Final Technical Report

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NATIONAL ENVIRONMENTAL RESPIRATORY CENTER

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I. Executive Summary

The National Environmental Respiratory Center (NERC) is a government- and industry-sponsored laboratory research program to improve our understanding of the relationship between complex mixtures of environmental air pollutants and human health. NERC was established in 1998 in response to the absence of research directly targeting the underserved issue of disentangling the contributions of the myriad man-made and natural air contaminants to the health effects associated statistically with exposures to complex mixtures of air contaminants. The program is based at the Lovelace Respiratory Research Institute (LRRI), which has uniquely well-suited experience, facilities, staff, and institutional commitment, but includes investigators in other institutions also. In addition to conducting a core program of novel research, the NERC provides resources to other researchers and maintains a large, web-based public literature citation database.

The NERC research strategy is aimed at constructing and analyzing a composition and health response database by conducting identically designed inhalation dose-response laboratory studies of the health effects of complex source emissions. This addresses the lack of an epidemiological or laboratory database allowing statistical analysis of the roles of individual air contaminants and their combinations in the different effects of inhaling complex pollution mixtures. A secondary, but important, goal is to make direct, detailed, contemporary comparisons among the health effects of common source emissions of public health and regulatory concern. To date, the exposures have included diesel emissions and hardwood smoke. Exposures to gasoline emissions, urban street dust, and coal emissions are being planned, and exposures to cooking fumes are envisioned. Each study will add a "layer" of data to the composite database. Parallel effort is underway to select strategies for analyzing the database.

The complex multi-year program is progressing well. Funding, rather than technical, limitations set the pace. The strategy was developed in concert with a highly qualified External Scientific Advisory Committee, which provides continuing, independent, active oversight. Non-EPA sponsors were recruited from federal and state agencies, industry groups, and corporations. Five pilot studies were selected competitively and conducted while the first "core" study was being developed. The experimental design and methods for each exposure have been developed on the basis of consensus input developed from expert workshops.

Exposures to contemporary diesel emissions and hardwood smoke have been completed, and exposures to gasoline emissions are imminent. Most, but not all, results from the diesel exposure are completed and analyzed, and publications are beginning to flow (results of pilot studies are published). Many, but fewer, results from the hardwood smoke exposure are complete. Results to date show that effects are modest within the plausible human exposure range and vary among exposures. Several important effects were detected. A reduced resistance to lung infection with respiratory syncytial virus is potentially the most significant effect identified at the lowest (i.e., approximately street-side) exposure level of diesel emissions. Dose-related inflammatory, cardiac, immunological, and DNA damage effects have been identified, but not all assays have detected significant effects.

II. Origin, Purpose, and Research Strategy of the Program

A. The "Multi-Pollutant" Dilemma and Development of the NERC Program

Our implementation of the Clean Air Act involves repeated cycles of review of the National Ambient Air Quality Standards (NAAQS) pollutants and reflexive single-pollutant research agendas that consume most air quality health research resources. Few resources are left to be directed toward the 33 "Urban Air Toxics" designated as most important among the 188 "Hazardous Air Pollutants" (HAPs); fewer yet are directed toward the remaining 155 HAPs; and ever-shrinking attention is given to the hundreds of other natural and man-made air contaminants that are not on these lists. In parallel, promulgation of source-specific standards (e.g., diesel engines, coal-fired power plants) tends to focus research attention on a few source types. For example, much attention has been focused on diesel emissions over the past 20 years, while almost no attention has been given to the even more ubiquitous gasoline emissions.

Moreover, hazards from complex source emissions are often conceptualized as resulting from a single, currently popular pollutant class, disregarding other classes that may be just as important or even more important hazards. An example may be the attention given to the soot fraction of diesel particulate matter (which of course is undoubtedly important) compared to the disregard of vapor-phase semi-volatile organic compounds (SVOCs) and elemental carbon-free organic nanoparticles, which overlap in composition with soot-borne organics and for which multiple lines of evidence strongly suggest are also inflammogenic, cytotoxic, and amplify development of allergic immune responses. It is not widely recognized among the health community that the mass emissions of SVOCs from contemporary (i.e., 1990 and later) on-road diesel engines are typically higher than mass emissions of particulate matter (PM), or that gasoline engines are also a major source of particulate and non-particulate organic emissions. Neither is it recognized that gradients of concentration with distance from roadways exist for SVOCs just as they do for fine or ultrafine PM. This is not to say that the air contaminants receiving attention are unimportant. Rather, it is to point out that researchers have incentives to demonstrate the magnitude, nature, and mechanisms of the effects of the pollutant or source "du jour," and much less incentive to place those effects into objective context among the effects of other air contaminants, other sources, or complex combinations of air contaminants.

Of course, people never breathe one pollutant, or pollutants from one source, at a time, but are always exposed to very complex, ever-fluctuating mixtures of hundreds of air contaminants from many man-made and natural sources. We have few biological markers that can link observed health effects to specific pollutants, classes, or sources. We know that many pollutants can cause the same effects (e.g., inflammation), that some pollutants can amplify the effects of others (e.g., ozone and diesel soot), and that the effects of some pollutants are less than additive when present together (e.g., mixtures of metals). It is plausible that a mixture of pollutants and their reaction products, each within its individually acceptable concentration, could present an aggregate health risk that is of an unexpected type or magnitude or that may be erroneously ascribed to the wrong pollutant or source. We have little understanding of the health hazards and risks of some ubiquitous air contaminant species (e.g., SVOCs) and source emissions (e.g., gasoline and cooking emissions, urban street dust), and very little understanding of the importance of exposures to complex combinations of air contaminants. We understand the shape of exposure-dose-response relationships at realistic exposure levels for only a few individual air contaminants and have scanty understanding of dose-response relationships for even simple combinations, much less complex mixtures, of contaminants.

To be sure, our past air quality research paradigm has brought us a considerable distance. It has resulted in much knowledge that has supported air quality standards that, in turn, have resulted in considerable improvement in air quality and (presumably) prevention of an otherwise greater health burden. The question is whether or not the same paradigm will serve us well in the future as currently regulated pollutants continue to decrease, pollutants and sources previously given little attention become relatively greater contributors to exposure, and further air quality gains become increasingly costly in the face of many competing public health needs. We cannot answer that question by asking how an improved understanding of mixtures will be applied in regulatory strategy; indeed, we cannot know what regulatory framework will make sense in the future until we develop an improved understanding. In a sense, we are talking about putting the "research horse" in front of the "regulatory cart," rather than compelling research to trail the regulatory cycle. The view underlying the NERC program, and by extension the proposed specific research, is that an increasing premium will be placed on "getting it right" with respect to the integrated relationship between air quality and health, and to do so, we need to complement (not wholly replace) the current research paradigm with new strategies, of which NERC is only one example. Indeed, the Clean Air Act foresees this need in its often-overlooked Section 103, which calls for research on "complex mixtures of air pollutants" and on health risks from exposures to both individual pollutants and "combinations thereof."

