State of California
AIR RESOURCES BOARD

Protocol Development for Vehicle Emission Toxicity Testing for Particulate Matter

RESEARCH PROPOSAL

Resolution 14-11

May 22, 2014

Agenda Item No.: 14-4-1

WHEREAS, the Air Resources Board (ARB) has been directed to carry out an effective research program in conjunction with its efforts to combat air pollution, pursuant to Health and Safety Code sections 39700 through 39705;

WHEREAS, a research proposal, number 2772-278, entitled "Protocol Development for Vehicle Emission Toxicity Testing for Particulate Matter," has been submitted by the University of California, Davis; and

WHEREAS, the Research Division staff has reviewed Proposal Number 2772-278 and finds that in accordance with Health and Safety Code Section 39701, research is needed to improve and standardize ARB's methodology for preparation of vehicle emission samples for toxicity testing. Results from this project will be used to establish a standard operating procedure for ARB researchers and improve the ability of staff to evaluate possible health risks associated with vehicle emissions. Research Division staff recommends this proposal for approval;

WHEREAS, in accordance with Health and Safety Code section 39705, the Research Screening Committee recommends for funding:

> Proposal Number 2772-278 entitled "Protocol Development for Vehicle Emission Toxicity Testing for Particulate Matter" submitted by the University of California, Davis for a total amount not to exceed $100,000.

NOW, THEREFORE BE IT RESOLVED that the Air Resources Board, pursuant to the authority granted by Health and Safety Code section 39703, hereby accepts the recommendations of the Research Screening Committee and Research Division staff and approves the following:

> Proposal Number 2772-278 entitled "Protocol Development for Vehicle Emission Toxicity Testing for Particulate Matter" submitted by the University of California, Davis, for a total amount not to exceed $100,000.

BE IT FURTHER RESOLVED that the Executive Officer is hereby authorized to initiate administrative procedures and execute all necessary documents and contracts for the

research effort proposed herein, and as described in Attachment A, in an amount not to exceed $100,000.

I hereby certify that the above is a true and correct copy of Resolution 14-11 as adopted by the Air Resources Board.

Tracy Jensen

Tracy Jensen, Clerk of the Board

Attachment A

"Protocol Development for Vehicle Emission Toxicity Testing for Particulate Matter"

Background

The Air Resources Board (ARB) evaluated the toxicity of Particulate Matter (PM) in numerous studies from a variety of sources, such as engine emissions, indoor air, and concentrated ambient air pollutant samples. Due to the lack of a standard operating protocol (SOP), individual researchers used different methodologies for PM sample preparation which can affect the toxicological properties of the sample. Toxicological variations arise from the different physical manipulations and chemical interactions that occur during processing which can preserve some critical PM components, eliminate others, and possibly introduce artifacts that could lead to false positive results. While possibly acceptable for comparison of samples for a particular assay, the different procedures used for PM sample preparations have compromised the ability to directly compare the toxicity of samples from different researchers even for the same PM sample. For example, some researchers use whole PM samples for *in vitro* studies while others use extracts from PM dissolved in either aqueous or organic solvents. It is not certain if any of these methods favor particular PM components or directly introduces toxicological artifacts, making result comparisons difficult. Best practices for sample preparation are needed, and there has not been a comprehensive evaluation of the merits of the various procedures.

The results of this research will contribute to the scientific baseline in the field of *in vitro* evaluation of PM by being one of the first studies to systematically compare a comprehensive panel of PM preparation methodologies and thus provide valid references for other researchers in the field. ARB staff plans to use the study results for standardizing toxicological evaluations in future engine emissions studies. Also, these results may be used to identify possible biased data in previously conducted *in vitro* toxicological studies and allow for reinterpretation of previous results. The knowledge gained and the improved methodology will help ARB in its current Vehicle Emissions Research Programs (VERP). VERP has been ongoing since 1998 and has included toxicological evaluation of PM from different fuels (biodiesel and renewable diesel), and from newer engine technologies (for both heavy duty and light duty vehicles). This project could affect the interpretation of these studies if it's shown that PM preparation methodology employed may have influenced toxicological results.

Objectives

The primary objective of this research is to develop a recommended SOP for the preparation of PM samples collected on filters for toxicological assays. This objective will be accomplished by investigating how different methodologies for isolating PM samples from filters can affect the results in standard toxicological screening assays. To the extent possible, the research will compare the recommended protocol with previously used procedures to assist ARB in interpreting the toxicity results from past studies. The adoption of a SOP would provide a streamlined methodology for future PM sample preparation for toxicological studies.

