

ABSTRACT

Project 5, the Toxicological Evaluation of Realistic Emission Source Aerosol (TERESA) study, will investigate the relative toxicity of primary and secondary particulate emissions from motor vehicles. The ventilation stack from a large tunnel within the metropolitan area of Boston, MA, will be used as the source of primary emissions. A sampling line will run from the plenum of the tunnel ventilation stack to a reaction chamber in a mobile chemistry laboratory, where the mixture of primary particles and gases will undergo photochemical oxidation to form secondary particle matter (PM). Simulations of different types of atmospheres will be conducted, including oxidizing, and oxidizing followed by neutralizing. This aerosol will then be delivered to a mobile toxicology laboratory for exposure of experimental animals. Five different exposure scenarios will be used, including filtered air, primary gas and particle emissions, primary plus secondary particles, primary plus neutralized secondary particles, and secondary particles formed in the absence of primary particles.

Normal laboratory rats exposed using each of the five scenarios will be evaluated for pulmonary, systemic, and cardiovascular effects using *in vivo* organ chemiluminescence, histopathology, bronchoalveolar lavage, blood cytology, and continuous measurements of cardiac and pulmonary function. The scenarios generating the largest and the least biological response will be further investigated using spontaneously hypertensive rats as a model of a susceptible population, using the same biological outcomes studied in normal rats.

Exposure atmospheres will be chemically and physically characterized using a wide range of continuous and integrated monitoring techniques. CO, NO_x, CH₄, NMHC, O₃, particle count, mass, and size distribution, and black carbon will be measured continuously. Particle elemental and organic carbon, elemental composition, ammonium, nitrate, sulfate, strong acidity, NH₃, HCHO, and VOCs will be measured using integrated sampling and analytical techniques.

For the biological effects observed during each exposure scenario, inter-group differences will be assessed using multi-way analysis of variance. To determine the effect of PM composition on biological response, linear regression models containing exposure concentrations as predictors will be fitted to each response outcome measure. Multiple pollutant linear regressions will be used to assess the independent effects of multiple pollution components on biological response.

The specific hypotheses tested by the proposed research are the following: i) Exposures to fresh and to photochemically aged mobile source emissions induce cardiovascular responses in normal animals; ii) Atmospheric photochemical processes enhance the toxicity of gases and particles emitted from motor vehicles; and iii) Animal models of a susceptible population (spontaneously hypertensive rats) will have greater biological responses to particles originating from mobile sources than the corresponding normal animal model.

1. OBJECTIVES

Because particulate and gaseous source emissions undergo many transformations once released into the atmosphere, it is likely that secondary and primary pollutants exhibit different toxicities. Since most of the source-specific toxicity studies to date have focused on primary pollutants, there remains a great need to investigate the relative toxicity of source-specific primary and secondary particles. We have recently begun to probe directly into this question with the Toxicological Evaluation of Realistic Emission Source Aerosol (TERESA) Power Plant study, a research project funded by the Electric Power Research Institute (EPRI) and the existing EPA PM Center. This study was designed to investigate the relative toxicity of primary and secondary particulate emissions from coal-fired power plants, *in situ*, and to explore the relationship between secondary particle formation processes and particle toxicity. As part of the TERESA study we have developed techniques and facilities to sample source emissions, form secondary particles inside a photochemical chamber, and expose animals to both primary and secondary particles. The TERESA approach forms an excellent foundation for future research, as it can readily be adapted to investigate other combustion sources. Using the developed technologies, we propose to extend this research to the toxicological investigation of primary and secondary pollutants from mobile source emissions released from the ventilation stack of a large roadway tunnel within the metropolitan area of Boston, MA. Subsequently, we will compare the relative toxicity of primary and secondary mobile source emissions with concentrated ambient particles (CAPs) and with primary and secondary coal power plant emissions from the current TERESA study.

The specific hypotheses of the proposed research are:

- Exposures to fresh and to photochemically oxidized mobile source emissions will induce cardiovascular responses in normal animals;
- Atmospheric photochemical processes enhance the toxicity of gases and particles emitted from motor vehicles, and;
- Animal models of susceptible populations (e.g., spontaneously hypertensive rats) will have greater biological responses to particles originating from motor vehicles than the corresponding normal animal model.

2. INTRODUCTION

The proposed study represents an extension of Project 4, which will investigate the relative toxicity of traffic and transported particles using normal and spontaneously hypertensive (SH) rats. Project 5 will employ the same animal models and biological outcomes to investigate the toxicity of primary and secondary traffic particles. Since the necessary background on selection of the animal model and health outcomes is presented comprehensively in Project 4, the introduction and approach sections of Project 5 will focus on the exposure aspects of the study rather the health outcomes. More specifically, this proposal will discuss the methods for: i)

exposure of animal subjects to representative vehicular emissions in the field, and ii) photochemical oxidation of primary emissions to produce secondary particles.

2.1 Linking Health Outcomes to Specific Sources: Understanding the relationship between source emissions and ambient PM-related morbidity and mortality outcomes is one of the fundamental issues of environmental health. Linking health outcomes to specific air pollution sources poses a great challenge, due to the complexity of ambient PM. Both primary and secondary ambient particles are largely internal mixtures of a wide spectrum of compounds originating from a myriad of sources, of many different types. The challenge of attributing the observed outcomes of PM exposure to specific particle components and subsequently linking them to source types is further complicated by the possibility of synergistic effects among particle components and gaseous co-pollutants. Consequently, many scientists have focused on the *in vitro* or *in vivo* biological effects of either: i) specific particle components such as sulfate, particulate acidity, carbon black, etc.^{1,2} or ii) specific sources including residual oil fly ash, diesel engine emissions, etc.^{3,4,5,6}. However, these toxicological studies are limited in that they rely either on artificial particles, ambient particle surrogates, or primary emissions. This is a serious shortcoming, since these pollutant mixtures are not representative of real world exposures, and may have significantly different toxicities than ambient particles.

