QUALITY MANAGEMENT PLAN (QMP)

University of Washington Center for Clean Air Research (Short title: UW CCAR)

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As Approved By:

3/22/11

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1 Management and Organization

1.1 Overview

Ultimately, the Center Director (Sverre Vedal) is responsible for all quality assurance and quality control (QA/QC) aspects of the study. Day-to-day QA/QC activities are managed by the Center Manager (Elizabeth Spalt) and the Quality Assurance Officers (QAOs) from each individual project.

The UW Center Quality Assurance Manager (QAM) (Amanda Gassett) ensures that the QMP is uniformly applied to the generation and processing of all exposure and health data collected under auspices of the grant. The QAM acts as an independent authority, organizationally removed from the grant's data generators and users. The QAM will report to the Center Director and QA/QC Subcommittee.

1.2 QA Policy

The University of Washington and the collaborating institutions in the UW CCAR are committed to producing quality environmental exposure and health data and analyses. As such, a QA program will be implemented for all monitoring, data gathering, sample and data transferring, laboratory analysis, data processing, and data analysis efforts. A key function of the QA program will be to document the methods used to collect and analyze data so that it will be scientifically valid and defensible. This Quality Management Plan (QMP) describes the elements of the quality system to be implemented by the UW CCAR and serves as the umbrella document under which individual quality activities are conducted. The QMP will be supported by the more specific Quality Assurance Project Plans (QAPPs), Standard Operating Procedures (SOPs), and QA/QC Reports.

The UW CCAR is an integrated multi-project research center focused on the cardiovascular health effects of near-roadway pollution. Individual projects use and generate data and other outputs that differ from those of the other center components. This makes for a complex quality management process, entailing a variety of QA/QC activities. Types of data include both primary data generated by our own air monitoring platforms and measurements and secondary, existing data obtained from many sources. Beyond data records, a program of QA/QC activities is also needed for monitoring, data gathering, sample and data transfer and storage, laboratory analysis, data processing and data analysis. The QA/QC needs are therefore varied and extensive.

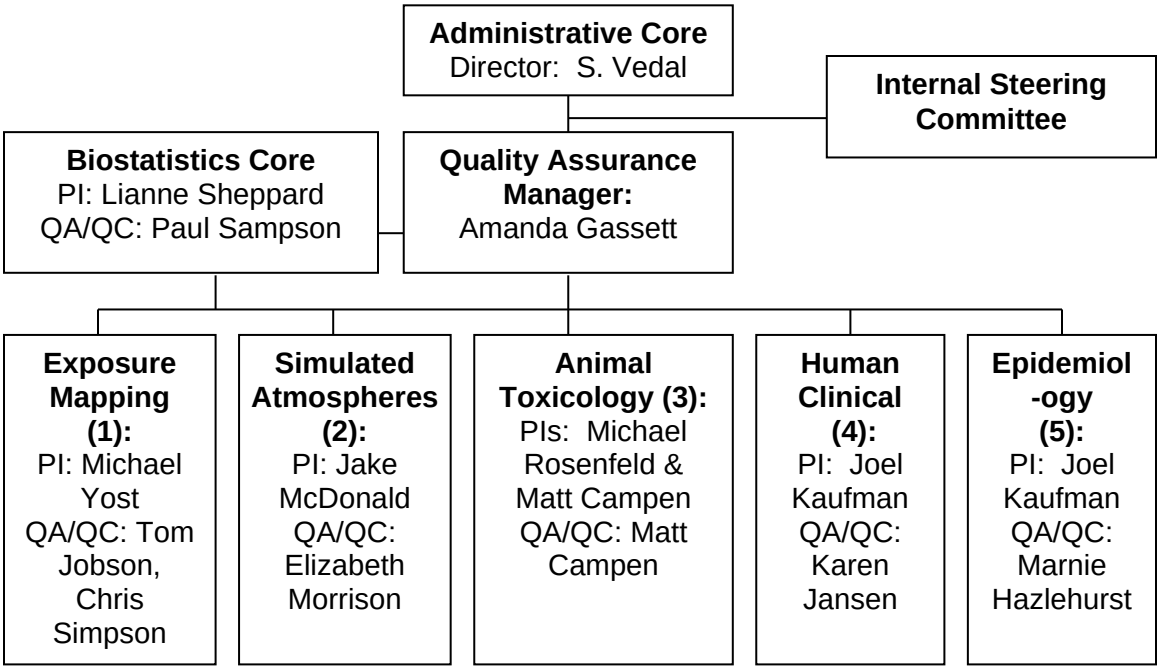
This proposed Center either utilizes study procedures closely related to those of ongoing research projects and centers, or exploits linkages with them. At UW, the most notably involved are: MESA (and its Coordinating Center [CHSCC] in Seattle) and MESA Air, the Tacoma and Seattle Area Air Toxics Community Scale Monitoring Program, the monitoring and epidemiology portion of the National Particle Component Toxicity (NPACT) study, and the NIEHS DISCOVER Center. At Lovelace Respiratory Research

Institute (LRRI), the most notably involved are the toxicology portion of NPACT, the National Environmental Respiratory Center (NERC), and the Advanced Collaborative Emissions Study (ACES). QA/QC protocols and other quality activities of these ongoing projects, that already have well-developed and documented QA/QC activities, will be exploited and integrated with the UW CCAR quality activities, as appropriate.

The policy of the UW CCAR is to conduct regular quality management oversight to verify that all research data collected and quality controlled are: scientifically valid; of adequate statistical quantity; of known precision and accuracy; and of acceptable completeness, representativeness, and comparability. This policy is achieved by carefully implementing quality management criteria at each stage in the research process.

The UW CCAR QAM will review the QMP and submit revisions to the Center Director and the UW CCAR QA/QC Subcommittee for review and approval on an as-needed basis. The QMP will be updated as required.

Figure 1. QA/QC Organizational Chart



1.3 Roles and Responsibilities

1.3.1 UW CCAR Quality Assurance Manager's Role

Amanda Gassett is the UW CCAR QAM for the exposure data. She currently serves as the QA Officer for MESA Air and the HEI/NPACT initiative and is familiar with all levels of quality assurance and control. This highly relevant experience is invaluable with respect to providing insight, knowledge, and guidance for the Center.

The QAM has the authority to review all field and analytical procedures, data sets, and analyses of exposure data to verify quality of data generation, compilation, and evaluation. The QAM is granted access to all aspects of the available data and materials to conduct comprehensive reviews. The QAM will prepare a written report to summarize findings and make recommendations in a timely manner, and the report will be available at the next scheduled meeting of the UW CCAR Steering Committee. The QAM's review extends to QAPPs and other quality documentation from all projects, regardless of the technical scope of that project.

1.3.2 Independence of QAM

For the UW CCAR QAM, three degrees of independence or separation can be defined to provide the opportunity for an impartial evaluation of all quality activities.

1. The Center's QAM will not be directly involved with the data collection, entry, or cleaning for any of the five UW CCAR projects.

2. Each project will provide a physical separation from the QAM. Project 1 will monitor remotely in the four MESA Air cities. Project 2 is based at Lovelace Institute in New Mexico. Project 3 will be conducted in an off-campus facility. Project 4 is also conducted in an off-campus facility. Project 5 will involve the CHSCC and monitor remotely in two of the MESA Air cities.

3. Each project has an appointed QA Officer, serving as the primary project-specific quality control administrator and contact. These individuals will provide materials and information, as requested to the QAM.

For efficiency and cost effectiveness, the existing MESA Air server system and database will be utilized for Center activities when possible. The system has the capability to house data generated from Project 1 and Project 5, which will be needed for cross-project integration. The QAM may be involved with developing the infrastructure for this data integration process, as the individual projects progress and closer collaboration is required.

Once data from the CCAR projects has been fully integrated into the database, the QAM will be responsible for reviewing these datasets. This should not present a conflict of interest, since review of the data at any time is within the scope of the QAM's responsibilities. Any quality issues found will be referred back to project-specific QA/QC staff.

The QAM will also not perform any health or exposure analysis with any of the projects' data products, nor author any manuscripts using this data.

1.3.3 Project-Specific Quality Assurance Officers (QAOs)

The Center is comprised of five distinct projects, conducting activities at multiple locations. To best maintain quality and consistency at the project level, each project will designate a Quality Assurance Officer that is responsible for oversight of the primary project activities and ensuring compliance with the protocol, the QMP, the QAPP, and the standard operating procedures (SOPs). These officers will work closely with the Center Manager to maintain overall consistency of quality standards, and participate as part of an internal QA/QC Subcommittee that meets and communicates on a regular basis. QA/QC Officers will facilitate the preparation of QA/QC Reports.

