Executive Summary

POTENTIAL HEALTH HAZARDS ASSOCIATED WITH PARTICULATE MATTER RELEASED FROM RICE STRAW BURNING

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Introduction

Historically, agricultural wastes have often been disposed of by burning. In California between 2-3 million tons of wheat, barley, and rice straw are produced annually. These straw wastes are often burned in the field after harvesting operations. The Sacramento Valley of California produces nearly all of the rice grown in California. A major portion of waste rice straw produced is burned each year in the fall after harvest or the following spring before planting. This investigation dealt with smoke from rice straw burning which has been perceived as a regional air quality problem. This is because of the large volume of rice straw burned on a seasonal basis, in a valley where topography and weather patterns can impede smoke dispersal.

Objectives

The potential health hazards associated with particulate matter released from rice straw burning were assessed by biological testing for two types of toxicity associated with organic substances contained in the particulate matter. Firstly, mutagenic activity was tested using the widely accepted Ames *Salmonella*/mammalian-microsome mutagenicity test. The results of this test allowed detection of substances in the smoke capable of causing genetic damage (mutations). Secondly, the effects on viability and physiological function of the pulmonary alveolar macrophage (PAM), a special class of cells defending the lung against foreign particles, were determined using a testing procedure developed on this campus. The PAM test offers an identification of the acute toxicity of rice straw smoke to an important class of cells involved in the protection of the respiratory system. In addition, rice straw smoke samples were analyzed for elemental composition, polyaromatic hydrocarbons, and other organic components to identify the chemical agents possibly causing these adverse biological activities. Finally, an estimation of potential exposure to pesticide residues in rice straw smoke was made even though the rice straw used in our tests was found to contain no detectable pesticide residues.

Rice Straw Smoke Sampling

Rice straw smoke was collected by dry filtration using high volume air sampling techniques (greater than 0.5 m^3/min flow). Samples were collected from (1) burning rice fields; (2) a small-scale incinerator combusting rice straw; (3) a smoke plum above and downwind from burning fields collected by an aircraft; and (4) a carefully controlled burning tower which simulated field burns, but concentrated the smoke so that substantial amounts of material could be trapped. Smoke particles were either trapped directly on a filter or passed through a cyclone preseparator prior to filtration. The latter configuration allowed collection of the respirable fraction of material, that would deposit in the alveolar regions of the lungs, for chemical and biological assays. In some cases a polymeric adsorbent (XAD-4) was used as a trapping medium for particles and vapor. The filters used were made of glass or quartz fibers, or acrylic polymer. These different materials were chosen based on their compatibility with different chemical analyses and biological tests.

Chemical Analysis

A rapid and efficient procedure was developed for extracting poly-aromatic hydrocarbons (PAHs) and heterocyclic compounds from samples of rice straw smoke. This procedure involved extraction by sonication of the samples in a benzene-methanol (1+1) mixture.

Smoke samples were fractionated by two different methods. Initially, smoke extracts were fractionated following the classical acid-base partitioning procedure to yield neutral (hydrocarbons, aldehydes, esters), basic (amines, N-heterocyclics) and acidic (carboxylic and sulfonic acids) fractions. Fractionation was also achieved by gel permeation chromatography to obtain aliphatic hydrocarbons (fractions 1 and 2), PAHs (fractions 3-5), and more polar constituents (fraction 6). Individual chemical species in these fractions were resolved and identified by capillary gas-chromatography (GC) and by computerized gas-chromatography-mass spectrometry (GC/MS).

Fractions 5 and 6 (gel permeation chromatography) contained the highest mutagenic activity; fluorescence spectrometry showed they contained a complex mixture of highly fluorescent compounds typical of PAHs. The presence of PAHs in these fractions was confirmed by GC/MS and a number of individual PAHs and heterocyclic compounds were tentatively identified. Elemental analyses of samples from burning rice fields revealed that the material trapped on the air filters consisted almost entirely of plant related materials as indicated by the potassium to iron ratio. Thus, particulate matter collected from burning rice fields contained little or no soil (potentially resuspended by the fire).

Pesticide Residue Analysis

Samples of unburned rice straw taken from rice fields immediately prior to burning and smoke sampling were analyzed for pesticide residues. The results showed that the residues of two chemicals applied to the rice early in the season, at least 3 months before harvest, were below the analytical detection limit--0.04 ppm for MCPA herbicide and 0.04 ppm for molinate (ordram) herbicide at time of harvest. Analysis of unburned straw for ethyl parathion also showed no residue at or above the detection limit of 0.01 ppm. Given the negative findings on the unburned straw, no pesticide analysis was carried out for smoke derived from the straw samples. Had the straw been contaminated with MCPA at the legal tolerance (2.0 ppm), calculations (see chapter 4) showed that the airborne residue in smoke taken just above the burning field would have been 140 ug/m^3 _if_ all the residue survived combustion and was emitted to the air (very unlikely). This value--a worst case estimate--is far below the threshold limit value (TLV) for 2,4-D (a close relative to MCPA) of 10,000 ug/m^3. It appears that, even under worst-case conditions, pesticide contamination of rice straw smoke will be far less than TLV values designed for protection of workers occupationally exposed to these compounds. For most of the chemicals used on rice in California, values for pesticide residues on the straw will be far less than 2 ppm; and residues in the smoke at the burn source will thus be far less than the value of 140 ug/m^3 calculated for MCPA using "worst-case" assumptions. Nevertheless, pesticide analyses should be conducted in rice straw smoke for the more persistent pesticides, and their toxic degradation products, especially under conditions where degradation of pesticides in the field might be slowed down.