Methods
This study will evaluate commonly used procedures for preparation of PM samples collected on filters, assess the relative merits of these procedures, and recommend a SOP for sample preparation. The initial phase of the study will involve a detailed literature review of recent studies which involved the isolation of whole PM and PM extracts for use in toxicological investigations. The evaluation will focus on which procedures are the best at protecting sample chemical integrity, retaining the most toxicologically relevant chemical components of the samples, and producing the fewest false positive toxicological artifacts. The results of this phase of the project will be a document detailing a review of all relevant studies, a summary of various methodologies with a critique on strengths and weaknesses. In addition, this document will outline the experimental protocol including five to six unique sample preparation methodologies to be used for this study.

Upon approval of the study document and experimental protocols, the investigator will be given archived filter samples from a previous dynamometer diesel engine study. To allow direct comparisons of various methodologies, all filters will be from the same test run. In addition, as a control, the investigator will obtain a standard diesel PM sample from the National Institutes of Standards and Technology (NIST) to be used for the study. Both whole PM and PM extract samples will be prepared from both the engine PM filter and the NIST standard using the methods outlined in the experimental protocol. The whole PM and PM extract samples along with relevant preparations from blank filters will be used in *in vitro* assays for inflammation, oxidative stress, and genotoxicity, the major physiological impacts related to engine PM health effects. The ability of the samples to elicit inflammatory and oxidative stress markers will be determined by measurement of the production of inflammatory biomarkers, such as COX-2, IL-8, CYP1A1, and HO-1, in a human macrophage cell culture. Oxidative stress potential of the samples will also be determined using a cell-free chemical assay. This will consist of measuring the ability of isolated PM or PM extracts to oxidize the chemical dithiolthreitol in an aqueous system. Genotoxicity of the PM sample will be assayed using the standard Ames assay which measures the ability of a sample to cause mutations in a bacterial sample.

Expected Results
Upon completion of the toxicological studies, the results will be analyzed using standard statistical methods in consultation with a staff statistician. The principal investigator, Dr. Keith Bein, will then summarize the results regarding which methodology produced the most optimal results for each toxicological assay. Based on these results the investigator will produce a SOP for sample preparation of PM collected on filters along with a final report detailing the study.

Significance to the Board
Research is needed to improve and standardize the ARB's methodology for preparation of vehicle emission samples for toxicity testing. Results from this project will be used to establish a SOP for Air Resources Board researchers and improve the ability of staff to evaluate possible health risks associated with vehicle emissions.

Contractor:
University of California, Davis

Contract Period:
24 Months

Principal Investigator (PI):
Keith Bein, Ph.D.

Contract Amount:
$100,000

Basis for Indirect Cost Rate:
The State and the UC system have agreed to a ten percent indirect cost rate.

Past Experience with this Principal Investigator:
ARB has not had any contracts with Dr. Keith Bein as a PI; however, the investigator has had ample experience in the field as demonstrated by his recent work on sample preparation techniques for two ARB-funded toxicological studies. Given his background in PM isolation techniques for both whole PM and PM extract samples, he is well-suited for this study which focuses on both preparation methods using methodologies similar to those he has previously employed.

Prior Research Division Funding to the University of California, Davis:

Year	2013	2012	2011
Funding	$ 1,131,716	$ 4,949,363	$ 1,394,560

BUDGET SUMMARY

University of California at Davis

"Protocol Development for Vehicle Emission Toxicity Testing for Particulate Matter"

DIRECT COSTS AND BENEFITS			
1.	Labor and Employee Fringe Benefits	$ 72,732	
2.	Subcontractors	$ 0	
3.	Equipment	$ 0	
4.	Travel and Subsistence	$ 1,000	
5.	Electronic Data Processing	$ 0	
6.	Reproduction/Publication	$ 0	
7.	Mail and Phone	$ 0	
8.	Supplies	$ 14,490[1]	
9.	Analyses	$ 0	
10.	Miscellaneous	$ 2,687	
	Total Direct Costs		$ 90,909
INDIRECT COSTS			
1.	Overhead	$ 9,091	
2.	General and Administrative Expenses	$ 0	
3.	Other Indirect Costs	$ 0	
4.	Fee or Profit	$ 0	
	Total Indirect Costs		$ 9,091
TOTAL PROJECT COSTS			**$ 100,000**

[1] Laboratory supplies for sample preparation tasks (chemicals, glassware, hardware, consumables) is $4590. Laboratory supplies for toxicological assays (cell culture supplies, biochemicals, assay kits, Ames assay supplies) is $9,900.