While it is conceptually straightforward to apply our current research approaches to an expanded list of air contaminants and source emissions one by one, it is more difficult to identify research strategies that could help us "get out in front of the mixtures problem" by substantially improving our ability to predict the effects of pollution mixtures from a knowledge of their composition. Epidemiology can only reveal effects of air contaminants that are measured; the "mixtures" can only be partially selected by time and location; area monitor data do not provide sufficiently accurate measures of personal exposure; and it is implausible to fully speciate personal exposures on a large scale or throughout chronic exposures. Controlled laboratory studies have tested binary and tertiary combinations of pollutants for effects that are more or less than additive. Because such exposures are not realistically complex, and testing permutations of pollutants becomes impractical beyond three or at most four components, this approach is best employed to resolve potentially important interactions once they are identified. Studies relating the composition of concentrated PM to effects are proving very useful for understanding that pollutant class, but only a portion of the pollution mix is concentrated. Studying exposures to mixtures such as combustion emissions are a step toward more realistic complexity and are relevant to source management, but previous studies have neither included detailed speciation of exposure nor attempted to disentangle the roles of more than a portion of the hundreds of individual components or combinations. In vitro assays offer rapidity and economy and are very useful for dissecting mechanisms, but they have yet to be shown to accurately predict the nature of inhalation exposure concentration-response curves or the relative ranking of lung responses to complex materials (Seagrave et al., 2003 offers a negative example). It seems unlikely that our ability to predict the health impacts of pollution mixtures will substantially improve unless other research strategies aimed specifically at that purpose are undertaken.

One theoretically sound strategy would be to conduct human studies to create a database containing precise, detailed, and uniform measures of personal exposures and health outcomes of large numbers of people exposed to different mixtures (combinations) of pollutants and to use compositional variations across the mixtures to disentangle the importance of individual pollutant species and classes and to point toward important combinations. Statistical strategies exist (and others would be adapted) to analyze such a database. One example of such a statistical strategy was recently demonstrated by our use of principal component analysis linked to partial least squares regression (aka projection to latent surfaces) to identify the compounds and classes responsible for rat lung inflammation and cytotoxicity among several collected diesel and gasoline emission samples that had overlapping but different compositions and a range of toxicities (samples and effects described in Seagrave et al., 2002; PCA/PLS results presented at June 2003 NERC advisory committee/sponsor meeting and manuscript in preparation). Because such a database cannot practically be constructed by epidemiology, NERC is undertaking that strategy in the laboratory using several animal response models in inhalation studies and key source emissions as the "mixtures." A valuable by-product will be detailed: contemporary comparisons among source emissions of regulatory relevance.

NERC resulted indirectly from congressional hearings surrounding the 1997 promulgation of revised NAAQS for PM and ozone, during which it was acknowledged that: 1) we had very limited ability to apportion the health impacts of air pollution among the myriad air contaminants and combinations, and 2) there was no research program aimed specifically at this problem. Congress designated funding in the FY-1998 EPA appropriation to initiate a multi-stakeholder, government-industry initiative termed by legislative language the "National Environmental Respiratory Center," with EPA as the lead Agency and the Lovelace Respiratory Research Institute (LRRI) as the host institution responsible for leading the design and conduct of the effort.

LRRI first undertook the task of identifying a research strategy that: 1) was plausible given current capabilities and very limited funding; 2) would provide information complementary to that from other research agendas and representing a meaningful, if limited, step forward in the understanding of health effects of complex inhalation exposures; 3) could be conducted with the input and sponsorship of multiple federal and non-federal entities with EPA as the lead sponsor; and 4) incorporated (to the extent plausible) consensus among the scientific community and sponsors. An External Scientific Advisory Committee (ESAC) was formed first (Table 1), and LRRI worked with the ESAC to formulate the strategy. In addition to traditional advisory roles, the ESAC was incorporated into NERC management by being given explicit approval authority over major decisions regarding NERC functions and experimental designs.

Table 1. Original NERC ESAC

Morton Lippmann, PhD, New York University (Chair)
Michael Bird, MSc. PhD, DABT, C.Chem, FRSC, Exxon Biomedical Sciences
Bill Bunn, MD, JD, MPH, Navistar
Glen Cass, PhD, California Institute of Technology
Jonathan Samet, MD, MS, Johns Hopkins University
John Vandenberg, PhD, U.S. Environmental Protection Agency
Ron White, MST, American Lung Association
Ron Wyzga, MS, ScD, Electric Power Research Institute

B. NERC Research Strategy

As cited above, the strategy addresses two interrelated information needs: 1) an exposure composition and concentration-response database encompassing several different, but overlapping, mixtures of air contaminants and 2) contemporary head-to-head comparisons of the health effects of complex source emissions of regulatory relevance (the ESAC originally recommended the 12 listed in Table 2). While it is implausible to generate exposure-response data for every possible combination of air pollutants, it is plausible to begin (and to test the strategy) by producing data encompassing a spectrum of pollutants relevant to source-based management of air quality.

Table 2. Research Matrix of 12 Exposures and 5 Categories of Health Outcomes Recommended Initially by the ESAC

	Cytotoxicity & Inflammation	Allergies & Asthma	Defenses Against Infection	Heart & Lung Function	Cancer
Diesel exhaust (contemporary, outdated)	+	+	+	+	+
Gasoline exhaust (on-road, off-road)	+	+	+	+	+
Road dust (paved, unpaved)	+	+	+	+	+
Wood smoke (hardwood, softwood)	+	+	+	+	+
Tobacco smoke	+	+	+	+	+
Cooking fumes (vegetable, meat)	+	+	+	+	+
Coal combustion emissions	+	+	+	+	+

Separate dose-response inhalation "core" studies are being done for each exposure atmosphere using a single core study protocol. The term, "core" is used to distinguish the studies used to build the database and compare source emissions from pilot or exploratory work ancillary to the database. Using a consistent protocol, whether or not all analytes or effects are expected on any single study, allows the data from all studies to be combined into a single database, with each study contributing a "layer" of composition-response data (Figure 1). The combined database will support analyses of the contributions of individual components, physical-chemical classes of components, and combinations of components to the health outcomes across exposures (i.e., as the "mixtures" change). Although this is largely a hypothesis-generating process, the underlying general hypothesis (which is also the hypothesis underlying interpretation of epidemiological results) is that *certain contaminants and/or combinations of contaminants will be shown to "drive" the different health outcomes, and these relationships will hold across different pollution mixtures.*

Animals are exposed to one of four concentrations (log dilutions) of each atmosphere, along with a clean air control group, and all exposure levels are intended to fall within the range of plausible human exposures (albeit perhaps occupational or "hot spot," rather than widespread environmental concentrations at the higher levels). Using five treatment groups within each study allows testing the significance of exposure-response trends and provides a view of the shape of the exposure-response curve, including potential identification of a no-effects level. A "significant" exposure-response relationship can thus be defined by a combination of trend tests and multiple comparisons among groups. The exposures are characterized identically for all atmospheres and at the greatest practical level of detail (equivalent, for example, to the speciation in the EPA Supersites program, environmental source apportionment studies, etc.). Exposure characterization encompasses the physical-chemical classes summarized in Table 3. Health responses are measured using a range of animal models and endpoints encompassing the general categories of health outcomes listed in Table 2. The health assays are summarized in Table 4.

