Toxicologic Pathways of Rail Yard Emission Exposure on Non-Cancer Health Impacts

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Introduction

The goal of this project was to utilize both quantitative chemical and cellular assays to assess the potential health effects of ambient air samples collected in residential neighborhoods surrounding rail yards. In the study, ambient aerosols were collected, their particle and vapor phases assayed for prooxidant and electrophile content and their effects on inflammatory and cytoprotective proteins determined.

The project had three objectives:
1. To determine the chemical properties of ambient air in residential neighborhoods near the three most polluted rail facilities in the state.
2. To establish a cellular assay for a stress response and for an adaptive response to ambient air pollutants.
3. To determine the cellular effects of the ambient air samples using the assays established in objective 2, and to analyze their quantitative relationship with the chemical assays performed in objective 1.
4. Engage local community residents living near three (3) rail yard facilities to determine sampling locations and share research results.

Summary

1. **Collections** Two collection campaigns were conducted, one examined sites neighboring the three major railyards in Southern California, Commerce, Long Beach and San Bernardino (Three Communities study). The second study examined aerosols from neighborhoods surrounding the Commerce Railyard (Neighborhood Sites study). Large scale samples of particles, collected on Teflon filters and vapors, collected by XAD resin beds were obtained. Collection sites were screened and chosen during community tours guided by East Yard Communities for Environmental Justice (EYCEJ) in Commerce, West Long Beach Neighborhood Association (WLBNA) in Long Beach and Center for Community Action and Environmental Justice (CCAEJ) in San Bernardino.
2. **Chemical characterization**
The long (48 hour) collection protocol resulted in a mixing of up and down wind aerosols, and reduced differences in the samples collected. As a result, only trends between the sites could be noted. However, some differences in the chemical and biological properties of the ambient aerosols were observed.

a. In the Three Communities study, particle prooxidant levels in the Commerce area tended to be higher than those for Long Beach and San Bernardino. In contrast, the electrophile content of vapor samples collected during the summer in San Bernardino was substantially higher than those for the other two sites (Table 1).

b. The focus on neighboring sites of the Commerce Railyards (Neighborhood Sites) proved to be more effective in assessing the differences in chemical properties of railyard emissions. Particle prooxidants were higher near the railyards than in a background site upwind from the yards. Although a small fraction of the total, the vapor phase prooxidants were higher in those sites near high locomotive and railyard activities (Table 2).

c. The particle prooxidants from all sites were mostly (>80%) metals, and most prooxidant activity was found in the particle phase, while most of the electrophiles were in the vapor phase (Table 1).

2. **Biological characterization**

The expression of two proteins, the inflammatory cytokine, tumor necrosis factor α (TNFα) and the cytoprotective or adaptive enzyme, hemeoxygenase-1 (HO-1) by macrophages in response to the particle and vapor phases of the ambient aerosols was monitored as measures of inflammatory and cytoprotective or adaptive responses, respectively. Quantitative enzyme linked immunosorbant assay (ELISA) procedures were established in the laboratory for these proteins with the objective of direct comparison with chemical reactivities. Selected samples from each of the two studies were then analyzed by these procedures and the results showed:

a. The summer samples from San Bernadino were the most active of the three community collections in terms of both TNFα and HO-1 expression,with TNFα induction caused only by the particles and HO-1 induction only by the vapor phase (table 1) . These results have been submitted for publication.
b. Preliminary data from the Commerce railyard Neighborhood Sites study showed that aerosols from the sites closest to the railyards, which included a main yard site and a maintenance site were the most active in inducing TNFα and were higher than a site reflecting truck traffic as well as the background site (table 2). As with the aerosols from the three community study, TNFα induction was caused only by particles and HO-1 induction only by vapors.
c. A correlation analysis was performed on the neighborhood study to assess the contributions of the chemical reactivities to the biological responses. The results, (table 3), showed the important influence of vapor phase reactivities to the cellular effects. As the analysis is based on preliminary data, the values are subject to change, but the trends should be valid; they indicate that vapor phase electrophile content correlated with both HO-1 induction and PM based TNFα induction. The latter correlation was better than that with PM prooxidant or electrophile content. Based on these data, vapor phase electrophile content would appear to be a good predictor of both proinflammatory and adaptive responses.