The development of particle concentrator technology has allowed toxicological assessment of real ambient particles. Exposures conducted with concentrated ambient particles (CAPs) have been used in conjunction with source apportionment methods in an effort to link biological outcomes to specific source classes such as oil and coal combustion, traffic or road dust^{7,8}. The CAPs approach has been refined and presented in Project 4 as a method to conduct exposure studies of local (largely traffic-related) and transported particles. The exposures proposed in this project, together with the targeted CAPs studies proposed in Project 4, will allow comprehensive and realistic inhalation toxicological assessment of primary and secondary particles from a representative urban traffic source.

2.2. Health Effects of Exhaust Emissions: Recent epidemiological studies supported by our existing PM center have shown that particle black carbon (BC), a marker of traffic, is associated with significant effects on heart rate variability⁹. CO, a gaseous pollutant emitted outdoors primarily by motor vehicles, showed similar patterns of association as BC, which became non-significant after controlling for BC. For subjects with prior myocardial infarctions, the effect of BC on heart rate variability was almost four times as great as for healthy subjects. These findings suggest that traffic-related particles harm the cardiovascular system by compromising autonomic control of the heart.

The exposure studies examining the health effects of exhaust emissions have focused on primary emissions, mostly diesel and to a lesser extent gasoline exhaust. For example, a study in which mice were exposed to diesel exhaust particles showed effects on cardiac function and on resistance to viral infection⁵. Similarly, an *in vitro* study using cultured rat alveolar macrophages found greater cytotoxicity in response to PM from gasoline vehicles than diesel engines. The same study showed that diesel exhaust at low concentration produced greater stimulation of peroxide production than gasoline exhaust samples⁴. Recently, researchers have stressed the need

to investigate the toxicity of aged diesel exhaust particles and the bioactivity of real world doses of diesel exhaust³.

Both gasoline and diesel exhaust are environmentally reactive and it has long been understood that the ambient interactions of hydrocarbons and NO_x result in the formation of ozone and other potentially toxic secondary pollutants. It has been shown that atmospheric photochemical reactions of vehicular emissions can result in the formation of secondary organic aerosol^{10,11}, as well as in the alteration of the toxicity and mutagenicity of polycyclic aromatic hydrocarbons (PAH)^{12,13}, most probably via the formation of nitro- or oxy-PAH derivatives. Therefore, the TERESA approach provides a unique opportunity to examine and directly compare the toxicity of both primary and secondary vehicular emissions.

2.3. Representative Source Emissions for TERESA Mobile Source Study: Motor vehicles are relatively inefficient combustion engines with fairly high CO/CO₂ emission ratios as compared to power plants. Primary vehicular exhaust particles are composed largely of both elemental carbon (EC) and organic carbon (OC). Gas phase organic compounds are abundant, including unburned fuel and products of incomplete combustion. NO_x concentrations are appreciable, but SO₂ is much lower, and is mostly emitted from heavy-duty diesel vehicles. In addition to combustion exhaust, motor vehicles also generate a variety of other emissions, including road dust, as well as evaporative emissions, brake dust, and engine lubricant, all of which will be present in the ventilation stack exhaust emissions.

Sampling and characterization of mobile source emissions has traditionally been conducted in three major ways: dynamometer studies (chassis or engine), car chasing studies (emission measurements behind a moving vehicle), or tunnel studies. The most frequently applied approach in both emission characterization and exposure studies has been the dynamometer method; many standardized driving simulations have been developed and used for testing tailpipe emissions. This approach has been favored because it is laboratory-based, allowing investigation of the effects of different fuels in the same engine, or the same fuel in different engines, under the same driving simulation conditions. However, selecting a “representative” engine, fuel type and dynamometer profile to use as the basis for a toxicological study poses a great challenge. Car chasing studies, though they reflect real world driving conditions, have similar difficulties with selecting representative vehicles and fuels. This approach is further limited by the technical difficulties of monitoring and conducting exposures behind a moving vehicle.

Of the three major approaches, the tunnel is of greatest interest for emission and toxicological studies, since it best represents the primary emission of a combined urban fleet. In addition, the tunnel captures all of the potentially toxic components, including tailpipe emissions, road dust and evaporative fuel losses. There have been a number of studies that characterize air pollution in tunnels reported in the literature, most designed to measure real-world emission factors for various gaseous and particulate pollutants. Earlier tunnel studies were directed more toward the potential for ozone formation from mobile source emissions and were not designed to investigate PM emissions or composition. However, neither photochemical nor toxicology studies of mobile source emissions utilizing tunnel ventilation have been conducted, to date. Table 1 below summarizes emission rates of key gaseous species and PM in several recent US tunnel studies.

It should be noted that particle and gas primary emissions from Light Duty Vehicles (LDV) are significantly lower in mass emitted per mile than those for Heavy Duty (Diesel) Vehicles (HDV), except for CO, for which the emissions from the two classes of vehicle are comparable. Fleet emissions reflect a mixture of HDV and LDV, with the average emissions in mass per mile determined by the proportion of HDV to total vehicles.