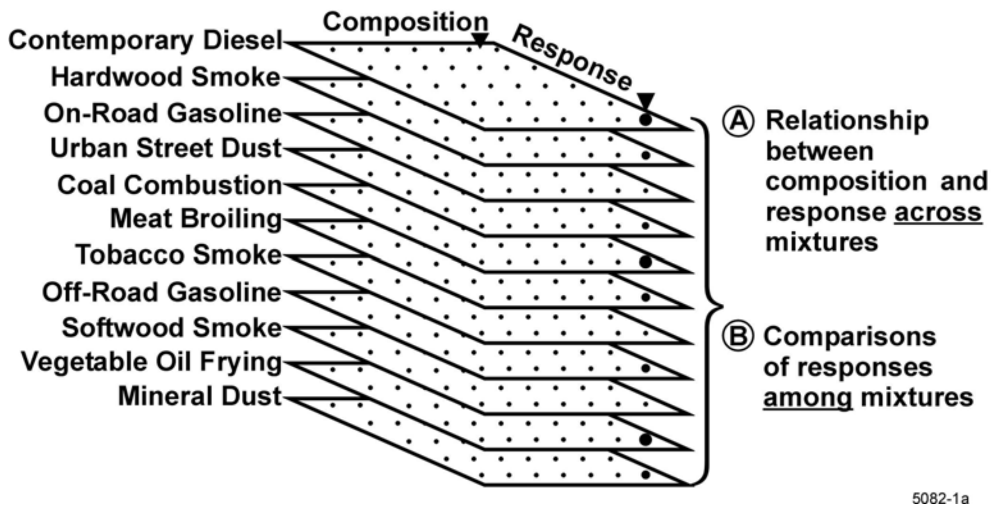

Figure 1. The Composition-Response Database Consists of "Layers" of Data from Individual Studies

Table 3. Characterization of Exposure

Particles:		Gases:
Mass concentration		CO
Size distribution		CO_2
Number counts		NO_x
Morphology		SO_2
Size-specific chemistry		HC
Extractable fraction (& OC/EC)		NH_3
Mutagenicity of extracts		
Particle Extract and SVOC:		
Ammonium	n-alkanes, cycloalkanes	organic acids
Sulfate	alkenes	alkaloids
Nitrate	Branched alkanes, alkenes	nitrosamines
Elements	Furans, benzofurans	PAHs (+oxy, nitro)
	Terpenes	Hopanes
	Volatile aromatics	Steranes
	Phenols (+methoxy)	Aliphatic alcohols
	Carbonyls	Carbohydrates

Table 4. Measurement of Health Responses

General toxicity:	Body & organ wt. Hematology, clinical chemistry, coagulation Bronchoalveolar lavage Histopathology
Pulmonary immune responses:	Development of allergic responses Enhancement of allergic responses
Resistance to respiratory infection:	*Pseudomonas aeruginosa* Respiratory syncytial virus
Cardiac effects:	Heart rate and variability Waveform abnormalities Heart and vessel histopathology
Carcinogenic potential:	DNA methylation Oxidative DNA damage Micronuclei Lung tumors in A/J mice Ames mutagenicity

The exposure atmospheres are prioritized for study, defined, approved, and the studies conducted individually as the program progresses; thus, the final list of exposures may differ in number or type from those recommended initially. This allows the program to respond to findings and current issues (e.g., evolving source types and emissions) as the construction of the database progresses.

Core research is conducted by a multidisciplinary team of nine LRRI and two external investigators who produce, analyze, interpret, and publish data, and four external contractors who perform additional analyses (Table 5). No team member works in isolation; each necessarily coordinates with others. The Study Director, Dr. Matt Reed, has responsibility for day-to-day coordination of study activities. Of course, the investigators supervise additional professional and technical staff to accomplish their work.

In addition to the core study protocol, lung gene alterations are analyzed using microarrays by Dr. George Leikauf, University of Cincinnati. While these analyses are being performed for each atmosphere, the protocol is exploratory, and thus not sufficiently standardized to be considered part of the "core" protocol.

The NERC management strategy aims to incorporate the best current thinking and broad consensus into the design of the research. This is accomplished by conducting peer expert workshops to review options and elicit recommendations for key components of the program. The ESAC and sponsors are also invited to attend all workshops. Based on input from the workshops, NERC management develops specific plans for ESAC approval. This process, which began with a workshop on optimizing core study experimental design to facilitate analysis of the resulting database, has continued with workshops to define each of the exposure atmospheres and methods for their generation. Summary reports were prepared following each workshop.

Table 5. NERC Core Investigative Team

<u>LRRI</u>	
Ed Barr, MSEE	Inhalation exposures
Ted Barrett, PhD	Immune responses
Steven Belinsky, PhD	DNA methylation injury
Matt Campen, PhD	Cardiac function
Andrew Gigliotti, DVM, PhD, DACVP	Histopathology, tumorigenesis in A/J mice
Kevin Harrod, PhD	Resistance to bacterial and viral infection
Jake McDonald, PhD	Exposure characterization, analytical chemistry
Matt Reed, PhD, DABT	Animal acquisition and management, body/organ weight, hematology, clinical chemistry, micronucleus
JeanClare Seagrave, PhD	Bronchoalveolar lavage
<u>Non-LRRI</u>	
Investigators	
Steve Seilkop, MS (SKS Consultants)	Biostatistics
Jim Swenberg, PhD (University of North Carolina)	DNA oxidative injury
Contractors	
Judy Chow, ScD (Desert Research Institute)	Analysis of ions
Eric Grosjean, PhD (DGA, Inc.)	Analysis of carbonyls & organic acids
Jamie Schauer, PhD (University of Wisconsin)	Analysis of elements
Barbara Zielinska, PhD (Desert Research Institute)	Analysis of organic compounds

In addition to conducting research, it is an explicit goal of NERC to leverage the investment by providing information and research resources to the broader community. A searchable public database of literature citations pertaining to the exposures and health effects under study by the NERC is maintained on the Center's web site, www.nercenter.org. The database contains citations from three sources: 1) established bibliographic services, 2) pre-database literature (i.e., pre-1980s), and 3) federal agency technical reports. Users access this information by either a user-directed search (i.e., entering search terms), or by viewing or downloading prepared lists of citations (topical reports) by categories replicating the cells of the research matrix shown in Table 2. The database is updated annually, and contains over 22,000 citations.