Conclusions and discussion

1. The overall observations made in this project indicate that the particle phase of the aerosols studied is responsible for the inflammatory response, with the vapor phase inducing a cytoprotective or adaptive response. Results of the correlation analysis (table 3) show that the chemical reactivity contributions are less clear because of the strong influence of the vapor phase reactivities which correlate with both HO-1 and TNFα induction. Thus, although the PM are primarily prooxidant in content, PM prooxidant content does not correlate as well with PM TNFα induction as do both prooxidant and electrophile content of the vapor phase. One possible explanation may be the nature of the prooxidants in the two phases. The PM prooxidants are mostly metals, as shown by the almost complete loss of activity by a metal chelator, whereas the vapor phase contents are all organic compounds. If the effects are due to the organic species of both phases, correlation of TNFα induction with the vapor phase reactivities may result. This possibility is being investigated in solvent based fractionation of diesel exhaust particles

2. Results from the Commerce Neighborhood Sites study suggested that the aerosols closest to the railyard had the highest proinflammatory actions, with the area close to the maintenance yard the highest. The vapors from the same aerosols exhibited the same tendencies, i.e. those closest to the railyards induced the highest levels of HO-1.

3. The distribution of the biological effects between the particle and vapor phase points out the importance of both phases in assessing the net health effects of ambient aerosols and the need for simultaneous monitoring of particles and vapors. The induction of the adaptive or cytoprotective protein, HO-1 by the vapor phase suggests that with repeated exposure, an attenuation of the inflammatory effects of particles may occur, thereby reducing the potential adverse health effects of the entire aerosol. This notion should be investigated as it would affect the interpretation of regulatory data based on particle count.
4. In January 2011, a day-long closed-door seminar was held at UCLA to share study results with and answer questions from the three community groups. The agenda for this meeting can be seen in Appendix 1. In May, sampling and toxicological analysis results were publicly shared in the three communities at task force meetings, whose attendees included U.S. EPA, Department of Toxic Substances Control, South Coast Air Quality Management District, staff and board members, California Air Resources Board, County Enforcement staff, local decision makers, including the new city council members, community residents and participants in the rail yard study (ie: those who offered their property as a sampling site). Products of these meetings are available upon request. [1,2]

Future directions and objectives

The data shown in tables 1 and 2 utilized samples collected in one week. Additional analyses will be performed to validate the conclusions made and to expand the correlation analysis to a larger number of samples.
Further studies of the properties of the samples need to be conducted. The issue of metal vs. organic based compounds in the particle phase will be addressed in organic solvent based extraction studies of these and other diesel exhaust samples to characterize the physical chemical properties of biologically active components of emissions. Feedback forms will be sent to the three community groups to learn from our interaction, hear what they found most useful and how they have advanced their rail yard pollution advocacy work as a result of our collective efforts.

[1] Powerpoint presentations from UCLA and community meetings, by Dr. Froines
[2] One page summaries of study findings for each of the three communities

Tables

Table 1 Aerosols from three communities: Chemical and biological properties

Site/Phase	**DTT Prooxidants (total)**	**DTT Prooxidants (metals)**	**DHBA Prooxidants**	**GAPDH Electrophiles**	**HO-1 expression**	**TNF α expression**
Units	**nmoles/min*m^3**	**nmoles/min*m^3**	**nmoles/min*m^3**	**NEM equivalents/m^3**	**pg/mg protein/m^3**	**pg/mg protein/m^3**
S-CM-2/particles	0.559 (0.475-.603)	0.559	0.379 (0.35-0.41)	0.033 ± 0.022	NS	51.1 ± 3.8
S-CM-2/vapors	0.066 (0.048-0.083	ND	ND	0.444 (average of 2)	34.5 ± 4.7	NS
S-LB-2/particles	0.371 (0.287-0.453)	0.371	0.606 (0.58-0.62)	0.000	NS	38.1 ± 1.8
S-LB-2/vapors	0.083 (0.069-0.099)	ND	ND	0.564 ± 0.019	43.2 ± 12.1	NS
S-SB-2/particles	0.651 (0.553-0.783)	0.651	0.477 (0.45-0.50)	0.011 ± .007	NS	463.9 ±40.9
S-SB-2/vapors	0.149 (0.104-0.194)	ND	ND	1.440 ± 0.155	181.0 ± 32.2	NS
Variance estimate	**95% CI**		**95% CI**	**SEM (N≥3)**	**SEM**	**SEM**

The values are from samples collected at the indicated sites on June 29, 2010. All values were normalized to volume of air in m^3. ND = not determined, NS = not significantly different from control. The estimates of variances are shown in the bottom row. The values for protein expression are the slopes of the log concentration vs. protein expressed dose response curves ± the estimated standard errors.