1.3.4 Center Manager

Elizabeth Spalt, the Center Manager, will work closely with the QAM, QAOs, field staff, and analysis laboratories to develop and maintain quality control guidelines and performance standards. This will include revising and reviewing standard operating protocols for field activities, as well as instrument and laboratory analysis and calibration performance. Additionally, chain of custody, documentation, and data security procedures will be reviewed and addressed if deficiencies are discovered. Regular reviews of all required certifications and training routines will be confirmed with the individual CCAR projects to maintain performance and consistency. As part of this process, the Center Manager will conduct regular communication with the project QA Officers to obtain updates on quality, data, documentation, and operational activities.

1.3.5 CHSCC Project Director

The Project Director for the CHSCC will be Kayleen Williams. The person in this position, as well as CHSCC staff, will work directly with Project 5 in the selection and recruitment of participants and provide follow-up activities relating to tracking consents and IRB approvals at participating MESA city collaborators. Oversight for study performance, data cleaning, and exposure and health dataset merges are also within the scope of work. In addition, the CHSCC Director will be the QAM for the health assessments from the MESA parent study.

2 Quality System Components

2.1 Quality Management Plan (QMP)

The backbone of the quality system for UW CCAR will be the QMP, supported by the QAPPs and SOPs. For UW CCAR, this document will provide the quality systems foundation for the five individual projects that comprise the Center itself and will define the quality standards and conditions that each individual project will be held to.

The QMP will include information pertaining to:

1. Management and Organization
2. Quality System Components
3. Personnel Qualifications and Training
4. Procurement of Items and Services
5. Documents and Records
6. Computer Hardware and Software
7. Planning
8. Implementation of Work Processes
9. Assessment and Response
10. Quality Improvement

2.2 Quality Assurance Project Plan (QAPP)

The QAPP integrates and documents all technical and quality aspects, including planning, implementation, and assessment, to assure that the research results obtained are of the type and quality needed and expected.

The QAPP will include information pertaining to:

1. Project Management
2. Data Generation and Acquisition
3. Assessment and Oversight
4. Data Validation and Usability
5. Data Quality Objectives (DQOs)

Each required QAPP will be reviewed and approved for completeness and compliance with the R-5 document[1] by the Center's QA Manager before any type of planned work with environmental data will be initiated.

Unlike the QMP, which provides a blanket document for the entire Center, each individual project within the Center will be responsible for creating and maintaining a project-specific QAPP.

The individual project QA Officers will be responsible for consolidating the appropriate materials and constructing their respective QAPPs. A preliminary QAPP document is submitted to the Center Manager to determine completeness and relevance and any revisions can be made at that time. A final QAPP document is submitted to the Center QAM for review. The QAM may request revisions before final approval and acceptance.

It is the responsibility of the project QA Officers to notify and forward to the QAM, revised versions of the QAPP for review and formal approval. Additionally, the Center Manager, as part of the QC Subcommittee meetings and the QC review, will confirm with the project QA Officers that all QC documents are current and on record.

A table will permanently reside on the UW CCAR server displaying the project, revision number, distribution, and the QAM approval date. This table will be maintained and updated by the QAM and/or Center Manager. The QAPP documents will also be placed on the UW CCAR server for reference.

2.3 Standard Operating Procedures (SOPs)

Each individual project and end-user will develop SOPs for all relevant processes and instrumentation. The project QA Officer will be responsible for the final documentation of the SOPs and providing all materials to the Center Manager and QAM for review. The Center Manager will collect and consolidate SOPs from external laboratories and other non project-specific processes.

Ultimately, all procedures will be assessed for the need of a standard operating protocol by the QAM. This need will be determined based on the potential for an adverse impact if the approved steps are not followed. A procedure demonstrated to have no impact on the quality of the product, or the product of others, will not require an SOP. If an SOP is required, it may be scaled to the complexity of the task and the importance of the outcome. Any field technician, process owner, or technical supervisor may recommend an SOP development to the QAM.

2.4 QA/QC Reports

All projects must submit a QA/QC report to the QAM and Center Manager once data collection is complete. This report will contain a summary of all of the data collected and will describe the overall quality of project data with regard to the DQOs. QA/QC report content will be project-specific but should include the following information, as applicable:

1) Data Summary
2) Comparisons between methods or to AQS data
3) Validity of samples and data flags
4) Treatment of non-detects
5) Discussion of duplicate and blank samples
6) Compliance with DQOs

2.5 Review and planning

Reviews of all quality documents (QMP, QAPP, and SOPs) and processes will be made by the QAM, with support from the Center Manager. A report developed from this review will be circulated to the Quality Control Subcommittees, the PIs and co-investigators, the Center Director, and the Biostatistics Core. The report will have findings, as well as recommendations. These groups will review the recommendations and generally make one of three decisions:

1) No action needed (maintain existing system), this requires written justification
2) Implement the recommended change
3) Implement another change. This last action also requires written justification. When changes are made to the system, outcome measures should be identified prior to implementation, allowing an evaluation of the effect.

3 Personnel Qualifications and Training

3.1 Training

Training will be a key element of the quality system used for UW CCAR. There will be training at two primary levels:

1. Training of all involved personnel as to the purpose and content of this QMP.
2. Training of all technical personnel on all aspects of Standard Operating Procedures for which they will be using.

3.2 Training Policy for Management and Staff

Staff will be selected and assigned to be responsible for specific activities or tasks because of prior appropriate training or knowledge, or they will be trained to bring them "current." If certification is required by a generally accepted body to complete a task, then this level of certification will be required for the person doing that activity.

It is the responsibility of each Project's Investigator and QAO to ensure that all personnel will have the requisite and appropriate education, training, experience, and supervision. In addition, all personnel will be required to successfully complete mandatory institutional safety programs, and remain current, before project activities commence or continue. Additional training needs will be identified on an on-going basis in response to changes in work activities and requirements, and through performance evaluations and career development plans.

The training process will use a phased approach, beginning with basic training, moving to on-the-job training, and finally working with close supervision. The time spent by project personnel within these training levels will differ depending on the initial skill set,

the complexity of the job at hand, the critical nature of the required task, and the evaluation of the trainer/supervisor.

Regular reviews by project officers will be conducted to verify adequate safety and quality. This includes personnel reviews, as well instrument function, data quality, documentation, and other associated activities. If deemed necessary, additional or remedial training for personnel will be required to maintain established standards.

3.3 Human Subjects Research Training (Institutional Review Board [IRB] training)

Requirements for human subjects research training differs between institutions. As necessary for staff that will interact with human subjects or human subject data, all personnel associated with the UW CCAR will be required to complete the human subjects training mandated by their individual institutions before any CCAR activities are initiated. Each project QAO will be required to save copies of certifications from all personnel from their project for documentation purposes, as well as confirming certification currency for all personnel. The Center Manager will also retain copies of these certificates.

University of Washington CCAR personnel will be required to have the training offered by the University of Washington (live session) or the web-based training offered by the University of Miami.

The Human Subjects Division offers tutorial sessions in ethical conduct. Certification of training is also available for researchers from the University of Washington and other collaborating institutions through web-based training [http://www.miami.edu/citireg] offered through the University of Miami. The University of Washington does not accept certification from any other online training programs. For University of Washington employees, this training is only required once, but other institutions may require training more often.

3.4 Animal Use Training - IACUC

As with human subjects training, requirements for animal use training differs between institutions. As necessary for staff that will be responsible for animal interaction, all personnel associated with the UW CCAR will be required to complete the animal use training mandated by their individual institutions before any CCAR activities are initiated. Each project QAO will be required to save copies of certifications from all personnel for documentation purposes, as well as confirming certification currency for all personnel. The Center Manager will also retain copies of animal use training certification.

UW requires adherence to federal regulations and the University's policies governing the humane care and use of laboratory animals[2]. UW's Office of Animal Welfare (OAW) Animal Use Training Program must provide appropriate training and instruction to all

individuals working with animals. The requirements include instruction on laws and regulations, species-specific hands-on trainings, and periodic re-certification. As part of the Institutional Animal Care and Use Committee (IACUC) protocol review process, the IACUC must ensure that personnel are qualified to perform the proposed animal procedures.