Mutagenicity Testing

The mutagenic activity of rice straw smoke was determined using the

Ames _Salmonella_/mammalian-microsome mutagenicity test, employing a battery of tester strains of _Salmonella typhimurium_. The strains TA98, TA1537, and TA1538 are frame-shift mutation testers while TA100 and TA1535 are base-pair substitution mutation testers. Chemicals that require metabolic activation to become mutagenically active are referred to as promutagens, and those requiring no metabolic activation are called direct acting mutagens. Metabolic activation is achieved by the use of a rat liver enzyme preparation.

The solvent extracts of rice straw smoke were shown to be mutagenic to strains TA98, TA100, TA1537 and TA1538, with greatest activity in strains TA1538 and TA98. Mutagenic activity detected with or without metabolic activation indicates that rice straw smoke contained both promutagens and direct acting mutagens. Mutagenic activity was associated with particulate matter. Owing to the difficulties encountered in the collection and testing of vapor phase materials in the field, we were unable to determine whether or or not rice straw smoke contains vapor phase mutagens (see chapter 5).

In an effort to characterize emissions produced by alternative methods of a rice straw disposal, aerosols emitted by a small scale incinerator (SSI) burning rice straw were tested. Particulate matter (fly ash) trapped by a baghouse fitted to the SSI as well as aerosols emitted by the baghouse were tested for mutagenic activity. Aerosols released into the atmosphere from the baghouse contained mutagenic materials. Rice straw fly ash trapped by the baghouse was approximately one-half as mutagenic (revertants/mg fly ash extracted) as coal fly ash of a similar aerodynamic size extracted and tested in a similar manner (see chapter 5). While these results are interesting it should be noted that 2 fold differences in mutagenic activity in the Ames test are not highly significant and clearly, further testing will be required to determine the significance of these differences in mutagenicity.

Effects on Pulmonary Alveolar Macrophage

The recognized significance of the pulmonary alveolar macrophage (PAM) in the biological defense of the lung against invading foreign particles have prompted development of assays for measurement of the cytoxicity of environmental toxicants to PAM. A bovine PAM *in vitro* test developed on this campus allowed simultaneous measurement of the effect of particulate matter on PAM phagocytic ability, attachment, adherence, and viability. PAMs were exposed to rice straw smoke particle samples in the respirable particle size range (< 3.8μ aerodynamic diameter), at the dose levels of 30, 10 and 3 μg/ml culture media for a 21 hr incubation time. At the 30 μg/ml level, significant toxic effects on the phagocytic ability of PAMs were observed but effects on adherence and viability were not evident. Of the end points tested using the PAM, phagocytic ability appears to be the most sensitive function in response to the action of rice straw smoke particles. Using this test, toxicity was compared for silica, rice straw smoke and coal fly ash. The relative potency of these particles in descending order is: silica > rice straw smoke > coal fly ash. Unfortunately the rice straw smoke sample from the baghouse exhaust of the incinerator was too small to allow PAM testing.

Significance

This study used *in vitro* bioassays in conjunction with chemical analysis as the first step in assessing a difficult question. Does agricultural burning of rice straw have a significant health impact?

Results from this investigation indicate rice straw smoke could have a health impact of unknown magnitude. Three areas of uncertainty are important in interpreting the significance of our results: (a) identification of the toxic chemical species in aerosols to which human populations are exposed, (b) the dose of aerosols human populations are exposed to and (c) extrapolation of in vitro results to human risks.

The first two areas of uncertainty impinge upon this study in several ways. As a first step in identifying toxic chemical species present in aerosols produced by rice straw burning, a limited number of PAHs emitted at the source of combustion were identified. However, long range transport of aerosols (such as rice straw smoke) could change the chemical composition of the smoke and are yet to be investigated. In addition, accurate determination of the concentrations of rice straw smoke which populations are exposed to has not been made.

The third area of uncertainty, extrapolation of in vitro results to human risk, is fraught with problems. In order to help reduce this uncertainty, comparison was made between aerosols whose risk is better understood with the unknown aerosol, rice straw smoke. For the Ames test the better characterized aerosols included coal fly ash, cigarette smoke and Los Angeles "smog" (see chapter 5) while the pulmonary alveolar macrophage assay employed coal fly ash and silica (see chapter 6).

Based on Ames test mutagenic response (revertants per plate per microgram of organic material extracted from particulate matter), rice straw smoke is similar to other aerosols we tested that are generated from incomplete combustion of carbonaceous materials. While a battery of short term genotoxicity tests are needed to confirm and more fully characterize rice straw smoke's genotoxicity, results in the Ames test indicate rice straw smoke is <u>not</u> an unusually mutagenic combustion generated aerosol.

Rice straw smoke particles (RSSP) also were toxic to a group of specialized cells (pulmonary alveolar macrophage) associated with the defense of the lung against foreign particles and inhaled microbial pathogens. RSSP demonstrated intermediate toxicity when compared with two other types of particulates known to adversely affect pulmonary alveolar macrophage function. Thus, while rice straw smoke particles were not shown to be exceptionally toxic in *in vitro* PAM studies, breathing air containing rice straw smoke cound conceivably weaken human resistance to infectious respiratory diseases. Much further work is needed to determine if indeed human PAM function is affected.

Conclusion

Results of these *in vitro* studies and chemical characterizations indicate that potentially toxic aerosols are produced by rice straw burning. For the endpoints tested, mutagenicity and PAM function, rice straw smoke did not exhibit unusual toxicity when compared to other aerosols derived from incomplete combusion of carbonaceous materials. Because of the three main areas of uncertainty previously mentioned (see significance, this executive summary) <u>no effects on human populations can be extrapolated from this study</u>. Further studies in the areas of: chemical characterization of aerosols (smoke) after atmospheric transport, actual doses human populations are exposed to, and *in vivo* testing (in sensitive human populations, if possible) are needed to assess any effects on human populations.