Table 1. Tunnel study vehicular emissions measurements reported in the literature

Tunnel, Year (REF)	Vehicle Type	PM _{2.5} g/mi	PM ₁₀ g/mi	EC mg/mi	OC mg/mi	NOx g/mi	CO g/mi	HCHO mg/mi	NMHC mg/mi
Tuscarora, 1999 (14,15 ^a)	Fleet	0.100	0.141	81.1	49.9	9.32 ^a		8.6 ^a	
	LDV	0.022	0.016	5.32	4.55				
	HDV	0.216	0.289	296	179.8				
Sepulveda, 1996 (16)	Fleet	0.083	0.110	50.1	40.3				
Caldecott, 1999 (17)	LDV	0.0115		6.72	6.96	0.8	6.38	5.11	
Caldecott, 1997 (18,19 ^b)	LDV	0.0087	0.0099	2.03	4.4				
	HDV	0.74	1.3	283.7	451.7				
	HDV ^b		1.43 ^b			23.8 ^b			
Cassiar, 1995 (20)	HDV					19.5	6.79		0.16
Caldecott, 1994 (21) ^c Summer; ^d Winter	LDV ^c					0.96	9.91	7.47	0.48
	LDV ^d					0.95	7.80	8.36	0.39
Van Nuys 1993 (22,23)	Fleet	0.066		15.8	19.3		17.5	17.2	1.2
Cassiar, 1993 (24)	Fleet					2.66	9.91		0.59
	LDV					1.15	8.23		0.58
Tuscarora, 1992 (25)	Fleet					6.1	5.8		0.48
	LDV					0.39	4.89		0.29
	HDV					19.46	6.03	43	0.68
Ft. McHenry, 1992 (25)	Fleet					3.3	7.4		0.76
	LDV					0.81	6.35		0.62
	HDV					14.43	9.84	53	1.54

3. PRELIMINARY RESULTS

3.1. TERESA Power Plant Study: The TERESA technologies and approach have been used successfully in the current field study at a coal power plant. Numerous technical challenges were overcome for this coal power plant study. These include: the development of stack sampling technology that prevents condensation of water vapor from the hot, humid power plant exhaust during sampling and transfer, while minimizing losses of primary particles; development and optimization of a photochemical chamber of sufficient capacity to provide an aged aerosol for animal exposures yet small enough to fit into a field laboratory; development and evaluation of a denuder system to remove excess gaseous components; and development of a highly functional mobile toxicology laboratory.

In the coal power plant field study, stack exhaust is sampled, diluted, and transported to the photochemical chamber. Oxidants are added and the mixture irradiated with ultraviolet light to accelerate the formation of sulfuric and nitric acid from SO₂ and NO_x in the exhaust. In the chamber, 30-35% of the SO₂ is oxidized to H₂SO₄. This simulates a realistic atmospheric

scenario, taking into account atmospheric transport, deposition, and typical oxidation rates. By converting a realistic fraction of sulfur dioxide in the chamber, we maintain an environmentally relevant ratio of metals to sulfate in the exposure chamber, representative of atmospheres downwind of power plants.

Preliminary results are available from the first set of exposure scenarios investigated in the field. Concentration data from a scenario simulating an aged plume consisting of a mixture of partially neutralized sulfate and secondary organic aerosol, without primary particles, are presented in Table 2. Since this is an ongoing study, health outcome data are just now being produced. Initial field operations of the mobile exposure laboratory are very encouraging: for all outcomes assessed in this field setting, standard deviations of both the sham control and the secondary particle exposure scenario (in the absence of primary PM) were never more than 10% greater than the standard deviations achieved using the same methods in our laboratory.

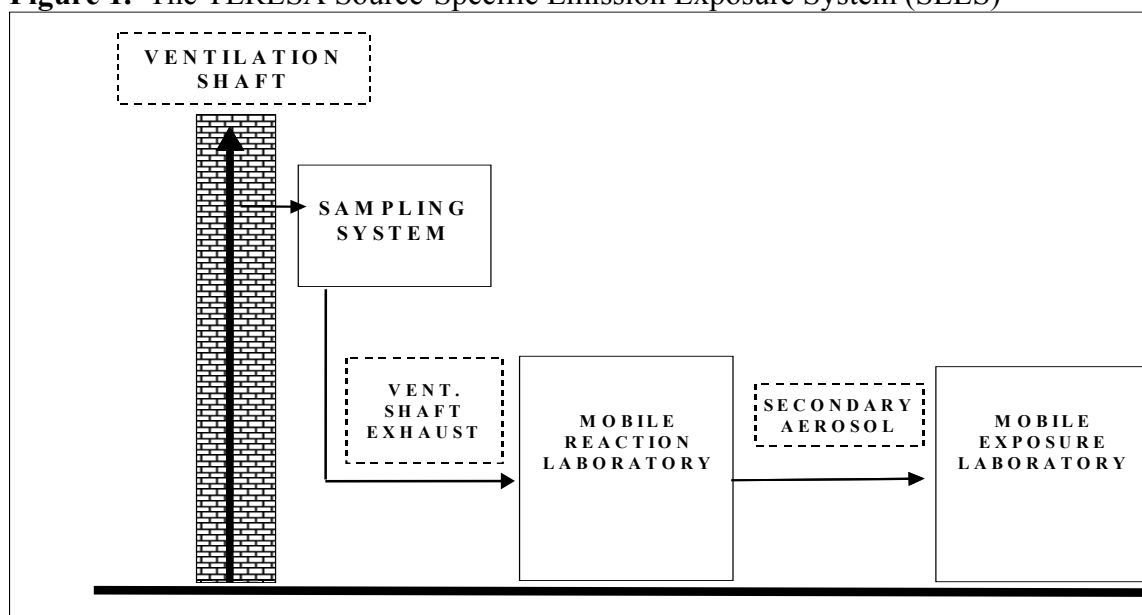
Table 2. Preliminary exposure characterization data: secondary (no primary) particle scenario