4 Procurement of Items and Services

To complete each individual Center project, equipment and supply purchases will be made prior to the commencement of project work, as well as throughout the length of the project period. Whether internal to the University of Washington, or through an external sub-award collaborator, it will be the responsibility of the end-user or their delegate to ensure that items and services purchased meet the required specifications and performance and quality standards for the task.

For routine supplies and consumable items, purchases may be conducted direct through each institution's individual purchasing process. For the purchase of technical equipment, including instrumentation, a purchase request form will be generated and sent to an individual with "signature authority." The signer of the purchase request should have sufficient experience to evaluate the requested purchase. The signed purchase request will then be submitted to the appropriate purchasing agent for processing.

After receipt of the item, the end-user will evaluate the item to the best of their ability. If the item meets their approval, they will sign the packing slip as received. If the item does not meet their approval, they should contact the vendor immediately to resolve the problem.

Monthly invoice reviews and reconciliation activities for all Center-related purchases, for each individual project, will be conducted by the Center Manager. When required by institutional guidelines, the purchaser must either develop an approved "sole source" justification or fully complete a competitive bid process. Preferred vendor status will also be reviewed when applicable.

5 Documents and Records

All documents and records will be prepared as far in advance as feasible. Time should be allowed for reasonable review and approval of the document prior to its use. Draft versions of documents will not be used except in exceptional circumstances. Such documents will be clearly marked as being "DRAFT" and will receive a designation as "Version 0.9".

5.1 Documents

Documents will be written by an individual with knowledge of the topic (the process owner, a supervisor, the QAM, etc.) and reviewed by others. The reviewers may include the process owner (if not the author), the QAOs, potential users of the document, the QAM, or a technical expert on the topic. Comments will be given to the author who will revise the document. Any disagreements between the author and a reviewer should be resolved with the involvement of an independent third party with relevant technical expertise. A second or third round of reviews may be required. A description of quality-related documents, their anticipated authors, reviewers and auditors is detailed in Section 2. The QAPP must be approved by the QAM and project PI's before data collection begins. SOPs should be validated during development and approved by the QAO. Process users and QAOs have the primary responsibility to identify the need for revisions to documents, and revisions will be reviewed and approved before implementation. Ideally, the original author of the document will make revisions to promote consistency between versions.

The first release of finalized documents will be numbered "Version 1.0". Documents that undergo minor text modifications or corrections will be assigned a higher version subnumber ("Version 1.1", "Version 1.2", etc.). Documents that undergo major changes, such as changes in operating procedures, or study design, will be assigned a higher version number (Version 2.0", "Version 3.0", etc.). Each version disseminated to process users will be archived by the project-specific QAO and by the QAM in a location that clearly communicates whether the document is obsolete or current. For example, documents may be filed within subdirectories labeled 'Archive' or 'Old' for the obsolete versions, and 'Final', or 'Current' for the most recent version. Current quality documents should be readily available to all process users on shared server space. The project QAO has the responsibility to ensure that project staff are aware of quality documents, alert staff to updates, and to ensure ready access to the documents.

When an updated version of a document has been completed, a notice will be sent to all users to destroy personal copies of the old version and implement the new one. The notice will include an overview of the document changes, the file name, and the document's location on the shared server space for that project. The QAOs are responsible for providing documents to process users and for delivering revisions to existing documents.

5.2 Records

Records (sample preparation records, field data sheets, laboratory analysis output, etc.) will be generated in a large number of processes. These records will be reviewed by the generator of the data for completeness and quality, entered into a computer database where applicable, and stored for future reference and review by the QAM and other staff.

When a record needs revision, the change must be completed so that it can be traced to the individual making the revision. The reason for the modification should be identified. For written records, the old item should be crossed out with a single line, initialed, and dated by the reviser. A note should be written on that record describing the reason for the change. Data entry databases or electronic files should include spaces for notes or comments so that any change that is made to an electronic record can be noted. After changes are made to the data entry records, any processing code that handles these records should be executed so that the changes are reflected throughout all databases and data files.

5.3 Confidentiality

The UW CCAR projects have undergone full review by the Institutional Review Boards at each of the sub-awarded institutions as well as at the University of Washington and the US EPA. All protocols and consent forms provide written assurance for the participants, that their identity, individual results, and residence location will be kept confidential. Access to forms, records, and data that contain potentially participant-identifying information will be restricted to staff with a demonstrated need for the use of such information, that have completed the required human subjects training, and that have signed any required confidentiality agreements.

6 Computer Hardware and Software

The UW CCAR information technology (IT) infrastructure is comprised of the hardware and software residing at the sub-awarded institutions (Lovelace Respiratory Research Institute, Washington State University, University of New Mexico, and during year 2, University of California at Los Angeles and Wake Forest University), the CHSCC (Coordinating Center), UW DEOHS (Environmental and Occupational Health Sciences), UW DCEE (Civil and Environmental Engineering) and with members of the UW CCAR Biostatistics Core.

Hardware and software, from all participating groups, are evaluated to meet the needs of the UW CCAR, including: data access, data transmission, secure storage, and reliable data collection and analysis software.

6.1 Hardware

UW CCAR will take advantage of the infrastructure that already exists at the University of Washington through DEOHS resources and existing MESA Air and CHSCC capacities. This will also apply to sub-award institutions, where existing resources and facilities will be used, when possible. Budgetary allocations have been included for upgrading software and hardware systems, where requested.

Initial UW CCAR data and documentation will reside on the MESA Air Intermediate Document Server (MAIDS) domain. Full documentation and data will be stored on a dedicated server partition that is allocated to CCAR, with access restricted to project staff according to role. If it is determined that additional capacity is required, existing hardware will be expanded or new technology will be explored. The DEOHS server is maintained by the DEOHS IT group and is routinely backed up every 24 hrs.

For data relating to subject information and recruitment, the well-defined CHSCC/MESA Air SQL Server Database will be utilized. Access to data resources is controlled through the use of Windows NT integrated security and SQL Server role mechanisms.

6.2 Software

Software needs are determined by the nature of individual project and by the associated project personnel at their respective institutions. This applies to staff computers, storage devices, and computers linked to monitoring or analysis instrumentation. All software used will be compatible with UW CCAR applications and provide the appropriate levels of integration, currency, and security.

For the CHSCC/MESA Air SQL Server Database, all software development tools and information delivery technologies are based primarily on Microsoft products, available through the Microsoft Developer Network (MSDN). Microsoft provides free, automated updates to the operating system and MSDN application suite.

CHSCC Software development tools and applications include:

- MS SQL Server – Database management
- MS Transact SQL – Primary development language for database related technologies
- MS Access – Primary development environment tool for custom stand-alone application development
- MS Visual Source Safe - Source code management
- MS Internet Information Server (IIS) – Web server
- SPSS – Statistical analysis

6.3 Integrated Computers

Computers of this type are integral to the instrument and do not operate independently. Equipment hardware and software are too closely integrated to allow separate evaluation. Initial evaluation of the instruments will help determine whether the hardware and software is appropriate for the specific application. If at all possible, the instrument systems will be evaluated independently of the computer hardware and software.

6.4 Interface Computers

For this type of system, an instrument may analyze a sample and either store the data in an internal logger or output the result directly to the computer for storage. The computer hardware for these systems would generally be desktop or laptop computers with either vendor-supplied or in-house developed interface programs. With either type of software, the entire system will have been validated when the process user carries out the various QA/QC checks. This allows a performance-based QC measure. Where possible, instrument performance and its internal data storage capabilities will be separated from computer hardware/software performance. This will facilitate the identification of a problem's source.

6.5 Data Transmission

Field sampling data or other electronic files will need to be exchanged between UW CCAR personnel. Files may be transferred via email or secure FTP. Files transmitted as email attachments will be AES encrypted. Options for compression and encryption utilities include the UW supported 7-Zip, v9.20 or WinZIP, v15.0. The FTP option would be managed with a password-protected utility such as the UW supported WinSCP, v4.3.2.

For cross-center collaboration, the above methods may be used for small-scale file transfers. Consideration will be given to a larger-scale file sharing platform, protected using password and/or group access.

6.6 Security

System security will be established and maintained according to University of Washington IT standards, specifically the DEOHS IT group. The university follows industry "best practices" and "open standards" to maintain a visible and secure computing domain.