The Center encourages and facilitates the use by internal and external investigators of exposures and biological samples to extend the scope and value of the program beyond the core study protocol. The resources are available on a case-by-case, non-interference, no-added-cost basis, and include: 1) exposure of ancillary, special-purpose animals or in vitro biological systems on a space-available basis; 2) provision of excess biological samples or samples from the exposure atmospheres; and 3) addition of sample collections or measurement of health endpoints in protocol animals. While additional costs incurred by LRRI to accommodate these collaborations must be paid from non-NERC resources, collaborators are not required to share costs that must be incurred for the core protocol (e.g., development, generation, and analysis of exposure atmospheres).

III. Summary of Progress by Project Year

Year-by-year progress was summarized in detail in Annual Progress Reports submitted in April 1999, April 2000, April 2001, May 2002, and May 2003. The reader is referred to those reports for detail (copies were provided previously). Key accomplishments during each project year are summarized in the following sections.

A. Progress During Project Year One, April 1, 1998 – March 30, 1999

Work during the first project year was focused in four areas: 1) assembling the ESAC and working with the ESAC to develop a fundamental strategy to accomplish the purpose of the program, 2) developing the protocol to be used in the core studies, 3) selecting and initiating a limited number of pilot studies to be conducted while preparations for the first core study were underway, and 4) developing funding from other federal and non-federal sponsors to cost-share the program.

The ESAC and strategy described in the preceding section were developed. The original ESAC included Dr. Jon Samet (epidemiologist) and Dr. Glen Cass (atmospheric chemist), who later left the Committee by rotation (Samet) or death (Cass) and were replaced by others. Together with the ESAC, alternate approaches to the "pollution mixtures" program were discussed, and the approach described above was adopted as the best, albeit difficult, strategy.

Five pilot projects were selected by the ESAC from applications submitted in response to an internal and local (including University of New Mexico [UNM] collaborators) solicitation. In addition to standing on their own scientific merit, these projects all addressed technical issues that were expected to be important in the core studies. The following five projects were subsequently completed; all resulted in scientific presentations, and all but one (Ménache, which was a statistical exercise and not a "study" per se) resulted in at least one publication in peer-reviewed journals:

Bice et al., "Role of Inhaled Ultrafine Particles in Exacerbating Asthma in Susceptible Individuals"

Ménache, "Exposure to Mixtures of Pollutants: An Empirical Approach to Predicting Effects of Mixtures Based on Toxicological Experiments on the Individual Compounds"

Muggenburg et al., "Effects of Metals on Electrocardiograms of Adult Beagle Dogs: Assessment of the Role of Metals in the Pathogenesis of PM_{10}-Associated Cardiopulmonary Disease"

Nikula et al., "Mixtures and Emphysema: Mechanisms of Possible Interactive Effects between Air Pollution and Cigarette Smoke"

Tesfaigzi et. al., "Effects of Subchronic Wood Smoke Exposure on the Respiratory Tract of Rats"

A web site for the program (www.nercenter.org) was established, and major elements of the site were put into place. A strategy for offering information services relevant to NERC topics via the web site was developed.

An approach was developed for incorporating non-EPA funding from government and non-government sources into the program, and solicitation of funds began. Cost sharing among stakeholders was envisioned from the genesis of the program, but an approach had to be developed that provided a degree of separation of the management of the program and interpretation of results from the diverse interests of the sponsors. The strategy involved setting a minimum amount of funding for full involvement in meetings and workshops and access to pre-publication results. It was made clear that support, regardless of amount, did not "purchase" membership on the ESAC or decision-making authority. A key feature was vesting approval authority for major decisions in the ESAC, a body independent of sponsorship. By the end of the project year, six non-EPA sponsors had been added.

A Quality Assurance Management Plan was developed for the program and implemented (provided previously).

B. Progress During Project Year Two, April 1, 1999 – March 30, 2000

Two workshops, both including a wide range of external experts, were held to: 1) optimize the general experimental design for its intended purpose (i.e., building a composition-response database suitable for analyses identifying air contaminants driving the various health effects) and 2) select the health assays for the core protocol. The success of the process of obtaining broad consensus on key experimental design issues and of the 1½-day discussion format set the paradigm for decision-oriented workshops in subsequent years.

A statistical design workshop involving seven external participants and several LRRI and UNM staff discussed all aspects of the statistical design of the core studies (e.g., balance between number of treatment groups and group sizes, exposure and sampling times, scaling of exposure concentrations, etc.), and also various statistical approaches that might be used to analyze the combined database resulting from layering of data from all of the core studies. The protocol that was adopted was based on the consensus conclusions from the workshop. Thus, attention was given to experimental design before core research was begun.

A health assay workshop involving 17 external and numerous internal participants focused on identifying the most appropriate animal/measurement models for addressing each of the five general categories of health outcome of concern (listed in Table 2). Working groups for each category identified plausible models and prioritized them in terms of current biological understanding, interpretive value, acceptance in standard-setting, and practicality within the constraints of exposure design and funding. Alternate options were then reviewed by the entire group, and a consensus list of assays was developed. Subsequently, assays were selected for the core study protocol from those recommendations.

It was recognized that health response assays would evolve during the course of the program. The strategy was to adopt a set of core health assays that would not change during the program (essential for collecting identical data among studies), and to allow for new or less well-standardized (exploratory assays such as gene microarrays, "proteomics," use of transgenic animals, etc.) to be applied in a "pilot study" manner and to be added to the core protocol if warranted. This approach is key to the program strategy but differs from the more typical research agenda of conducting short-term studies with exploratory models and measurements. The latter approach is useful for many purposes, but has not, and cannot, produce a database of the sort necessary to accomplish the program's ultimate analytical agenda. Indeed, this is why the program was developed in the first place—explicitly to undertake a research approach that was needed but would not otherwise be accomplished because it ran countercurrent to the typical research paradigm.

In addition to the formal on-site workshops, two other information-gathering and consensus-building activities occurred during the year. Discussion with the Emissions Committee of the Engine Manufacturer's Association and the DOE-sponsored government-industry Diesel Crosscut Team resulted in unanimous recommendations for the engine, fuel, and operating cycle to be used in the core study of diesel emissions. The difficulty, but remarkable success, of this very open and inclusive information gathering and consensus building effort established the general framework for selecting the parameters of subsequent studies. The involvement of technical experts along with participants from both the regulated and regulatory communities in discussing and recommending experimental design parameters, with final approval by the ESAC, is one reason that the program has broad support among both the scientific community and sponsors.