Exposure Parameter (units)	Secondary PM Scenario 6 Concentration Average (N=8)	Secondary PM Scenario 6 Concentration Standard Deviation
SO ₂ (ppb)	31.4	3.9
NO (ppb)	3.3	1.6
NO ₂ (ppb)	20.3	5.3
O ₃ (ppb)	32.1	4.0
PM _{2.5} (µg/m ³)	202.1	45.1
Total Particle Count (#/cm ³)	44,942	3,777
Temperature (°C)	24.2	0.9
Relative Humidity (%)	43.8	8.7

4. APPROACH

4.1. Overall Study Design: Since tunnel studies provide the best representation of the fleet emissions, we propose to utilize the ventilation exhaust from a large tunnel near downtown Boston. Using a TERESA study approach, we will investigate the relative toxicity of primary and secondary particles from mobile source emissions, and the role of atmospheric conditions in secondary particle toxicity. We have been given permission by the state agency regulating the specific tunnel to use it for the proposed study. The mobile reaction and exposure laboratories, developed for the TERESA power plant study and described in section 4.2 below, will be installed at the base of the selected tunnel ventilation stack building. Figure 1 is a schematic diagram of the field system for conducting this project. A sampling line will run from the plenum of the tunnel ventilation stack to the photochemical chamber in the mobile laboratory where it will be photochemically oxidized to form secondary PM. Simulations of several different types of atmospheres will be conducted, including oxidizing, and oxidizing followed by neutralizing. Both the incoming mobile source emissions and photochemical chamber output will be extensively characterized, and animal tests will be conducted for on-site evaluation of toxicity using normal and compromised laboratory rats.

Figure 1. The TERESA Source-Specific Emission Exposure System (SEES)



There is a seasonal difference in gasoline formulation.²¹ During the winter, gasoline is formulated with additional oxygenated compounds, to reduce the formation of the winter “brown cloud” effect. The most common additives are alcohols and methyl t-butyl ether (MTBE). Since emissions can be influenced by fuel composition, there will be both winter and summer components to the field study.

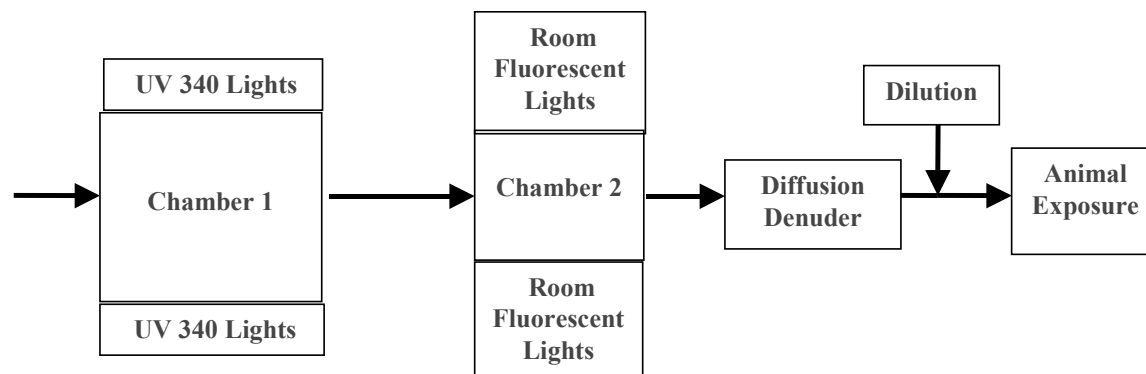
Upon completion of the study, the toxicity of fresh primary, aged primary and secondary PM from mobile source emissions will be compared with the toxicity of concentrated ambient particles and primary and secondary particles from coal power plants.

4.2. TERESA Study Source-Specific Emissions Exposure System (SEES): As suggested by Figure 1 above, the SEES developed for the TERESA study is a complex system designed to sample source emissions, simulate atmospheric photochemistry, generate an exposure, expose laboratory animals, provide exposure characterization, and determine biological outcomes of exposure. The component systems of this SEES are presented below in subsections 4.2.1 through 4.2.3.

4.2.1. Mobile Reaction Laboratory: This mobile facility is designed to transform particles and gases contained in the primary emissions from mobile sources to realistic atmospheric mixtures of primary and secondary particles for toxicological studies. For exposures to primary particles the gaseous co-pollutants in the direct emissions are reduced to concentrations that have negligible toxicity using dilution and diffusion denuders (described in section 4.2.2 below). Realistic formation of secondary particles is achieved using photochemical reaction chambers that combine primary emissions with atmospheric conditions that parallel those present in the troposphere. In addition, there is a full array of instruments/equipment to measure the composition of the ventilation stack gas, as well as the intermediate stages of the exposure atmosphere reactions. As demonstrated in the preliminary results section above, these techniques have been successfully applied to direct emissions from a coal-fired power plant and can readily

be adapted for mobile source emissions, as detailed below in Section 4.4. The simulated atmospheric reaction scheme is based on a two-stage model. This model assumes that the oxidation of NO_x and reactive organic gases (ROG) to form ozone, nitric acid, and secondary organic aerosol (SOA) takes place primarily in the plume that is formed from the initial dispersion of the ventilation stack emission. The second stage occurs when the HNO₃ mixes with and is neutralized by ammonia from ground level sources. The reaction scheme requires two separate reaction chambers, as shown in Figure 2 below.

Figure 2. The TERESA Photochemical Reaction Simulation System



In the first chamber, vehicular emissions are irradiated with UV light to form SOA and HNO₃, using a residence time of about one hour. The photochemical reaction chamber will use UV light with relatively low intensity in the short wavelength end of the spectrum, to prevent formation of reaction products that do not occur in typical mobile source emission plumes in the atmosphere. The reaction mixture then enters the second reaction chamber where, for some toxicological exposure scenarios, the acidic aerosol is neutralized with ammonia to produce ammonium nitrate particles. From the second chamber, the reaction mixture passes through a parallel plate diffusion denuder (see description below) to reduce reactive gas concentrations, while allowing the particles to pass through unchanged. Particle formation, composition, and toxicity are compared for the different scenarios corresponding to realistic atmospheric conditions, providing information on the effects of atmospheric chemistry on the formation of secondary particles and their health effects.