In addition to a comprehensive "NetFilter," and Windows Internet Connection Firewalls, Snort, an open source intrusion detection system is now being used. If required, a VPN will be configured for remote server access from outside the University. Access will be controlled by limiting outside computer connections by IP address or other unique identifier, such as password authentication or group membership.

As with other technological concerns, participating institutions will be required to provide adequate security measures and will be evaluated in meeting the standards of the UW CCAR applications and activities. Reviews relating to effectiveness and currency of technology will be conducted regularly. If it is determined that additional resources are required, those needs will be addressed.

6.7 Data Entry

A strict method of version control will be maintained for the data entry software programs. These programs will also have a number of QA checks on data entry, such as requiring specific data types, flagging values greater or less than specified values, flagging blank values, etc. Data entry will be directed by its own SOP.

6.8 Data Analysis, Modeling, and Simulations

A strict method of version control will be maintained for analytic packages, developed analysis code, models, and simulations. Most exposure modeling code will be written using R, though supporting or pre-processing code may be written in SAS. Health analyses may be performed using Stata, SAS, or SPSS according to analyst preference. These activities will also be overseen by the Biostatistics Core.

7 Planning

The UW CCAR uses a systematic planning process to ensure that appropriate and adequate detail and preparation is developed at every level of operation. The process is based on the scientific method, common sense, and the graded approach. This standardized planning process maximizes quality and efficiency and minimizes error, duplication, and waste of resources.

The planning process requires participation at all levels of the study organization as well as input from sponsoring institutions, collaborating programs, and independent scientific experts. The tools used in the process include: periodic assessments of system and data quality, annual internal audits, regular committee and team meetings, and open channels of communication between and across all levels of the study. The immediate products of the planning process include: thorough documentation of quality management plans and technical standard operating procedures, proper training of all technical and management personnel, suitable equipment and resources, realistic timelines and milestones, and efficient communication throughout the study program.

7.1 Roles and Responsibilities

Effective planning for the UW CCAR requires participation at all levels of the organization's structure. The basic roles and responsibilities within the study are summarized in Section 1.

All personnel listed in Section 1 have the authority and responsibility to call meetings, write reports and assessments, review reports, and conduct audits in their fields. The QAM and QAOs have primary responsibility to develop and update the quality-related documents. All process users have the responsibility to report quality control issues to the QAO or QAM. If a need for a revision to a QAPP or SOP is identified, these revisions should be submitted to the QAM for approval expediently.

7.2 Study Schedule

The original planned study schedule for all CCAR projects is provided in Table 1. Some of the work illustrated in this table is being conducted during the approved two-year no-cost extension period (Years 6 and 7). All data collection and health analyses for Project 4 are occurring during Years 5 through 7, and exposure and health analyses for Projects 2, 3, and 5 are also extending into these years. Work for Project 1, other than manuscript preparation, concluded by the end of Year 5.

Table 1. Original schedule for all projects. The timeline for Project 4 has changed substantially due to differences between the proposed and actual scopes of work.

	Year 1	Year 2	Year 3	Year 4	Year 5
Project 1 - Exposure Mapping					
Pilot Testing of Mobile & Stationary Monitoring					
Characterization of UW Exposure Atmospheres					
Characterization of LRRI Exposure Atmospheres					
Field Sampling					
Development of Spatial Models					
Factor Analysis of Compositional Data					
Project 2 - Exposure Generation					
Characterization of laboratory inhalation atmospheres					
Develop and generate atmospheres for use in Project 3 animal exposures					
Generate atmospheres for use in Project 4 human exposures					
Project 3 - Toxicology					
Exposures, Aim 1					
Generation of Animal Models Aim 2					
Exposures, Aim 2					
Generation of Animal Models, Aim 3					
Exposures, Aim 3					
Assays					
Project 4 - Human Clinical					
Customize exposure conditions					
Finalize protocols, hire staff					
Recruit Subjects					
Data collection					
Sample analysis by subcontractors					
Health Modeling					
Project 5 - Epidemiology					
Finalize protocols, obtain IRB approvals					
Recruit Subjects					
Field Sampling					
Sample analysis and quality control					
Outdoor spatio-temporal model development					
Individual-level exposure predictions					
Integration of personal monitoring data					
Health analysis					

7.3 Overview of Data Processing and Assessment for All Projects

7.3.1 Data Processing and Verification

Data processing includes collection, validation, storage, transfer, and reduction. Precautions will be taken each time the data are reduced, recorded, calculated, and transcribed to prevent the introduction of errors and the loss of information. The methods of data processing, reduction, and management will either be documented in a project-specific Data Organization and Operating Procedures (DOOP) if extensive, or in the QA/QC Report otherwise. Quality issues that arise during data collection, entry, cleaning, or processing will be documented, tracked, and reviewed by the QA/QC Subcommittee.

7.3.2 Data Quality Consistency

Prior to primary data collection, all relevant instrument and procedural protocols (SOPs) will be fully developed and validated to ensure consistent and effective methods and performance. All projects will submit the SOPs for these processes for review before data collection and use begin. All SOPs must be reviewed, revised, and approved by the individual project Quality Assurance Officer, the Center Manager, and possibly the QAM. These SOPs will be strictly maintained and enforced, and all relevant training and certifications will be provided by the appropriate institution. All SOPs will undergo regular review to verify currency and relevance.

For all projects, NIST-traceable standards will be used when available for calibration of sampling or analysis equipment. Otherwise, alternative documented standards will be used according to the SOP for that process.

7.3.3 Data Quality Assessment

The methods of data quality assessment will be detailed in the project-specific QAPP. All projects will develop QAPPs before data collection begins. These documents will detail all of the quality assessment criteria for each type of data to be used. For example, data quality objectives (DQOs) for the precision and count of QC samples will be established in this document.

Each project will regularly report on data quality to the QA/QC Subcommittee, Center Manger, and QAM. These entities will provide recommendations for quality improvements and audit the adherence to SOPs, the comparisons to performance criteria, and the implementation of process improvements. During intensive data collection

periods, each project should provide summary results as batches of data become available to the Center Manager and QAM to confirm quality and progress. All projects must submit a QA/QC report to the QAM and Center Manager once data collection is complete. This report will contain a summary of all of the data collected and will describe the overall quality of project data with regard to the DQOs.

7.4 Project-Specific Planning

7.4.1 Project 1

7.4.1.1 Project Objectives

Roadway-source particulate and gas phase pollutants are transformed by chemical and physical reactions as they age in the environment. Human exposures to these pollutants can range from relatively fresh to highly aged components that vary with respect to particle size and chemical composition. Mobile and fixed site monitoring will be employed to assess this aging process from roadway sources to population areas to provide a more comprehensive understanding of the temporal, seasonal, and spatial variability.

The main project objectives are: 1) Characterize spatial and temporal gradients of selected air pollutants along roadways and within neighborhoods in MESA cities using a mobile platform; 2) Measure spatial variation in concentrations of selected air pollutants at two-week average fixed sites in coordination with the mobile measurements; 3) Characterize aging of air pollutant components transported from roadway sources to neighborhood receptor locations; 4) Provide detailed characterization of laboratory exposure conditions available for toxicology testing, and identify likely conditions that mimic those found in urban settings.

7.4.1.2 Project Data (and Instrumentation)

- Primary Data
    - Mobile Monitoring
        - Particles (Continuous Measurements) – Light scattering coefficients; Particle diameters (0.025 um – 30 um), number concentrations and mean particle mobility diameters (0.025 um – 0.3 um) – *Nephelometer, PTRAK, AeroTrak, Aerosol Spectrometer, NanoCheck*
        - Particle light absorption (dual wavelength) – *Aethalometer*
        - Particle-bound PAHs – *EcoChem*
        - Gases (Continuous Measurements) – O_3, NO, NO_2, NO_X, CO – *2B Technologies, Langan*
        - Indicator measurements for vapor phase organic compounds - *PHOTOVAC FID and PID*
        - GPS (Continuous Measurements) - Coordinates and route logging – *Garmin and/or Trimble*
        - Multichannel Data Acquisition System

- Stationary Reference Site – Supporting Mobile Platform
  - Particles: Continuous Measurements, light absorption, and particle-bound PAHs
  - Indicator measurements for vapor phase organic compounds
  - Gases (Continuous Measurements)
- Stationary Site(s) – Measurement of Coarse Particles and Gases
  - Gases (Integrated Measurements) – O_3, SO_2, NO, NO_2, VOCs – *Ogawa and 3M Passive Badges*
- Characterization of Exposure Atmospheres
  - Particles: Continuous Measurements, light absorption, and particle-bound PAHs
  - Gases (Continuous Measurements)
  - Indicator measurements for vapor phase organic compounds
  - Multichannel Data Acquisition System
  - HR-ToF-AMS (Continuous Measurements) – Time of Flight Mass Spectrometry for size and chemical composition of organic aerosols
  - PTR-MS (Continuous Measurements) – Proton Transfer Reaction for high sensitivity Mass Spectrometry information of volatile organic compounds

- Secondary Data
  - AQS data from any co-located sites for particles and/or gases used for comparative and quality control measures.
  - Laboratory IC, GC/MS, UV and microscopy analyses for passive samples. Most of the analysis for passive badges will be provided by the accredited on-campus UW Environmental Health Laboratory (EHL).