Advice garnered from site visits by several experts resulted in a strategy for making changes to the Institute's engine emissions exposure system and for the characterization of all NERC core study exposure atmospheres. One of the participants was Dr. Jake McDonald of the Desert Research Institute, who was later recruited to lead the analytical effort. These discussions resulted in modifications to the engine emissions dilution/distribution system (which had been unchanged since the early 1980s) in accordance with the latest understanding of "real-world" emissions and the effects of dilution-time-temperature profiles on the composition of exhaust.

A standardized multi-step strategy for collecting, conducting quality assurance audits, and archiving core study data was developed. Dedicated computer equipment was obtained, and the strategy was implemented.

Dr. Gerald van Belle, a renowned biostatistician with particular interests in complex environmental problems (i.e., mixed exposures), was added to the ESAC. Along with the Committee's epidemiologist members (Samet, Wyzga), this addition bolstered oversight of statistical and experimental design issues.

The NERC web site was updated, and a public, searchable literature citation database was established. This citation database fulfilled an aspect of the program's intent to not only produce information, but also serve as a public source of information related to the exposures and health outcomes addressed by the program. By the end of the project year, over 22,000 citations had been entered, including many older citations and technical reports that were not accessible by standard electronic searches or library holdings.

Sponsorship was expanded to include 17 non-EPA agencies, trade associations, companies, and individuals.

C. Progress During Project Year Three, April 1, 2000 – March 30, 2001

The first core study, contemporary diesel emissions, began during the project year. Prior to initiation, the scope of the health measurements was scaled back from the original protocol in view of funding constraints. This was done in concert with the ESAC and reflected a normal balance between the ideal scope of work and that practical within the funding bounds.

Although deleted from the core study protocol for financial reasons, the effect of exposure on resistance to lung infection with respiratory syncytial virus (RSV) was conducted as a collaborative pilot study with other funding. The remaining equipment necessary for exposure characterization and assessment of health responses was obtained. Techniques were refined, standard operating procedures were developed, and the exposures began.

In keeping with consensus recommendations, diesel emissions were generated alternately from two 2000 model year Cummins 5.9-L ISB engines operated on test stands and controlled by computer on continuously repeating EPA heavy-duty certification cycles burning certification fuel representing national average composition. Animals were exposed in whole-body inhalation chambers 6 hours/day, 7 days/week for up to 6 months to whole exhaust diluted to particle (PM) concentrations of 1000, 300, 100, and 30 $\mu g/m^3$ or to clean air as negative controls. The exposure time varied among the different health outcome models from 3 days to 6 months.

Exposure to hardwood smoke was selected for the second core study exposure, and a workshop of nine external technical experts and several NERC investigators was held to discuss parameters of the exposure. The workshop resulted in consensus recommendations on wood, stove, burning cycle, and other operating conditions, based on demographics of wood stove use. A protocol was proposed to the ESAC, refined, and finalized, and work began to obtain the components and assemble the exposure system. In contrast to research on diesel emissions, there had been very few studies of wood smoke, and none that attempted to simulate actual burning conditions.

In discussion with the ESAC, simulated on-road gasoline engine emissions were selected for the third core study exposure.

The web-based literature citation database was updated. An e-mail newsletter was distributed to over 200 health researchers to make them aware of opportunities to conduct ancillary studies making use of the NERC exposure and biological samples that were excess to the core study protocol.

A total of 20 non-EPA sponsors had been enlisted by the end of the project year, some new, and many retained from previous years.

D. Progress During Project Year Four, April 1, 2001 – March 30, 2002

The exposure to diesel emissions was completed, and many of the results were also completed and analyzed. A strategy was implemented for statistical analysis of exposure-response relationships within individual core studies, and a biostatistician was recruited as a NERC investigator. A key problem was the likelihood of finding significant differences from control among the very large number of outcome variables by chance. All health data were analyzed by a multi-step screening process that met two criteria for significant exposure effects. A significant exposure effect required a trend across all treatment groups and also that one or both of the two highest-level exposure groups differed significantly from controls. This strategy proved effective in limiting the “positive” results to those sufficiently robust to warrant analysis and interpretation.

Many preliminary results from the diesel study were presented at scientific meetings, while study data were undergoing the quality assurance and statistical evaluations that would make them "final" and therefore suitable for publication.

Preparations for exposures to hardwood smoke were completed. A non-certified heating stove was located and sent to a stove certification lab in Washington State. There, the performance of the stove was evaluated, and operating parameters were developed. A building of the appropriate size for the stove to heat was moved to a location adjacent to the exposure laboratory, insulated, and fitted with air conditioners to create an appropriate (and realistic) year-round heat sink. The building was fitted with a cupola that maintained a constant environment (e.g., draft) around the top of the stack. A load of split wood consisting of a mixture of two species of oak from Missouri was obtained and placed in a walk-in freezer that had been converted to a repository for maintaining the wood at the proper humidity. A three-phase burning cycle was standardized, and a daily operating protocol was developed.

The hardwood smoke study was initiated. In view of the finding in the pilot study (mentioned above) that diesel emissions retarded clearance of RSV from the lung, the assay was re-instated into the core study protocol.

A decision was made in discussion with the ESAC to conduct a short-term follow-on diesel exposure using the original engines, fuel, and operating conditions so that the RSV assay could be applied to all exposure concentrations, and thus the database would be complete for that assay. It was also decided to repeat a portion of the assay of exposure effects on respiratory immune responses, in view of uncertainties left from the results of the original assay.

Dr. Judy Chow, a renowned atmospheric chemist from the Desert Research Institute, was added to the ESAC in view of the death of founding member Dr. Glen Cass.

A workshop of technical experts was held to discuss the generation of a gasoline emissions exposure atmosphere. The parameters of the exposure were discussed at length at the annual ESAC/sponsor meeting, and an approach was approved. Based on consensus recommendations, the emissions were to be generated from properly functioning, mid-life 1996 model intermediate-sized (approximately 4.0 L) automobile engines operated on test stands on the Unified Driving Cycle, which is a cycle for vehicles on chassis dynamometers. This necessitated measuring performance parameters of vehicles on chassis dynamometers and programming controls to simulate the same operation on engine test stands. Fuel was to be a national winter-summer average blend without added oxygenates. Stock, seasoned exhaust catalytic converters would be used to simulate typical vehicle emissions. Efforts to obtain the engines (vehicles) was initiated, and a contract was developed with the Southwest Research Institute (SwRI, San Antonio, TX) to test the vehicles, configure the engines for test stand use, and program the operating cycle.

Two new non-EPA organizations were added to the list of sponsors.