4.2.2. Parallel Plate Membrane Denuder: This novel denuder was developed by our group for use in the TERESA coal power plant study to reduce concentrations of un-reacted combustion gases to acceptable exposure levels, while allowing particles to pass virtually unchanged. Figure 3 below shows a schematic diagram of the parallel plate membrane denuder. The reaction mixture passes through an inner channel, while clean air is passed in a counter-flow fashion through two outer channels. Micro-porous PTFE Teflon membranes are placed between the inner and outer channels, which allow the diffusion of gaseous species, while particles pass through the inner channel. Laboratory and field evaluation showed that typically, gas concentrations are reduced by 80-90%. By using clean, humidified air in the outer channels, the denuder will achieve a humidity of about 50%, which is most appropriate for animal exposures.

Figure 3. Parallel Plate Diffusion Denuder

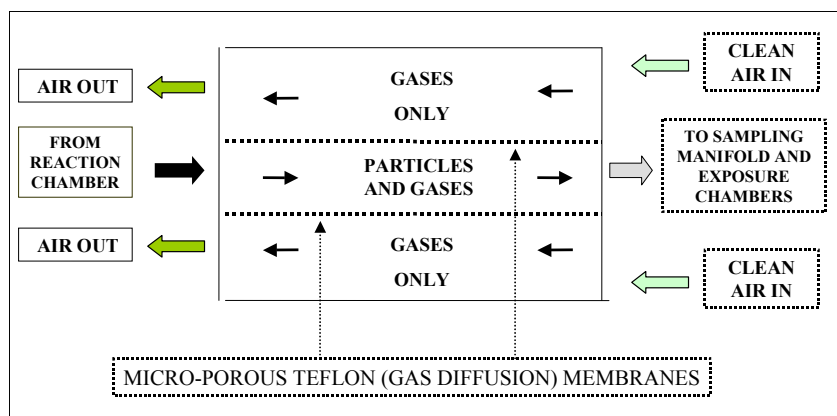
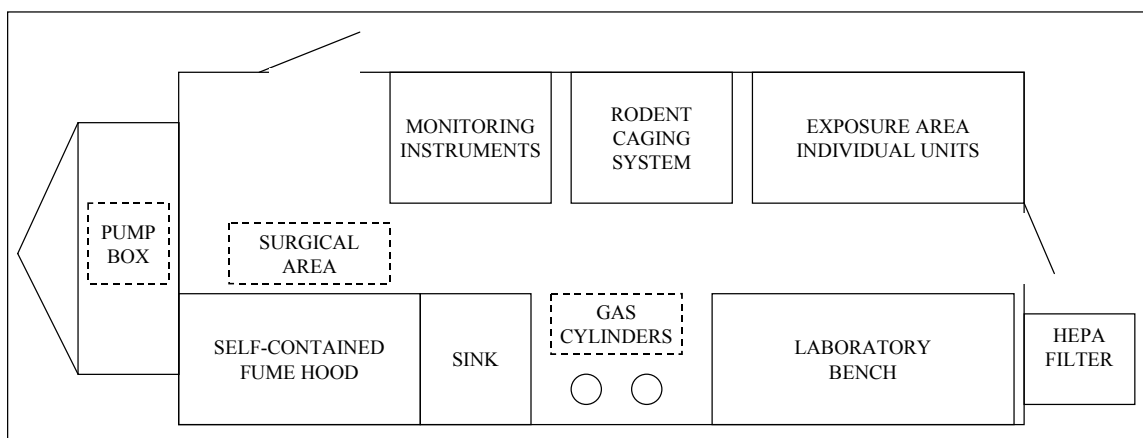


Figure 4. The TERESA Study Mobile Exposure Laboratory



4.2.3 Mobile Exposure Laboratory: A stainless steel tube is used to transport (fresh or aged) emissions from the reaction laboratory to the mobile exposure laboratory. The mobile exposure lab, shown schematically in Figure 4 above, contains all of the facilities necessary for toxicological testing, including the animal exposure system, pulmonary monitoring (BUXCO) and remote monitoring of blood pressure and cardiac function using surgically implanted telemeters (see Project 4, Section 4.2.7). The mobile exposure laboratory is a self-contained laboratory, requiring only electric power and telephone connections for operation. Water and waste are self-stored. An electric heat and air conditioning system control temperature and humidity to the specifications required for housing animals. An ADT alarm system monitors the trailer environment (temperature, humidity, ventilation in the animal housing unit) and trailer access. The alarm also has a motion sensor for movement within the trailer. A Thoren rat caging system provides proper rodent housing, with particle- and pollutant gas-free ventilation air provided through HEPA and charcoal filtration systems. The laboratory also contains a hot and cold water sink, a self-contained fume hood, a refrigerator/freezer, as well as equipment necessary for animal surgery, conducting *in vivo* chemiluminescence measurements, collection of blood, dissection of animals, collection of fixed tissues, liquid nitrogen storage of frozen tissues, and BAL analyses. In addition, there is a full array of instrumentation and samplers for full characterization of exposure atmospheres at the point of exposure.

4.3. Tunnel Characteristics and Concentrations: The tunnel selected for the proposed study is a tunnel with moderate-to-heavy traffic density, with a small positive grade (approximately 2°) over the portion of the tunnel served by the ventilation stack. Measurements in the ventilation stack plenum indicate that the exhaust, though significantly more dilute than vehicular emissions, will be suitable for this study. Average measurements for pollutant concentrations and traffic characteristics, recently collected over a 4-week period, were provided to us by the state agency that oversees the tunnel and are presented in Table 3 below. The tunnel ventilation system was designed to keep the concentrations of NO_x and CO within a specific range by adjusting the ventilation rate in response to CO levels. The relationship between CO and NO_x concentration in the tunnel is evaluated on a recurrent basis.