7.4.1.3 Data Collection and Use

Objective 1

Mobile monitoring with an instrument platform designed to measure concentrations of particles and gases continuously while on the move will be used. In addition, a stationary "central" site will be established in each city to capture variations over time during the mobile monitoring periods. Sampling sites will be selected to represent different tertiles of ambient NO_X levels based on existing MESA-Air models. For each city, multiple traffic intersections [sites] will be sampled in a "cloverleaf" pattern for two seasons; heating and non-heating. Three sets of measurements will be collected at each site in a given season. Measurements will be taken both in the afternoon and again in the evening.

In addition to visiting the intersections, transit measurements will be collected while traveling between these locations. The monitoring locations in each MESA city will be selected with these additional between-intersection routes in mind. Specifically, travel demand model shapefiles for all the MESA cities and will be used to identify well-traveled commuter routes. The sampling routes and intersection locations will be established prior to sampling and visited following one of two different specific sequences.

The mobile platform measurements will initially be adjusted for temporal variation using the fixed site data. This data will be used by the Biostatistics Core to develop multivariate spatial models of selected roadway-source air pollutants for use in health studies, and to characterize the aging of air pollutant components as they are transported from sources to populated areas.

Objective 2

The primary specific aim of the passive monitoring in this project is to measure spatial variation in concentrations of selected roadway-source air pollutants. Passive monitoring at approximately 20 stationary sites in each of the four MESA cities will be used to measure concentrations of coarse particles, gases (O_3, SO_2, NO, NO_2), and selected volatile organic compounds (VOCs).

Objective 3

The combination of mobile monitoring and stationary site monitoring will be used to evaluate the characteristics of air pollutants at near-roadway (un-aged) and neighborhood receptor (partially aged) locations. In order to account for dilution of the near roadway plume with "background" air, CO will be used as a conservative tracer of urban emissions and results will be normalized to CO concentration.

The comparison of these measurements will provide insight into the degree to which aging modifies the chemical and physical characteristics of the exposures for the participant locations. The mobile monitoring will be conducted on a neighborhood scale (1-10km), and will sample spatially around a stationary reference site with continuous time-resolved data. The stationary site samples will provide a suite of measurements similar to the mobile measurements.

Objective 4

Characterization of conditions in the toxicology exposure chambers (Project 2), will be achieved by deploying the same instruments used in the mobile monitoring platform, along with LRRI instruments, and additional high sensitivity mass-spectrometer instruments (Aerosol TOF-MS and PTR-MS). These high-resolution instruments will provide reference measurements and this mass spectrometer data will be correlated to the mobile monitoring and LRRI instruments. Characterization will involve a parametric study where the LRRI and UW laboratories will be asked to systematically vary their exposure system conditions while analyzing the gas and particle compositions of the mixtures with the instruments.

A factor analysis of the laboratory data will be conducted to decompose the complex signals into different gas and particle components and correlate these with the mobile instrument signals. Using these same factor components, the results of these laboratory measurements can be compared to the data obtained in the field studies, in terms of VOC composition, organic and oxygenated aerosol components. This will help to identify laboratory test conditions that are most similar to the exposures found in near-roadway and residential-receptor locations observed in the MESA-air study cities.

7.4.1.4 QA/QC Activities and Performance Assessment

Mobile monitoring continuous data collected by instrumentation will be stored within each instrument's internal memory, as well as actively consolidated into a central data acquisition system. Collected data will be downloaded from the acquisition system and backed up on a central data download computer, with external hard drive, and onto the UW CCAR sever routinely for added security. Additionally, data collected from collaborator instrumentation will be stored in the same manner to preserve integrity and security.

Sample analysis data obtained from the UW EHL, or any other internal or external facility, will be subject to internal QA/QC methods and standards, and will also be required to produce and deliver a quality assurance summary report per analysis batch. Sample analysis results will be further reviewed internally according to well-established MESA-Air data quality and processing standards. Likewise, any secondary data obtained from collocated monitoring agency AQS sites will be subject to internal QA/QC methods and standards and will be further reviewed according to well-established MESA-Air data quality and processing standards.

7.5 Project 2

7.5.1 Project Objective

An integral component of the Center is the development of laboratory-generated atmospheres for experimental exposures of animals. This Project will develop these atmospheres, with the primary objective of simulating environments containing key components of roadway emissions and the products of environmental factors that transform them. The exposures will help determine air contaminants that cause or potentiate the toxicity of roadway emissions or confound interpretations based on roadway proximity alone.

7.5.2 Project Data

Data for this project will come from the following sources:

- Primary data
    - Exposure atmosphere composition-real time data
    - Exposure atmosphere-detailed analysis of integrated samples

The purpose of the exposure data is to define the content and composition of exposure atmospheres that are used to expose animals for UW CCAR Project 3. The characterization of the exposure atmospheres will involve a partnership with Project 1 in order to directly mirror the characterization of ambient exposures. Table 2 summarizes the full suite of analyses to be conducted on laboratory inhalation atmospheres. Many of these samples will be collected in real time and archived/analyzed on a secure server that stores the data. Some samples are collected for subsequent chemical analysis. In this

case, the raw data may be stored on the local system, but will be transferred to the secure server and audited to 10 % to ensure the data transfer did not have any unintended errors.

Table 2. Summary of PM and co-pollutant properties that will be utilized to characterize exposure atmospheres

PM/Co-pollutant Properties	Method of Characterization
Physical Characteristics	
Size (coarse, fine, ultrafine)	Impactor, differential mobility
Number	Condensation particle counter
Surface area	Differential mobility/calculations
$PM_{2.5}$ mass concentration	Gravimetry
Chemical Characteristics	
Total metals and elements	X-Ray fluorescence
Carbon	
Black carbon (elemental carbon)	Thermal/optical analysis
Organic carbon	Thermal/optical analysis
Organic carbon class/species	GC/MS and LC/MS/MS
Biogenic	Luminol, PCR, GC/MS
Ammonium	Colorimetry
Sulfate/nitrate compounds	Ion chromatography
Co-Pollutants	
Nitrogen oxides	Chemiluminescence
Ozone	UV absorption
Gas phase hydrocarbons	
Total hydrocarbons	Flame ionization
Speciation of gas hydrocarbons	GC/MS and LC/MS/MS
Carbon monoxide	Infrared
Sulfur dioxide	Sorbent/ion chromatography
Ammonia	Sorbent/ion chromatography

Abbreviations: GC: gas chromatography; MS: mass spectrometry; LC: liquid chromatography; PCR: polymerase chain reaction; UV: ultraviolet

This Project benefits from the significant exposure, analytical, health assessment, and QA infrastructure that is already in place at the LRRI facility. The Project will also benefit from the many standard LRRI exposure management policies and procedures, which include (but are not limited to) annual validation of the performance of all equipment (e.g., flow measurement and environmental monitoring [e.g., temperature, humidity]), documentation of equipment maintenance, maintenance and documentation of calibration/verification tools in a secure calibration laboratory, and overall facility oversight by the QA Unit. LRRI's Animal Care Unit and animal facilities are integral to the Project, and the staff is accustomed to close coordination of animal care and exposure operations.

Once an exposure system has been developed, the conduct of the study includes: development of standard operating procedures (SOPs) for system operation and safety,

pre-study exposure system validation; training and training certification of system operators; and conduct of exposures. SOPs document the conduct of all aspects of the study and the step-by-step procedures for operating the generation system, collecting environmental or exposure monitoring data, and safety. Institutional SOPs are reviewed and approved by LRRI management, and study-specific SOPs are approved by the Principal Investigator who oversees each function. Once an SOP is developed, each technician participating in that portion of the study is trained, and training is documented.