E. Progress During Project Year Five, April 1, 2002 – March 30, 2003

Exposures to hardwood smoke were completed without incident. Despite uncertainties about the reproducibility of smoke generation by an "uncontrolled" stove, the exposures proved to be well controlled and very reproducible. Effort was initiated to summarize, analyze, and report the results. Although analysis of results is not yet complete, it is clear that there were differences between responses to diesel emissions and wood smoke.

The short-term "repeat" exposure to diesel emissions was conducted to repeat some of the immune response assays and to conduct the assay of resistance to RSV infection at all exposure levels (the pilot study had used only three of the five treatment groups).

Multiple papers from the diesel study were in preparation, and some had been submitted before the end of the project year.

Considerable effort was expended to prepare for the gasoline emissions study. Three Chevrolet S-10 pickup trucks with 4.3-L V-6 engines and 49,000 to 71,000 odometer miles were purchased from individual owners after evaluation by an independent shop that the vehicles were operating properly and emissions were in the normal range. It was necessary to obtain three engines because two were needed on each exposure day to provide two "cold starts" per day, and thus an additional engine was required as a spare. The vehicles were fitted with new oxygen sensors, oil and filters were changed to those to be used during the study, and the vehicles were driven to the SwRI in San Antonio. SwRI verified that the emissions of the vehicles were normal and very similar when operated on the Unified Driving Cycle on chassis dynamometers and then removed the engines from the vehicles. The engines were re-configured for use on test stands, and one was mounted on a stand to develop the hardware necessary to connect them to dynamometers. Work on programming the cycle began.

Fuel specifications were finalized, and providers were contacted about blending the special fuel. An agreement was reached with the ConocoPhillips plant at Borger, TX, to provide fuel meeting the specifications, which were an average of national average summer/winter blends. Arrangements were made with a local company dealing with hazardous materials to store the fuel in drums under controlled conditions and to transport drums to the laboratory on demand.

Many modifications were made to the engine room in view of the greater safety hazard of operating gasoline engines within the facility than was the case for diesel engines. Most of the modifications and procedures were developed from information gathered by the Institute's safety and engineering staff to multiple engine laboratories (including the EPA Ann Arbor group and other laboratories in the Detroit area). The fire rating of the walls of the engine room was bolstered by adding layers of sheet rock, and new doors were installed. A CO_2-based fire suppression system was selected over alternative approaches, purchased, and installed. A strategy for transfer of fuel to the engine room was developed and a mobile system was constructed that incorporated flexible fuel bladders inside of a safety cabinet suitable for inflammables. Heat and chemical sensors were installed and mated to a computer-based alarm and shut-down system. The room ventilation equipment and control system was modified to provide multiple levels of ventilation, or a shut-down as necessary, depending on the nature of the emergency.

Dr. Severre Vedal, a renowned clinician/epidemiologist was added to the ESAC to replace Dr. Samet, who rotated off of the Committee but remained interested and available in an "emeritus" status.

Effort continued to identify the most appropriate statistical approaches to analyzing the composite core study database to determine composition-response relationships. A pilot study testing the utility of one likely statistical approach for identifying the components of complex exposures driving health effects was initiated. This study used a combination of principal component analysis and partial least squares regression (also known as projection to latent surfaces) (PCA/PLS) and results from testing the mutagenicity and lung toxicity of complex emission samples from normal and high-emitting gasoline and diesel vehicles in a DOE-funded study. The PCA/PLS approach gave very promising results and was able to provide high-quality statistical models revealing the components driving the different biological responses. These results were discussed with the ESAC and sponsors.

It was decided that the most appropriate next step in planning the database analysis would be to hold a workshop with the aim of developing a statistical working group that would be involved in both advising the activity and participating in the analysis. It is presumed that no single individual will conduct the analyses, but rather, it is most appropriate that multiple approaches be tried. The analyses will be conducted in a collaborative manner by multiple groups (e.g., universities) and coordinated by a NERC investigator. It was agreed that a workshop will be conducted during project year six, chaired by ESAC member Gerald van Belle.

A workshop of technical experts was held to discuss options for generating a coal emissions exposure atmosphere. Consensus was reached on the general parameters of the desired final exposure atmosphere, regardless of how it was generated. Two alternate strategies were identified and were subsequently discussed with the ESAC. Development of the exposure was estimated to involve considerable effort, and the ESAC recommended that exposures to urban street dust might be moved ahead of coal emissions on the study schedule.

The NERC Quality Management Plan was revised, and a Data Policy was drafted, reviewed, revised, and approved by the ESAC. A copy of the Quality Management Plan, which contains the Standard Operating Procedure for data management, was submitted previously. A copy of the Data Policy also was submitted previously. The Data Policy formalizes existing practices for the flow, auditing, statistical analysis, archiving, and public release of NERC core study data. The Data Policy was reviewed in draft form at the 2003 Annual ESAC/sponsor meeting, revised, reviewed with all investigators, and approved by a subcommittee of the ESAC.

IV. Summary of Key Achievements of the Program During the Five-Year Term of the Assistance Agreement

A. Successfully Established an Unusual, Highly-Targeted, Multidisciplinary Research Program in an Area of Unmet Need

Arguably, the greatest achievement was the successful development and implementation of a substantive, credible research program to directly and explicitly address the "pollution mixtures" dilemma in a research environment in which there was growing recognition of the need but no normal (i.e., RFA) mechanism for establishing such a program. No program had produced a composition-response database of sufficient size and complexity and encompassing sufficient different mixtures to allow the kind of analyses that could suggest causal roles of individual components across changing mixtures. There is no expectation that the NERC program will fully meet the need or answer all the important questions, but there is every expectation that the program is a step in the right direction and will provide relevant new information. Through Congressional appropriation and development of co-funding from a broad range of government and non-government sponsors, a program was created that had not been possible otherwise. In large part, the difficulty lay in the fact that a hypothesis-generating program was needed that was at once complex, long-term, expensive, and risky (i.e., the strategy is unusual, and its success is likely but not assured). What was needed was a program that complemented, rather than duplicated, the smaller, more technique-focused hypothesis-testing research approaches typically supported by grants, yet more exploratory and evolutionary than those typically supported by contracts.

The nature of the work ran countercurrent to typical research strategies yet had marked implications for regulatory strategies. Its success depended on successful identification and implementation of a unique combination of creative multi-stakeholder funding, a management strategy that isolated the program from diverse sponsor interests, facilities that were not widely available, the assembly of an appropriate investigator team, active scientific management balanced with high-quality science, building constituency within and among sponsors as well as the scientific community, and production of results and objective interpretations commensurate with the nature of the program's strategy. Those issues have been addressed successfully, although certainly not without difficulty.

