-to-12 months will be compared between the treatment and control groups. Data from the questionnaire and baseline home visits will be analyzed for each group to determine PAPM stages and transitions. The results from this study will be used by NIEHS to plan future primary and secondary asthma prevention trials.

Title: Cockroach Allergen Reduction by Extermination Alone in Low-Income, Urban Homes-A Randomized Control Trial

PI: Darryl C. Zeldin, M.D. (NIEHS/DIR/LRB)

Precis:

Evidence suggests that exposure to cockroach allergen might be the most important risk factor for asthma in inner-city households. Contrary to other students in the literature, we recently reported that cockroach extermination alone-without professional cleaning or occupant education-reduced cockroach allergen levels in inner-city homes. This was an important finding because extermination alone would be much less expensive and easier to implement than a more comprehensive intervention. The primary objective of this study is to validate our previous finding that cockroach extermination alone can significantly reduce cockroach allergen levels in inner-city homes. The secondary objective is to determine the level of expertise and effort in extermination that would be required to achieve significant allergen reductions. The study design will be a 3-arm, randomized control trial. Sixty cockroach-infested, multi-unit rental homes will be randomly assigned to either a control group or one of two treatment groups. The Treatment-1 group, which will test the efficacy of extermination, will receive insecticide bait placement by staff from the Urban Entomology Department at North Carolina State University (the gold standard for this study). The Treatment-2

group, which will test the effectiveness of extermination, will receive extermination from 1 to 5 commercial pest control companies randomly assigned to the homes. Study staff will assist home occupants in obtaining a 12-month, prepaid contract. Periodically in all homes, study staff will administer a questionnaire, set cockroach traps to monitor cockroach numbers, and sample dust to monitor cockroach allergen levels. Treatment-1 homes will receive exterminations by NCSU staff at baseline and then as needed, as determined by cockroach trap counts. Treatment-2 homes will receive extermination by commercial applicators according to the terms of the contract. In the NIEHS laboratory of Dr. Zeldin, dust samples will be analyzed for concentrations of cockroach allergens Bla g 1 and Bla g 2. Statistical analyses will compare cockroach allergen concentration changes in each of the treatment groups relative to changes in the control group. The percentage of homes for which concentrations are reduced below 8.0 and 2.0 units of allergen per gram of dust-the proposed thresholds for asthma morbidity and allergic sensitization, respectively-will also be compared between groups. The results from this study, if they prove to be consistent with our previous work, will be used by NIEHS to plan future primary and secondary asthma prevention trials. With the exception of NCSU staff, the field and laboratory work will be carried out by staff from Dr. Zeldin's clinical program.