7.6 Project 3

7.6.1 Project Objective

This project will use complex roadway mixtures as generated and characterized in the laboratory. Aim 1 will ascertain 1) the potentiating effects of physical and photochemical aging on fresh emissions and 2) interactions of vehicular emissions with pertinent copollutants (ozone, road dust), both in terms of driving systemic vascular oxidative stress. Aim 2 will examine effects of the emissions-induced oxidative modifications to endogenous phospholipids, in terms of activating immune-modulating receptors such as LOX-1, CD-36, TLR-2, and TLR-4. This Aim will utilize transgenic models to examine the roles of these receptors, as well as characterize the lipidomic alterations in various tissues. Lastly, Aim 3 will further explore the role of specific immune cell populations as participants in the innate and adaptive responses to emissions-induced phospholipid modifications. This Aim will utilize mouse models of immunodeficiency, including SCID and B-Cell deficient models. Additionally, bone-marrow transplants will be pursued from mice lacking those receptors described in Aim 2 to mechanistically establish the involvement of the oxidatively-modified phospholipids.

7.6.2 Project Data

The following primary data will be collected in Project 3:

- Markers of aortic lipid peroxidation and mRNA expression
- Aortic histopathology
- Plasma biomarkers
- General toxicology indicators (body weight, clinical observations, pulmonary inflammation)

Lipid peroxidation reflects an increased oxidative stress and damage in the aorta. Furthermore, we believe that certain oxidatively modified lipids (measured in bulk by this assay) are central to the immune-activating pattern recognition receptor-mediated progression of atherosclerosis. This assay has been found to be the most reliable and robust in making comparisons between the relative toxicities of different pollutant atmospheres.

Following exposures, aortas will be collected and frozen in liquid nitrogen and stored in a -80c freezer until the assay is conducted (no more than 5 years of storage). The assay is most commonly referred to as the Thiobarbituric Acid Reactive Substances or TBARS

assay. Values generated with a standard TBARS kit (Zeptometrix) are derived from a spectrophotometer. They will be distributed to the QA/QC manager (Campen) and stored digitally on a network computer that is backed up on a monthly basis. After review of the data, files containing the original data and any QA notes will be distributed to the other investigators (Rosenfeld)

The data will be compared with historical values to ensure that the range and distribution of data is consistent with previous studies. Outliers will be assessed with a Grubb's outlier test, but decisions to exclude such values will ultimately be based on agreement of all key investigators (Rosenfeld, Campen). Data will be analyzed by ANOVA, with consideration for whether data are normally distributed, or require some transformation (eg, Log10).

Other biochemical data include mRNA expression of specific markers (MMP9, LOX-1, ET-1) that will be determined by real time PCR using taqman chemistry. Data will be generated in duplicate on a Roche Lightcycler 480 machine and handled similarly to the TBARS data.

Histopathology will be conducted in the Rosenfeld laboratory. Tissues harvested after exposures will be prepared for sectioning according to protocols developed at UW and transferred to LRRI. Methods for staining and scoring are protocolized and will be conducted accordingly. Data from the pathological scoring will be reviewed by the QA/QC manager for consistency with historical data and distribution, then saved on the network drive and distributed to co-investigators.

Plasma biomarkers are essential for linking findings between projects 3 and 4. Soluble LOX-1, MMP-9, and ET-1 will be assessed in plasma using standard commercially-available kits and read by a UV-Vis plate reader. Data will be reviewed by the QA/QC manager for consistency with historical data and distribution, then saved on the network drive and distributed to co-investigators.

Similarly, body weights and clinical observations will be noted by trained animal care staff at LRRI throughout exposures, and all data will be compiled for trend analysis (2-way ANOVA). Pulmonary inflammation will be assessed by bronchoalveolar lavage in the high dose subjects. Assuming no overt inflammation is observed at the experimental levels, this effort will not be continued for all studies (i.e., this will be used to verify that the exposures will not induce pulmonary inflammation). However, lavage fluid will be collected and preserved for potential assays at a later date.

7.7 Project 4

7.7.1 Project Objective

The main project objective is to use physiological measurements, biological samples, and in-vehicle monitoring in order to determine the effect of traffic-related air pollution on

the cardiovascular system. The double-blind, randomized, controlled crossover trial will measure various cardiovascular health endpoints, before and after a scripted 2-hour commute on I-5. Subjects will be randomized to three exposures and each exposure will be separated by a wash-out period of 3 weeks. The trial will test whether exposure to a traffic-derived mixed pollution atmosphere causes an increased vascular response (brachial artery vasoconstriction, increased blood pressure, reduced retinal arteriolar diameter) compared with filtered air (FA). The nested aims include: whether specific exhaust-related monocytic gene expression effects are mediated by lipid peroxidation; whether traffic-related pollutants' vasoconstrictive effects are increased in subjects with a common SNP variant in the gene coding for TRPV1; and whether monocyte DNA methylation in specific genes is decreased with exposure to typical, roadway-derived exposures..

7.7.2 Project Data

Data for this project will come from the following sources:

- Primary data
    - Health measurement data
        - Health and mood questionnaires
        - Vital signs (blood pressure, heart rate, respiratory rate)
        - Serial blood draws (DNA methylation, gene expression, ANG-2, endothelin-1, IL-6, homocysteine, MPO, TNFα, TGFβ, IL-1, MDA, HMOX1, GCLC, PPARA, CD14 positive cells, FOXP3)
        - 24 hour Finometer (hemodynamic measurements)
        - 5-lead EKG (Holter monitor recording)
        - Brachial artery vasoconstriction (endothelial function)
        - Urine collection (markers of diesel exhaust exposure e.g. urinary metabolites of 1-nitropyrene)
    - Sample analysis by subcontractors
        - Serial blood draws (HDL, LDL, cholesterol; MMP9; catecholamines; PON; NPY)
    - Monitoring samples of the in-vehicle environment

7.7.2.1 Primary Data

Sixteen participants will participate in a double-blind, controlled exposure crossover clinical trial in 16 subjects, randomized to order. Eligible subjects are randomized to three 2-hour commutes that travel I-5, extending from North Seattle to roadways in South Seattle (e.g. Duwamish Valley). During each drive, subjects are accompanied by research staff responsible for collecting subject health measurements and monitoring conditions of the drive. Each drive is separated by at least 3 weeks. During drives, the cabin air and HEPA filters are configured to reflect the randomized exposure conditions (i.e., on-road ambient or filtered air exposure). The cabin ventilation controls are adjusted such that air is entrained and directed to the floor vents, and the temperature inside the vehicle is comfortable for the occupants. Van windows remain closed during

the drive and subjects wear N95 masks while transitioning from the lab to the UW van regardless of drive condition.

Subjects complete health measurements at baseline, during the drive, immediately after the drive, 3 hours later, 5 hours later and 24 hours later. These health measurements include: questionnaires, blood markers, Holter ECG, ambulatory blood pressure, 24-hour urine, brachial artery reactivity, retinal photography, and Finometer measurements. The frequency of health measurements are shown in Table 1. All subjects provide a urine sample for a cotinine test and, if female, a pregnancy test.

This study involves in-vehicle monitoring for 48 drives involving 16 participants in Seattle. Each day of monitoring will include the following suite of monitors in order to collect real-time measurements of the pollutants: $PM_{2.5}$ (Nephelometer, Radiance Research), black carbon (microAethelometer, Aeth Labs), particle count (P-Trak, TSI Inc), PAHs (PAS 2000CE, EcoChem), NO_2 (CAPS, Aerodyne Research Inc), NO_X (UV absorbance Model 410, 2B Technologies), ozone (chemiluminescence 3.02P, Optec), CO (CO T15n, Langan), CO_2 (CO_2 K-30-FS Sensor, CO_2 Meter.com), temp/RH (Precon HS-2000, Kele Precision Mfg), location (GPS BU-353, US GlobalSat). Filters and air monitors inside the car are powered by gel cell batteries connected to power inverters.