The program is widely known and has considerable need-based and science-based philosophical support among both the scientific community and sponsors. It has broad support within EPA management, despite the obvious and understandable handicap of its funding to date by appropriation. The credibility of the program is demonstrated by its broad (and expanding) base of continuing sponsors, none of which have the program as a "line item" in their institutional budgets (i.e., except for EPA, all funding is voluntary and on a year-by-year basis). NERC is now widely recognized as one of the approaches, albeit certainly not the only viable approach, that should be undertaken as a new generation of integrative research paradigms that might move us away from the limitations of a single-pollutant view of the air quality-health relationship.

B. Established a Source-Based Health Research Program

It is ultimately pollution sources, rather than individual physical-chemical air contaminant species, that are targeted to manage air quality. Certainly, regulations may be expressed in terms of concentrations or emissions levels of individual pollutants, but it is important to EPA and to states to understand the contributions from, and comparative hazards presented by, pollution sources. The NERC research strategy uses common emission sources as tools for generating exposures to varying complex mixtures of air contaminants, rather than creating the exposures from artificial mixtures of specific pollutants. While this is not the only strategy that should be pursued, it provides the extremely valuable "by-product" of direct, detailed, contemporary comparisons of the health effects of common source types. Importantly, the source types include both those studied often (although not in such detail), such as diesel emissions, and those that have never been studied rigorously, such as wood smoke, gasoline emissions, cooking fumes, or street dust. An important feature of the program is that, regardless of the success of its fundamental long-term (combined database) strategy, the program is certain to pay off in terms of source comparisons—and has already begun to do so.

The differences between the information provided by this program and that provided by previous studies of source emissions are several. The methods for generating the exposures are selected on the basis of their representing typical emissions and thus significant population exposure scenarios. While the time and funding requirements of the strategy limit the program to one, or at most two, cases of each source, there is pre-existing consensus that the cases studied are reasonable. The exposures are characterized at the greatest level of detail allowed by contemporary analytical capabilities, and involve the capabilities of multiple institutions. It seems astonishing from our present vantage point, for example, that no previous study of diesel emissions has included full speciation of the exposure atmosphere; indeed, many still equate exposures to whole diesel emissions as exposures to "diesel particles" despite the fact that particles comprise a small portion of the total mass emissions! No previous program has applied such a broad (yet selective) spectrum of health outcome assays in an identical manner to different source emissions; yet, that is the only way to compare directly among them.

C. Produced Several Important Specific Findings from Core Studies Conducted to Date

Each of the two core exposures conducted to date (contemporary diesel emissions and hardwood smoke) generated a very large amount of data that is still being analyzed. Although no attempt will be made to report all findings herein, the following are examples of key summary findings to date:

- Exposures to even high concentrations of contemporary diesel emissions and hardwood smoke relative to environmental exposures (i.e., up to 1000 µg PM/m^3) cause only modest effects in common indicators of toxicity (e.g., morbidity, serum chemistry, bronchoalveolar lavage, histopathology). Indeed, exposures daily for six months to even the highest concentrations caused no light microscopic lesions other than a physiological increase in the number of alveolar macrophages.

- It is not possible to produce exposure atmospheres of different dilutions of diesel emissions at a wide range of concentrations in inhalation exposure chambers without inducing slight differences in composition. This is due to the background emissions from the animals in the chamber. Previous studies had not demonstrated this because the exposure atmospheres were not characterized in sufficient detail.

- At dilutions producing equal concentrations of particles, the effects of diesel emissions and hardwood smoke overlap, but differ in nature and magnitude.

- Hardwood smoke causes greater bronchoalveolar lavage indications of lung inflammation than diesel emissions.

- Exposure to diesel emissions for either one week or six months causes a dose-related reduction of serum clotting factor VII—a finding that was unanticipated at the initiation of the study but that has since proven to have parallels in both epidemiology and human experimental exposures.

- Diesel emissions at environmentally relevant concentrations reduce the ability to clear RSV from the lung and increase RSV-induced pathology. This finding, confirmed by reproducing the effect at the same low concentration, is the first evidence for amplification of viral infections and has important implications for susceptibility to this widespread infection of infants. Preliminary data indicate that hardwood smoke has some, but less, effect at equivalent particle concentrations.

- Exposures to diesel emissions for up to six months does not induce clastogenic changes (micronuclei) in circulating erythrocytes of mice or increase lung adenoma development in strain A/J mice, but does cause both oxidative damaged and increased methylation of genomic DNA.

- The effect of exposure to diesel emission on exacerbation of airway responses to allergens depends on the exposure order. Responses to allergens are amplified if the subjects are challenged with the allergen before diesel exposure, but reduced if the exposure order is reversed.

- Exposure to diesel emissions causes subtle, but measurable, changes in the heart rate and electrocardiogram pattern of hypertensive rats, but exposure to hardwood smoke at the same particle concentrations does not cause significant effects.

- Exposure to diesel emissions causes a dose-related activation of numerous rat lung genes, many of which are "clock genes" that are related to functions having diurnal cycles.

D. Provided Research Resources to the Scientific Community that Leverage the Value of the Program Beyond its Core Goals

To leverage the investment in this program, experimental resources are offered to the scientific community for collaborative work on a case-by-case basis. The resources primarily include exposures of animals ancillary to the core protocol, measurement of ancillary endpoints, and provision of biological samples (e.g., serum, tissues) that are not consumed by the core protocol measurements. A special effort is made to collect and archive biological samples for such use. The availability of these resources is communicated via the web site, through formal presentations at other institutions and informal discussions with colleagues, and by periodic e-mail newsletters. Although the usage of these resources has not reached its potential, many useful collaborative activities have been accomplished. The following are examples.

1. Lung Gene Alterations: George Leikauf, University of Cincinnati

Dr. Leikauf is a leader in the field of using lung gene micro-array techniques to identify and interpret up- and down-regulation of genes in response to toxicant exposures. Although the analytical methods are relatively standardized, the field is still learning how to interpret gene changes in terms of human health hazards. For this reason, the assay was not included in the NERC core study protocol. Regardless, there is considerable interest in evaluating gene changes, and interpretive ability is improving. We did not want to lose the opportunity to apply the assay on an exploratory basis. Dr. Leikauf is planning to evaluate gene alterations in lungs of rats in of all the NERC exposures, using samples extracted at LRRI and conducting analyses with funding from his NIEHS center grant. He found numerous exposure-related alterations in diesel-exposed rats and is now completing the analyses and summarizing them for publication. This is the first time the micro-array assay has been applied in a dose-response study of inhaled engine emissions.

2. Resistance to RSV Infection: Kevin Harrod, LRRI

As described above, the effects of diesel exposure on clearance of RSV from lungs of rats was first evaluated in an exploratory pilot study. The exposures were provided by NERC, and the other costs were covered by other funds. The finding that clearance of RSV was delayed and RSV-induced pathology was increased at even the lowest exposure level had important previously unknown implications for the potential effect of pollutants in general and diesel emissions in particular to increase the incidence or severity of this important infection of infants. The pilot study also provided insights into the mechanisms by which normal protection was diminished by demonstrating concurrent reductions in production of mucus and Clara cell secretory protein. These findings led to incorporation of the assay into the core study protocol and to a funded grant to determine the mechanisms.