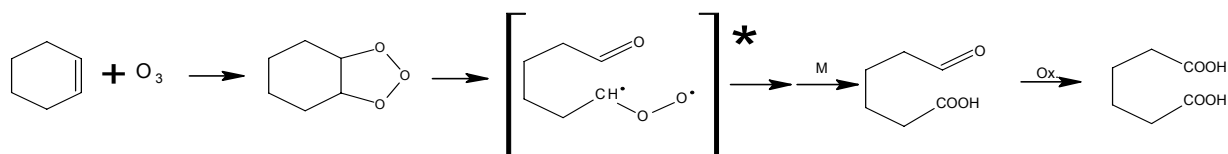
Table 3. Characteristics of Boston roadway tunnel traffic and ventilation exhaust at stack.

Parameter	Range	Average (approximate)
PM10 Concentration (µg/m ³)	120 - 200 µg/m ³	150 µg/m ³
NO _x Concentration (ppm)	1 – 2 ppm	1.5 ppm
CO Concentration (ppm)	12 – 20	15
Traffic Count (vehicles·day ⁻¹)	40,000 – 60,000	45,000
HDDV/Total Vehicles	-----	10%

The proposed study aims to investigate the relative toxicity of primary and secondary particles from mobile sources. Since the tunnel ventilation pulls ambient air in to dilute automobile exhaust, experiments will only be conducted during days when Boston air pollution levels are less than 10% of those in the exhaust air. For this reason PM₁₀, CO and BC concentrations in the ambient air outside the tunnel at ground level will be monitored before and during experiments.

4.4. Photochemical Reaction Simulations: Due to the presence of gas phase organics in the tunnel exhaust, ·OH will be needed only to initiate the reactions, since it will be subsequently regenerated in the chamber through photochemical chain reactions. A generalized (and simplified) mechanism for photochemical smog formation is included below in Table 4. It should be emphasized that the reactions summarized in the table are gas phase reactions. To initiate the photochemical smog mechanism an initial source of radicals is required. In the atmosphere, the photolysis of aldehydes is a significant source of free radicals; the photolysis of formaldehyde (HCHO) to form CO and 2 HO₂· radicals occurs several times faster than the generic photolysis of RCHO to form CO, HO₂·, and RO₂· in the table below. It should be noted that HCHO and CH₃CHO are relatively abundant in mobile source emissions, as indicated in Table 1. If necessary, formaldehyde and/or hydrogen peroxide can be added to the chamber as an initial source of free radicals. In other studies of gasoline vapor, propene has been added to initiate formation of ·OH radicals.²⁶ These reactions, in a chamber or the atmosphere, will eventually result in the accumulation of O₃, peroxyacetyl nitrate (PAN), and other PAN-like compounds. The rate of O₃ accumulation and photochemical oxidation within the chamber is determined by a number of factors, including the relative amounts of aromatics and aldehydes among the total volatile organic compounds. Aldehydes and aromatic compounds can result in increased radical concentrations, since they or their major photochemical oxidation products undergo photolysis to generate free radicals.

highest SOA yield compounds, it is also understood that the oxidation of gas phase cyclic alkenes by ozone can result in the formation of dicarboxylic acids and other dicarbonyl compounds, via a Criegee biradical intermediate, as illustrated below:



As the hydrocarbons become more oxidized, they tend to become less volatile and increasingly partition to the particle phase, both by condensation and nucleation. In addition, recent research has found that small dialdehyde molecules may readily polymerize to increase formation of SOA.³² Although SOA yield may be fairly low in terms of percent of reactive organic gases (ROG) oxidized, it is essential to remember that particulate phase organics in primary emissions are also expected to be altered in the atmosphere or reaction chamber, as discussed previously. This is especially true for PAHs, which are expected to form various nitro- and oxy-PAH derivatives.^{12,27,29}

The projected PM concentrations in the vent stack are expected to be approximately $150 \mu\text{g}/\text{m}^3$, representing mobile source primary emissions. OC and EC together should account for at least 80% of the total fine primary PM. We plan to increase the particle mass by about 30-35% with secondary organic aerosol. Of the tunnel studies summarized in Table 1 above, the overall NMHC/NO_x ratio is on the order of 1:4 for fleet vehicles. Applying this ratio to the selected Boston area tunnel, we estimate that NHMC concentrations would be approximately 350 ppb. Converting 4% of the gas phase organics to SOA would result in more than $45 \mu\text{g}/\text{m}^3$, assuming a molecular weight of $80 \text{ g}\cdot\text{mol}^{-1}$.

4.6. Planned Exposure Scenarios (Photochemical Simulations): Photochemical conditions to be simulated in producing aged emissions for exposures are summarized in Table 5 below.