Table 2 Aerosols from neighborhoods near the Commerce Railyard

Site	DTT Prooxidants	Metal DTT Prooxidants	TNFα Expression	GAPDH Electrophiles	HO-1 induction (@ 0.5 m^3/mL)
Site 1 (main yard) PM	0.606 (.54-.67)	0.606	44.01 ± 4.56	0.116 ± .006	
Site 2 (maintenance) PM	0.496 (.48-.55)	0.412	59.70 ± 13.88	0.122 ± .008	
Site 5 (trucks) PM	0.433 (.40-.47)	0.433	10.71 ± .565	0.043 ± .012	
Site 6 (Bkg) PM	0.084 (.066-.102)	0.084	2.043 ± .028	0.000 ± .012	
Site 1 (main yard) Vapors	.038 (.032-.044)	ND	NS	0.557 ± .009	16.43
Site 2 (maintenance) Vapors	0.101 (.082-0.120)	ND	NS	1.178 ± .015	44.39
Site 5 (trucks) Vapors	.038 (.031-.045)	ND	NS	0.277 ± .023	17.47
Site 6 (Bkg) Vapors	.021 (.016-.027)	ND	NS	0.119 ± .007	3.69

Results from a set of samples collected over the same period at the sites indicated are shown. All values were normalized to volume of air in m^3. ND = not determined, NS = not significantly different from control. The values for TNFα expression are the slopes of the log concentration vs. protein expressed dose response curves ± the estimated standard errors. The values for HO-1 expression are the levels found after incubation of the cells with samples at a concentration of 0.5 m^3/mL

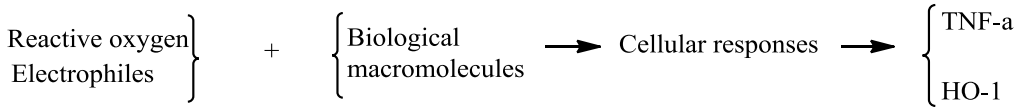
Table 3 Correlation analysis of the Neighborhood Sites data

Variable 1	Variable 2	Correlation coefficient	p Value
Vapor GAPDH	Vapor HO-1	0.961	0.038
Vapor GAPDH	PM TNF α	0.943	0.057
Vapor DTT	Vapor HO-1	0.989	0.011
Vapor DTT	PM TNF α	0.828	0.172
PM DTT	PM TNF α	0.757	0.243
PM DTT	Vapor HO-1	0.576	0.423
Vapor GAPDH	Vapor DTT	0.966	0.034
Vapor GAPDH	PM GAPDH	0.854	0.146
Vapor GAPDH	Total PM DTT	0.599	0.400
PM GAPDH	PM DTT	0.897	0.106

The data of table 2 were subjected to a correlation analysis. Note the high correlation between vapor GAPDH and the cellular responses. The somewhat higher correlation between the PM TNFa and vapor DTT compared with PM TNFa and PM DTT is consistent with the notion that organic species, reflected by their content in the vapor phase samples, are responsible for the biological effects.

Background

The overall hypothesis of the project is summarized below:



Reactive oxygen and electrophiles generated or present in the air pollutant mixture interact with biological molecules to modify key functional groups causing the initiation of inflammatory or adaptive responses. The objective of this research project was to determine levels of the reactive chemical species and compare those levels with the biological response to the mixture. By measuring these species and responses in quantitative terms, we hoped to characterize the chemical species involved in the health effects by correlation analyses.

Rationale and nature of the chemical assays used.

The particles and vapors that make up the atmospheric aerosols are exceedingly complex mixtures containing chemical species capable of catalyzing the reduction of oxygen to reactive oxygen species (prooxidants) and of forming covalent bonds with nucleophilic functions on biomolecules (electrophiles). As an approach to measuring the capacity of a given sample to carry out these two reactions, we developed two assays that assessed these reaction capabilities without identifying the specific chemical species involved. The DTT based prooxidant assay determines the rate at which the components of the test sample reduce oxygen, using DTT as the electron source. A multitude of chemical species including transition metals and quinones are capable of the reaction with a rate that varies with the specific chemical. As a result, the assay would be more sensitive to some compounds. For example, the three membered quinone, 9,10-phenanthroquinone is about 5 times faster than 1,4-naphthoquinone in this assay and if both compounds were present in the sample at the same concentration, the overall rate would be dominated by phenanthroquinone. Thus, the results cannot be interpreted to reflect a particular chemical species but the sum of all prooxidants with contributions according to their particular reactivity. Likewise, the GAPDH assay for electrophiles measures the content of chemical species capable of forming covalent bonds with the enzyme thiol. Different chemical species will interact with the function with differing affinities, so again, the assay results reflect general reactivities, not specific compounds.