3. Cystic Fibrosis as a Susceptibility Factor: Eric Sorscher, University of Alabama

Dr. Shorscher was working on the mechanisms and implications of cystic fibrosis using a transgenic mouse model (CF) of cystic fibrosis, and wanted to know if the specific gene alteration reflected in the model increased the response to inhaled toxicants. He shipped CF mice to Albuquerque, and with appropriate attention to the biological integrity of the NERC animals, his mice were exposed to the NERC diesel atmosphere for a few days. The CF mice did not prove to be more susceptible to the effects of diesel emissions than normal mice.

4. Effect of Diesel Emissions on Development of Respiratory Immune Responses in Newborn Mice: Ted Barrett, LRRI

The NERC core assay of the effects of exposure on the development of immune responses to an inhaled allergen uses young adult mice. Because the core diesel study did not demonstrate a significant effect, it was questioned whether there might be an effect if the exposure occurred when the mice were newborn. Effects of environmental exposure on infants are of considerable current interest. A study was conducted with shared funding using the NERC diesel exposure. The study demonstrated that the difference in age did not change the outcome of the exposure.

5. Effects of Diesel Exposure on Response of Splenic Lymphocytes to Mitogenic Stimulus: Scott Burchiel, University of New Mexico

Dr. Burchiel conducts research on the effects of environmental exposures on the immune system, and he hypothesized that exposure to diesel emissions and wood smoke might increase the proliferative response of splenic lymphocytes in response to stimulus with a mitogen. Because the spleen is only collected for histopathology in the NERC core study protocol, which only requires a small amount of tissue, portions of fresh spleen were provided to Dr. Burchiel. His results from diesel-exposed animals showed a biphasic proliferative stimulus, which he has interpreted and is preparing for publication.

6. Validation of Responses of Cells Exposed in Culture to Airborne Emissions by Comparison to Responses of Animals Exposed by Inhalation: JeanClare Seagrave and Jake McDonald, LRRI

Improved techniques were recently developed for exposing cultured cells to gases, aerosols, and mixtures (e.g., emissions) in "real time." The assay had been attempted several times in the past but was hampered by methodological problems. The new approach resolves many of those problems. If validated against responses to inhaled agents, the approach could be a considerable advancement in the quest for faster, less expensive, sub-animal toxicity assays. The approach was refined with funding from a DOE project, and NERC provided a source of exposure to diesel emissions. The responses of cells can be compared to the responses of animals that are measured in the NERC core study protocol. The full series of validation tests are nearly complete, and will represent one of the very few attempts to directly compare in vitro and in vivo responses.

7. Effect of Hardwood Smoke on Inflammation and Respiratory Function of Rats: Yohannes Tesfaigzi, LRRI

Dr. Tesfaigzi conducted a study of the effects of wood smoke exposure on lung inflammation and respiratory function of rats as one of the NERC pilot studies. Based on those published findings, he proposed a follow-up study to explore further the inflammatory, immunological, and respiratory function effects of inhaled wood smoke. A study was conducted using available space in the NERC hardwood smoke exposure chambers, with the non-exposure costs paid from Dr. Tesfaigzi's NIH RO1 grant. Useful findings resulted, and a publication is in preparation.

8. Effect of Exposure to Diesel Emission on Nasal Epithelium: Jack Harkema, Michigan State University

Dr. Harkema is a leader in investigating the effects of inhaled materials on nasal epithelium, and he wanted to evaluate changes in the noses of rats exposed to diesel emissions in the NERC study. Techniques for proper collection of nasal tissue were added to the standard NERC necropsy protocol, and Dr. Harkema and his technician came to LRRI and collected tissues for his research.

9. Microstructure of Carbon Particles in Diesel Emissions and Wood Smoke: Gerald Huffman, University of Kentucky

Dr. Huffman is an expert in the use of specialized electron microscopy to determine the microstructure (i.e., crystalline lattice structure) of carbonaceous particles. Dr. McDonald provided samples of particles from the NERC diesel and wood smoke atmospheres for inclusion in Dr. Huffman's structural comparisons. This was a unique opportunity to examine two very different types of carbonaceous particles collected using identical dilution/collection methods, thus avoiding methodological artifacts that had interfered with previous studies.

10. Bioavailability of Iron in Particles in Diesel Emissions and Hardwood Smoke: Ann Aust, University of Utah

Dr. Aust is a leader in the field of characterizing the specific chemical forms of iron in environmental particles and determining their bioavailability. This is important because different forms of iron differ greatly in their potential participation in oxidation reactions that are thought to be important to several cellular responses. Samples provided from the NERC exposure atmospheres were added to her comparisons. The diesel particle sample was shown to have much less total and bioavailable iron than did some previous samples from older technology engines. This finding expanded the information base and demonstrated that diesel particles could vary widely in iron content.

11. Translocation of Inhaled Pollutants to the Brain and Induction of Brain Pathology: Lillian Calderon-Garcidueňas, University of North Carolina

Dr. Calderon-Garcidueňas is conducting pioneering research on the links between air pollution and lesions of the central nervous system that resemble those seen in early stages of Alzheimers disease. She has identified lesions in humans and dogs from Mexico City in comparison to individuals from cleaner areas. This is a new finding, and the implications are far-reaching. Brains from diesel-exposed rats were provided to Dr. Calderon-Garcidueňas, and, although only very preliminary findings have been communicated, she has found exposure-related alterations.

E. Resulted in Numerous Scientific Presentations and Publications

The program has produced numerous presentations at scientific meetings and publications in both meetings proceedings and peer-reviewed journals. The results of the pilot studies conducted during the first year of the program were published. The rate of publication from the core studies has increased recently, but has been slower than might be expected to result from an equal amount of funding spent on more typical small, individual investigator-driven grants. In large part, that is a natural outcome of the program's core strategy. The core studies are large and complex, and each takes approximately a three- to four-year cycle from the initiation of planning to the completion of all the data and statistical analyses. For example, in part because of required technique development and the staged approach to analyzing groups (i.e., analyzing samples from controls and high level exposed before deciding whether to analyze the remaining groups), the DNA damage analyses from the diesel study are not yet complete. Regardless, the pace of data completion and analysis has increased during the past year and should increase further in each subsequent year as multiple studies are completed and comparisons are made among the exposures.

The following lists include publications and published abstracts of presentations resulting wholly or in substantial part from the program as of the date of this report. The items are listed in descending order from the most recent to the earliest.

1. Papers

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