Table 5. Exposures and photochemical simulation scenarios for mobile source study

Scenario	Simulated Atmospheric Condition	Exposure
1	Sham Exposure	Gas and Particle Free Air
2	Primary Mobile Source Emissions	50 ppb NO_2 , $150 \mu\text{g}/\text{m}^3$ primary PM
3	Aged plume: oxidized emissions, secondary organic aerosol formation with primary particles	$<50 \text{ ppb NO}_2$, $150 \mu\text{g}/\text{m}^3$ primary PM, and $45 \mu\text{g}/\text{m}^3$ secondary organic aerosol
4	Aged plume: primary particles with secondary organic aerosol and ammonium nitrate formation	$<50 \text{ ppb NO}_2$, $150 \mu\text{g}/\text{m}^3$ primary PM, $45 \mu\text{g}/\text{m}^3$ SOA, and $25 \mu\text{g}/\text{m}^3$ ammonium nitrate
5	Secondary organic aerosol, ammonium nitrate formation without primary particles	$<50 \text{ ppb NO}_2$, $45 \mu\text{g}/\text{m}^3$ SOA, and $25 \mu\text{g}/\text{m}^3$ ammonium nitrate

The control scenario, repeated with each exposure, will be a sham exposure to gas- and particle-free air. The primary emissions exposure, scenario 2, will be tunnel ventilation stack effluent reduced to 50 ppb NO₂ concentration using the parallel plate membrane denuder (described previously) as well as dilution with clean air, if necessary. Scenarios #3, 4, and 5 will simulate photochemically oxidized mobile source emissions. Scenario #3 will result in exposure to mobile source emissions plume consisting of primary particles and secondary organic aerosol formed in an oxidizing atmosphere. Scenario #4 will simulate exposure to an aged plume, containing primary particles with both secondary organic aerosol and ammonium nitrate formed by photochemical oxidation followed by neutralization with ammonia. An additional scenario (#5), consisting of secondary particles without primary particles, will use the same conditions as scenario #4 on tunnel ventilation exhaust filtered to remove primary particles. This scenario will be conducted to further investigate the relative toxicity of secondary PM as compared to photochemically aged primary PM. Scenario #5 is particularly important in this vehicle exhaust study, since primary particulate organic compounds may undergo gas-particle reactions in the atmosphere or the chamber.

4.7. Animal Exposures and Toxicological Measurements: Animal exposures will be performed using normal and compromised laboratory rats in temperature- and RH-controlled chambers located within the mobile exposure laboratory. Each exposure will include at least 6 exposed and 6 sham-exposed rats. This is the number of animals that can be optimally studied per experiment in the field laboratory. Repetitions of experiments are used to obtain the numbers of animals needed per outcome group. Photochemically aged air will be drawn from the atmospheric reaction chamber into a manifold, then through individual exposure chambers in parallel. Exposures will be six hours in duration. The mobile exposure laboratory contains the animal exposure apparatus, animal housing, and surgical and laboratory facilities that will allow on-site biological sample collection and toxicological evaluation. Selection and assessment of biological outcomes is presented comprehensively in Project 4, and briefly summarized below.

4.7.1. Stage I Toxicological Assessment: For each of the scenarios in Table 5 above, normal male Sprague-Dawley rats will be exposed and subsequently undergo toxicological assessments, as summarized in Table 6 below. Each scenario will be repeated at least 6 times, so that sufficient numbers of animals are achieved for each outcome, and each repetition will consist of 12 (6 exposed and 6 sham exposed) rats. Of the 6 exposed rats in each group in each repetition, 2 will be used to assess organ chemiluminescence, 2 for cardiac and pulmonary histopathology and morphology, 2 for bronchoalveolar lavage (BAL), and 4 for blood cytology. Pulmonary function measurements will be made in all rats.

4.7.2. Stage II Toxicological Assessment: The scenarios inducing the greatest and least effects will then be investigated in a Stage II toxicological assessment using a rat model of a susceptible (hypertensive) population. These two scenarios should be sufficient to test the hypothesis of whether enhanced outcomes will be observed in this susceptible animal population. Susceptible animal models mimic human diseases or conditions that may make humans more sensitive to the effects of air pollution. These models can help determine which population subgroups are at highest risk and also provide additional insight into the mechanism(s) of PM effects. This stage will be conducted using male spontaneously hypertensive rats and their Wistar Kyoto strain

related controls. The toxicological evaluation will include the same biological endpoints summarized in Table 6 below. The rationale for selecting the spontaneously hypertensive rat model is discussed in Project 4.

4.8. Chemical and Physical Characterization of Exposure: Chemical and physical characterization of exposures will closely parallel the previous study. For each scenario, exposure atmospheres will be comprehensively monitored for pollutant gases, particle number and size distribution, and inorganic and organic particle composition using an array of continuous and integrated methods, detailed in the Particle Technology and Monitoring Core, Section 4.4.

Table 6. Stage I toxicological assessment of normal rat exposures to each scenario

Evaluation Method (Detailed in Section)	Effect Assessed	Specific Parameters	Measurement Period
BAL ^{8,33,34,35,36,37,38} Project 4, 4.2.11	Pulmonary Inflammation and Injury	cell viability, total cell count, cell type, lactate dehydrogenase, β-n-acetyl glucosaminidase (βNAG), total BAL protein	24 hours post exposure
Histopathology and morphometry of lung and heart vessels ^{8,39} Project 4, 4.2.12	Pulmonary and cardiac injury, evidence of vasoconstriction	3 randomly selected slices of fixed lung and heart tissue	24 hours post exposure
BUXCO ^{7,33} Project 4, 4.2.6	Pulmonary Function	peak inspiratory flow (PIF), peak expiratory flow (PEF), tidal volume (TV), minute volume (MV), respiratory frequency (F), end inspiratory pause (EIP), end expiratory pause (EEP), Derived parameter PenH	Continuously during exposure
Organ Chemiluminescence ^{40,41} Project 4, 4.2.8	Pulmonary and Cardiac Oxidative Stress	<i>in vivo</i> whole organ chemiluminescence of heart and lung	Immediately post exposure
Blood Cytology	Systemic Effects	total white cell counts, differential profiles	24 hours pre- and post- exposure
ECG telemeter Project 4, 4.2.7	Cardiac Function	heart rate, heart rate variability (SDNN),* ECG morphology	Continuously during exposure
Pressure Telemeter Project 4, 4.2.7	Blood Pressure	Systolic pressure Diastolic pressure	Continuously during exposure
Blood Chemistry Project 4, 4.2.10	Systemic Effects	complete blood count, cytokines (interleukin-6), atrial natriuretic peptide (ANP), vasoactive mediator endothelin-1	Post exposure

*SDNN: Standard deviation of the normal beat-to-beat intervals

Sampling will be conducted at three locations: (1) photochemical reaction chamber inlet (primary emissions); (2) outlet of the photochemical reaction chamber (aged emissions); and (3) from an empty animal chamber (exposure). The first two sampling locations will be used to monitor effectiveness of the photochemical reaction simulation system, looking at changes in

concentration of gas and particle concentrations and particle size distribution. The third sampling location will be used for exposure characterization, with the sampling location as comparable to the exposure conditions as possible.