However, if the sample is fractionated by for example, volatility, a physical chemical property can be attributed to the reactive chemical involved. Thus, as organic compounds would be expected to localize in the organic extracts of particles and of XAD resins which trapped volatile organic compounds, the organic extracts provide additional information regarding the chemical species involved.

Rationale and nature of the cellular assays.

Macrophages can respond to chemical insults in two ways, one is the expression and release of agents that promote the inflammatory response and the second is the intracellular expression of enzymes that reduce the intensity of the insult by inactivating the insulting chemical. TNFα is one of multiple proinflammatory proteins that trigger the inflammatory response in responsive tissues and HO-1 is representative of a group of enzymes that inactivates reactive chemicals such as hydrogen peroxide, quinones and some electrophiles. These proteins were selected as markers

for the inflammatory and adaptive responses by cells to the reactive chemicals in air pollution mixtures for two reasons. Both TNFα and HO-1 have been shown to be highly responsive to challenges from air pollutants in terms of their intensity. One, their biochemistry is clearly associated with inflammatory and cytoprotective responses so the interpretation of changes is relatively straightforward. Second, as ELISA assay kits are available for their quantitative analysis, dose response curves of their expression can be obtained for quantitative comparison with the chemical reactivities.

The assessment of the role of particles and vapors on cells adds another dimension to their properties, i.e., the toxicokinetics, or the ability of the reactive species present in the two phases to gain access to the cell interior. The vapor samples contain volatile organic chemicals, defined by their trapping by polystyrene resins and subsequent extraction with dichloromethane. Their movement into cells can occur by passive diffusion or, if they are charged, by anion or cation transporters. Organic compounds can also be trapped in cell membranes and removed by the multidrug resistant protein, a reverse transport carrier. Cellular entrance of metal ions is carefully regulated and tends to be specific for different metals, so their extracellular concentration may not be good predictors of intracellular effects. These toxicokinetic issues may interfere with correlation attempts between the chemical reactivities and cellular responses; the results of the correlation analyses, in particular the weak association of particle prooxidant content with may reflect these issues. .

Appendix

January 12, 2011 WEDNESDAY • UCLA School of Public Health Building • Room 16-145

9:30-9:45 **Welcome and Introductions**

9:45-10:30 **Introduction and Background Information (John Froines, PhD)**
Background to the Southern California Particle Center
Goals of the AQMD-BP Rail yard project (toxicology basics)
Overview of the AQMD-BP Rail yard project Sampling
Q & A (throughout)

10:30-11:30 **Presentation of AQMD-BP Rail yard Research Results (Arthur Cho, PhD)**
Presentation of results from all three communities
Description of sampling challenges
Opportunities and Next Steps for future research
Q & A (throughout)

11:30-12:30 **Discussion**

12:30-1:30 **LUNCH BREAK**
Elina to spend the lunch break with Long Beach to discuss Community Activities to Share Research Results

1:30-2:30 **COMMUNITY RAIL YARD UPDATES (Policy, Other Research/Sampling & on Railyard Construction/Activities)**
John Cross, Update on the Long Beach Rail yard(s) & Policy Updates
Angelo Logan, Overview of the ARB Rule Making Efforts and Policy Updates
Penny Newman, Update on the San Bernardino/Riverside Rail yards & Policy Updates

***2:30-3:30** **Next Steps**
Discussion of Community Activities to Share Research Results
*Time permitting: We want to ensure that the meeting answers all critical questions about the science. If there is time to discuss future activities to share research results, we will.

Attendees:

Center for Community Action & Environmental Justice (CCAEJ) in San Bernardino/Riverside
Penny Newman & Sylvia Betancourt

East Yard Communities for Environmental Justice (EYCEJ) in Commerce
Angelo Logan, Joceyln Vivar, Isella Ramirez, Debbie Vongiviwot

West Long Beach Neighborhood Association
John Taelifi, John Cross (leaving at 2pm)

UCLA-USC Southern California Particle Center
John Froines, PhD, Arthur Cho, PhD