Plasma *endothelin-1* will be measured using ELISA techniques (R&D Systems, Minneapolis). The measurement of plasma *malondialdehyde* (MDA) concentration, a marker of lipid peroxidation, will be assessed using a method based (Bioxytech, Oxis International, Portland, OR) on the reaction of a chromogenic reagent, N-methyl-2-phenylindole (R1, NMPI), with MDA at 45°C in HCL with Probucol to minimize the reaction of 4-hydroxyalkenals. This technique provides estimates for total MDA that are similar to levels obtained using HPLC[3,4,5,6,7]. The measurement of *oxidized LDL* is by means of a monoclonal antibody–based ELISA (Mercodia, Uppsala, Sweden).

Instruments used to monitor health effects will be calibrated according to the manufacturer's description and these calibrations will follow a set schedule to ensure consistent, effective methods. Technician proficiency will also be monitored for quality control.

7.7.2.2 Analysis of Samples by External Laboratories or Institutions

For sample analysis that cannot be done in-house, subcontractors with reputations for high quality analysis will be selected. Several subcontrators have collaborations with our lab at the UW: Jesus Arajo, UCLA; Matt Campen, UNM; Charles Wilkinson, VA, Seattle). No DQOs will be established for data analysis by subcontractors, but quality reports from those laboratories will be reviewed and will be made available to the QAM or EPA quality auditors upon request.

Extraction of nuclear material from the blood samples for the gene expression studies and the DNA methylation studies will be managed by the Functional Genomics Laboratory

(Fred Farin, director). The methods will use commercially available kits and manufacturer's established methods.

Antibodies that recognize oxidized phopholipids will be measured by Dr. Rosenfeld's lab (as in Project 3) in the serum according to the method of Rolla et al.[8]

7.7.2.3 Secondary Data

Characterization of the in-vehicle exposure conditions including measurements and data cleaning will be conducted following the same methods as Project 1.

7.8 Project 5

7.8.1 Project Objective

Project 5 has three primary objectives. (1) Employ the small-scale gradient data acquired as part of the mobile monitoring campaign in Project 1 in conjunction with central fixed site data, regulatory monitoring data, and geographic covariates to build a multi-pollutant exposure model for traffic-derived air pollutants. This model will incorporate complex spatial information on primary and secondary traffic-derived particles and gases. (2) Develop and validate individual-level exposure estimates for traffic-derived air pollutants, integrating the outdoor residential concentration estimates from the multi-pollutant model; ii) estimates of residential infiltration rates; iii) road class- and traffic condition-specific estimates of on-roadway concentrations; and iv) individual-level questionnaire-derived time-location information. These individual-level exposure estimates will also utilize personal monitoring data designed to clarify the in-transit component of total exposure. (3) Estimate the effect of individual-level exposure to traffic-derived air pollution on subclinical cardiovascular disease, using these exposure models. Health outcomes will include left ventricular myocardial mass as ascertained by MRI, arteriolar diameters as measured by retinal photography, coronary artery calcium as ascertained by CT, intima-medial thickness as measured by ultrasound, and DNA methylation.

7.8.2 Project Data

Data for this project will come from the following sources:

- Primary data
    - Passive sampling data (Ogawa samplers for NO, NO_2, NO_X, SO_2, and O_3; 3M 3520 Organic Vapor Monitors for benzene, isoprene, toluene, n-decane, n-nonane, 2-methylpentane, m-xylene, undecane, i-pentane, n-pentane, and o-xylene) collected for 144 participants. This data includes:
        - Indoor residential samples
        - Outdoor residential samples
        - Personal samples
        - In-vehicle samples.

      All samples will be two-week time integrated measures.

    - Location data from GPS data-logging trackers worn by the participants during the study
    - Time-activity diary data
- Secondary data
    - Project 1 Monitoring Data
    - AQS monitoring data
    - Geographic data including roadways (line lengths and proximities), land use, emission sources, census variables (e.g., population density), and other locations (e.g., ports, airports, city center, etc.)
    - Health data (stored at the MESA Coordinating Center), including LV mass, retinal arteriolar diameters, CAC scores, IMT thickness, and DNA methylation
    - Personal covariates (also stored at the MESA Coordinating Center, e.g., age, race, gender, medical history, smoking status, diabetes, cholesterol, etc.)

7.8.2.1 Primary Data

All primary data will be collected as part of Objective 2A. From 144 participants, during each of two seasons, 2-week integrated passive samples will be collected using Ogawa and 3M badges indoors, outdoors, in vehicles and on persons. GPS tracking data of the participant's locations and time-activity diary data will also be collected. All air monitoring data will be collected according to validated SOPs, and data entry will be verified by quality control research staff. Data quality objectives for the precision, count of QC samples, and limits of detection based on field blanks will be established in the QAPP.

GPS data will be reviewed for data gaps and other unusual features. Criteria will be established for the completeness of this data, which will be outlined in the QAPP.

7.8.2.2 Secondary Data

This project will integrate data on traffic-derived pollutants from the novel, state-of-the-art mobile monitoring campaign (Project 1) into a multi-pollutant model, incorporating participant-specific time-location information, to provide individual-level estimates of exposure. A longitudinal analysis will examine the relation between these exposure predictions and subclinical cardiovascular disease in a large and well-characterized cohort. This project will be the first application of a multi-pollutant approach to a large-scale air pollution epidemiology study.

Project 1 data will have been thoroughly reviewed for quality by staff on that project. Similarly, health data collected through the CHSCC has undergone thorough quality checking and pre-processing by CHSCC staff. Project 5 will also leverage the MESA Air quality control process by obtaining GIS variables and AQS monitoring data that has been cleaned and processed by the MESA Air data core. However, analysts will perform due diligence to verify that data transmitted to them are sensible and complete.

7.9 Biostatistics Core

7.9.1 Core Objective

The overall aim of this Core is to support the database management and statistical needs of all Center activities. This will be achieved through the following specific objectives:

1. Advise Center projects on data management and compilation
2. Ensure quality statistical design and analysis of Center research
3. Implement novel statistical methods that are required for Center projects: Develop an analytical framework for quantifying the health effects of different mixtures of air pollution components in a cohort study (Project 1 and Project 5)
4. Identify additional statistical methodological research that will advance Center projects
5. Communicate and disseminate Center findings

The Biostatistics Core will provide a data management advisory role to all projects as needed. This may include database design, data entry form design (including QC checks such as double entry of particular fields), and validation checks. For example, procedures may be recommended to detect out of range or illegal values. Project data may require a system of flags to mark data that are questionable for some reason (e.g. below the LOD); the Core may help identify the types of flags that are necessary. All procedures and databases, including variables, values, valid ranges, etc. should be documented. Members of this Core will be available to advise on data documentation.

As Center needs evolve, the faculty and staff of this Core will consult with project investigators on the design of new studies. For large or complex studies, this Core will enable sample size calculations using more sophisticated approaches such as simulation. As requested by project investigators, this Core will provide the statistical perspective to data collection planning, documentation, and ongoing monitoring of data collection. The Core will also aid analysts in developing Statistical Analysis Plans (SAPs). The SAP will be an important tool in the Biostatistics Core's efforts to encourage sound statistical practice. Each proposed analysis (e.g. for a paper) should be pre-specified in a SAP. Oversight of analyses will be provided by Core faculty through the regular Core research meetings and in other forums. All analyses are strongly encouraged to have a SAP. Many analyses will be affected by subtle features of data, models, and analysis approaches that can affect the interpretation of results. This Core will provide the statistical perspective and expertise on results interpretation.

The major methodological objective of the Core is to develop a set of tools that allow an analyst to specify a baseline multi-pollutant mixture of components and to calculate relative risks/hazard ratios for alternative multi-pollutant mixtures. These methods will

be incorporated into Project 1, Objective 2 (mobile monitoring) and Project 5, Objectives 1 and 3 (epidemiological analysis). Code for this purpose will be written in R and encapsulated in an R package for general use.

Verification of results and validation of models and simulations will be conducted when possible, and may include cross-validation or re-analysis of the same data using a different statistical package. All analyses should be performed using a clearly documented script that would allow an analyst unfamiliar with the project to reproduce the original results. The documentation should indicate whether the script is the final version used to generate results for publication. Ideally, interim versions and the final version of analytical code will be stored and version-controlled in a Subversion repository. Final versions of analytical code should be archived on CCAR server space.

8 Implementation of Work Processes

Ensuring that work is performed according to planning and technical documentation should be seen as a tiered approach. Development and understanding of documentation and performance standards is established at the project or end-user level and review and enforcement of these operational procedures is conducted through to the audit level. Between these actions are many steps of review by differing members and committees, formed to cover all aspects, at all levels, of quality and performance.