4.9. Power Calculations: This project uses the same animal models, biological outcomes, exposure metrics, and sample sizes as Project 4. See discussion in Project 4, Section 4.2.1.1.

4.10. Data Analysis and Comparative Toxicity Analysis: Data analysis will begin with application of traditional exploratory techniques (univariate exploration, distribution checks, outlier identification). Based on these data summaries, data transformations will be considered so that the data satisfy modeling assumptions. Multi-way ANOVA will be used to assess differences among groups defined by type, source, and season (summer versus winter) of pollution, as well as rat type (normal versus hypertensive). Analyses will be conducted using PROC GLM in SAS to first assess overall differences among levels for each factor, and then use multiple comparisons procedures, such as Scheffe's multiple comparison procedure for cohort groups or Dunnett's procedure when interest focuses on making comparisons against the filtered air control, to assess differences between pairs of factor levels. Residual analysis and other diagnostic tools will be used to confirm model fit and identify highly influential data points.

Although there is a target dose of pollution for each exposure, there will be some variability in these levels across experimental runs. If variability of concentrations among repeated experimental runs of a given exposure scenario is significant, linear regression techniques will be used to assess dose-response slopes for each exposure scenario. These analyses will estimate toxicity by using total mass or the concentration of a particular component of exposure as the predictor in the linear regression. The comparative analysis involves the integration of air quality and toxicology data collected under each component of the study (summer and winter mobile source emissions) with similar data generated for coal-fired power plant emissions and CAPs. Because the proposed study uses the same protocol and biologic outcomes as the current coal-fired power plant study as well as the CAPs studies proposed in Project 4 of this proposal, the ANOVA and linear regression models will be extended to include exposure by study interactions in order to assess differences in toxicity from PM generated by the different sources. Techniques are described in detail in Sections 3.1.1 and 4.2.5 in the Biostatistics Core.

4.11. Animal Subjects: Animals are maintained and studied in accordance with the National Institutes of Health guidelines for the care and use of animals in research; the mobile exposure laboratory and all protocols have been approved by the Harvard Medical Area Standing Committee on Animals. In addition, field operations will be monitored by an independent veterinarian reporting regularly to the committee. Selection and handling of animal subjects is discussed in Project 4, Section 6.2.

4.12. Timeline: The proposed TERESA Mobile Source study will begin during the second half of Year 3, following the Project 4 Animal Toxicology study, and conclude in Year 5 of the PM Center. Preparation of the chamber and modification of the photochemical reaction system will be conducted in the laboratory during the second half of Year 3 of the PM Center. Two exposure studies will be conducted during the winter and summer of Year 4 of the proposed Center. Data analysis will be completed during Year 5 of the Center.

5. EXPECTED BENEFITS

The vast majority of the population is exposed to traffic particles; therefore, assessing the toxicity of both primary and secondary particles from mobile sources is critical. This project uses an innovative approach to investigating this very important source of ambient particles. In addition, using tunnel emissions offers a unique opportunity for investigating representative emission samples as compared to that from a single-vehicle tailpipe. The findings from this study are therefore likely to be of great public health significance. Examining the toxicity of tunnel ventilation exhaust has a distinct advantage within this proposal, in that it will strengthen the relationship between the Bus (Project 2) and NAS (Project 1) studies, which investigate the effects of traffic emissions on human health using the corresponding inflammatory and vascular outcomes. Furthermore, results from this project in combination with the Animal CAPs (Project 4) study will investigate the differences in biological effects between exposures of ambient CAPs enriched in local traffic emissions compared to primary and secondary vehicular emissions.

6. GENERAL PROJECT INFORMATION

6.1. Personnel: The Principal Investigator of this Project will be Dr. Petros Koutrakis who will be assisted by Co-Principal Investigator Dr John Godleski and Co-Investigators, Dr Beatriz Gonzales-Flecha, Dr. Joy Lawrence and Dr. Jack M. Wolfson. Petros Koutrakis will oversee all technical and administrative aspects of the Project. Because of his background in atmospheric chemistry and air quality he will be responsible for the design of the exposures scenarios. John Godleski will oversee the animal exposures and biological measurements. Beatriz Gonzales-Flecha will oversee the *in vivo* chemiluminescence studies. Joy Lawrence, atmospheric chemist, will be responsible for the reaction chamber and characterization of the exposures. Finally, Dr. Brent Coull, Center Biostatistician, will participate in the data analysis.

6.2. Facilities: The mobile reaction and exposure laboratories developed, equipped, and deployed for the original TERESA study, funded by the existing PM Center and EPRI, will be available for this study. Development of these facilities was conducted within the scope of the existing PM Center, and is presented within the PTM Core of this proposal. Our mobile facilities already contain equipment and instrumentation required to conduct the proposed study. Modifications to the existing facilities for this project will be conducted, including adaptation of the photochemical reaction simulation system described above in section 4.4. In addition, the sampling and dilution system will require modification, since the tunnel exhaust ventilation is already sufficiently diluted and exists at ambient temperature and humidity.

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