1. End User/Project Level – Development, use, and revision of standard operating protocols (SOPs) relating to activities, procedures, and equipment.
2. Quality Assurance Officer/Project Level – Determine what processes and equipment require SOPs and include them in the QAPPs. Verify that new SOPs are written and that existing SOPs are relevant and current. Facilitate preparation of a QA/QC report. The QAO will conduct annual reviews of all operational protocols, training procedures, and certifications to confirm completeness and currency.
3. Project Subcommittees – Each individual project is expected to conduct regular meetings to discuss operational and quality activities. These meetings will be used for frequent updates on documentation and training requirements, as well as verifying all procedures are being properly followed.
4. Center Manager – The Center manager will communicate closely with the project-specific QAOs. A quarterly review will occur by the Center Manger to confirm with each project that standard operating procedures cover all processes and equipment and that the QAPPs are formally updated to reflect changes. This will also be an opportunity to remove SOPs that cover obsolete processes. The Center Manager will also collect and store all relevant protocols from each project to form a reference "library." Additionally, protocols will be collected and

reviewed for currency and application from any laboratories used for sample analysis.

5. Quality Control Subcommittee – A committee of members from each project, including QAOs, who meet on a quarterly (or as needed) basis to discuss and review quality evaluation activities. During these meetings documentation requirements will be discussed as well as training and certifications. As the Center projects progress, this forum will be used to review compiled summary statistics for performance and corrective measures.
6. Quality Assurance Manager – The QAM provides an overall review of the implementation and assessment of the documentation process.
    - Assessing whether work is performed according to approved documents.
    - Ensuring documented procedures are standardized at each level of the study organization.
    - Controlling, assessing and maintaining documented procedures, whether old, new or revised.
    - Recording and tracking steps to document changes, e.g.: identification, reviews, recommendations, approvals, additions, removals, alterations, confirmations of implementation, follow-up assessments.
7. Biostatistics and Exposure Working Group – Two informal groups of university biostatisticians and DEOHS participants who meet weekly to discuss relevant issues. These groups provide problem solving and peer review feedback and are outstanding resources that the Center has immediate access to.
8. UW CCAR Investigators Committee – A formal group of the UW CCAR investigators and principal people that meets monthly (or as needed) to discuss and review all matters related to the science being conducted within the Center. This group provides an opportunity to get feedback from a varied but relevant audience.
9. UW CCAR Internal Steering Committee – A formal group of the Center's investigators who define and shape the research being conducted. This committee will evaluate the QAM's findings and provide feedback as to corrective measures or significant changes in focus.
10. QAM Review – As discussed, the QAM will provide comprehensive reviews assessing the quality activities and performance relating to the Center's projects.

9 Assessment and Response

The Center Director will determine the suitability and effectiveness of the QMP system and the quality performance of the environmental research programs to which the quality system applies by:

- Assessing the quality system through the QAM's review. This review will be received by both the Quality Control Subcommittee and the Science Advisory Committee through a written report.
- Placing reviews on the QC and SAC committee agendas and requesting the QAM attend the meetings to verbally report findings.
- Recording receipt and review of quality reports in the committee meeting minutes as well as in the QMP manual, and making these materials available to the EPA Project Officer.
- Ensuring the QAM continues to be qualified to conduct assessments, has no direct involvement or responsibility for work being assessed, nor any conflict of interest.
- Ensuring that the documentation provided by the QAM addresses whether sufficient authority, access, and freedom to identify quality problems have been achieved.
- Ensuring the QAM proposes recommendations and independently confirms implementation and effectiveness of solutions.
- Requiring the QC Subcommittee to review the QAM's reports and respond to assessment findings.
- Providing a process to ensure corrective actions are made promptly, to confirm implementation and effectiveness of corrective actions, and to document actions on a case-by-case basis.
- Notifying and requiring the QC Subcommittee to address disputes that may arise.

9.1 Assessment and Response

Monthly (or as needed) QA/QC meetings allow the administrative core and QAM to keep abreast of the status of data quality and provide an opportunity for projects to receive feedback on an on-going basis. In-depth Technical System Audits (TSA) will be scheduled by the QAM as necessary, following EPA assessment guidelines.

The results of these reviews will be detailed in the QAM's assessment reports.

This document will also include:

- The status of and compliance with all quality management documents, noting any revisions, additions or corrective actions;
- Any outstanding quality issues and recommendations;
- Any disputes affecting technical or quality management; and
- An assessment of the QAM's access to needed information and personnel.

These reviews ensure that work is conducted according to operation protocols and quality management documents. The review will be circulated to the Biostatistics Core and the Principal Investigators of all projects. If reviewers indicate that work is not adhering to the quality management standards, the QAM will conduct an inquiry to establish the basis for the disparity. If reviewers indicate that a new SOP is warranted, the QAM will duly note the change in circumstances. The QAM will follow-up with recommendations to correct the operation by developing a new SOP, or revising the quality management document. The recommendation may also include improving the compliance of the associated staff. The QAOs and project managers will oversee the implementation of those recommendations and the QAM will re-assess their effectiveness.

The UW CCAR Quality Control Subcommittee will promptly address and respond to all issues reported in the assessment, and will seek input from the Center Director and Science Advisory Committee when necessary.

Data quality assessments may be conducted as a part of the QA/QC meetings, by members of the Biostatistics Core during the initial stages of data analysis and model building, and as a part of the annual review of data quality by the Biostatistics Core. Data quality assessments for the sub-awarded institutions may be conducted at the Center Director's discretion after intensive collection periods or at recognized milestones.

9.2 Stop Work Order

If the QAM finds that the quality of a process is unacceptable, that a minor alteration in the procedure would not fix the problem, or that staff are not adhering to a valid SOP, the QAM will contact the Center Director to determine the necessary action. A stop work order may be issued by the QAM until revisions to the process are made and an acceptable level of quality for the product can be documented.

10 Quality Improvement

Through the procedures outlined in this plan and the QA guidance documents provided in the references, communication across elements of the UW CCAR regarding quality control is ensured. All investigators, regardless of assigned QA status, are expected to identify, plan, implement, and evaluate effectiveness of quality improvement activities, and they are expected to maintain the goal of continuous quality improvement. UW CCAR staff, when directed by their principal investigator, the QAM or the Quality Control Subcommittee are expected to fulfill their responsibilities in preventing, correcting and identifying quality problems. Staff are expected to correct quality problems as soon as possible, and to document and track their activities.

11 References

US EPA, Office of Environmental Information, *Guidance for Developing Quality Systems for Environmental Programs, EPA QA/G-1*, EPA/240/R-02/008, November 2002.

US EPA, Office of Environmental Information, *Guidance for Preparing Standard Operating Procedures (SOPs), EPA QA/G-6*, EPA/240/B-01/004, March 2001.

US EPA AirData : Access to Air Pollution Data, http://www.epa.gov/air/data/.

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[1] US EPA, Office of Environmental Information, *EPA Requirements for Quality Assurance Project Plans EPA, QA/R-5*, EPA/240/B-01/003, March 2001.

[2] University of Washington, Human Subjects Division Grant and Contract Services Office of Research, Training website, http://depts.washington.edu/hsd/INFO/train.htm

[3] Block G, Jensen CD, Morrow JD, et al. The effect of vitamins C and E on biomarkers of oxidative stress depends on baseline level. Free Radical Biology and Medicine 2008;45(4):377-84.

[4] Li X-Y, Chow C. An improved method for the measurement of malondialdehyde in biological samples. Lipids 1994;29(1):73-5.

[5] Richard MJ, Guiraud P, Meo J, Favier A. High-performance liquid-chromatographic separation of malondialdehyde thiobarbituric acid adduct in biological-materials (plasma and human-cells) using a commercially available reagent. Journal of Chromatography-Biomedical Applications 1992;577(1):9-18.

[6] Suttnar J, Cermak J, Dyr JE. Solid-phase extraction in malondialdehyde analysis. Analytical Biochemistry 1997;249(1):20-3.

[7] Young IS, Trimble ER. Measurement of malondialdehyde in plasma by high-performance liquid chromatography with fluorometric detection. Annals of Clinical Biochemistry 1991;28:504-8.

[8] Rolla R, Vidali M, Serino R, Pergolini P, Albano E, Bellomo G. Antibodies against oxidized phospholipids in laboratory tests exploring lupus anti-coagulant activity. Clin Exp Immunol 2007;149(1):63-9.