COMPREHENSIVE RESEARCH ON RICE
ANNUAL REPORT

January 1, 1981 - December 31, 1981

Project Title: CHARACTERIZATION OF HAZARDOUS CONSTITUENTS IN RICE STRAW SMOKE

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Level of 1981 Funding: $15,000.00

Objectives and Experiments Conducted by Location to Accomplish Objectives:

I. Objectives:

1. To characterize biological activity, through laboratory mutagenicity assays of particulate samples of rice straw smoke collected from open-burning.

2. To identify, through chemical and instrumental techniques, major classes of biologically active constituents of whole and fractionated smoke samples.

3. To determine changes in biological activity and chemical constituents in rice straw smoke as a function of air residence time and air quality.

II. Experiments:

In order to accomplish objectives one and two, and eventually the third, it was necessary to develop a successful analytical method for fractionating the whole smoke extract, obtained from high volume air sampler filters, into specific classes of compounds. Once developed, it was possible by use of the Ames mutagenicity assay to determine which types of compounds are primarily responsible for the mutagenicity noted in the whole smoke extract. The methodology and the results achieved to date will be described.

Smoke from rice straw burning was collected using high volume (HiVol) air sampling techniques employing filtration through glass fiber filters (GFF) and quartz fiber filters (QFF). Since field samples supplied too little material for method development, a specialized

burn facility at University of California/Riverside was used to burn baled rice straw. HiVol air samplers were placed at the top of the teepee shaped chimney and were run during the entire time required to burn twenty (20) pounds of rice straw (Fig. 1) (Hsieh, et al. 1981).

Glass or quartz fiber filters containing ca. 0.5 gm particulate matter were extracted three times with 250 ml benzene:MeOH (1:1) using a Branson Model J-32A sonifier for 2 min. per extraction. The three extracts were then combined and concentrated to ca. 30 ml on a rotary evaporator, filtered through a 0.7 m filter two times to remove insoluble carbon particles, and blown down under dry N_2 to a final volume of 20 ml (Fig. 2).

The whole smoke extract at this point is a very complex mixture which shows about 1.5% of the activity of benzo(a)pyrene in the Ames mutagenicity assay (Fig. 3). In order to determine the degree of potential health hazard of the rice straw smoke it is desirable to identify the compounds or classes of compounds that are responsible for that mutagenicity. Since the whole extract is too complex to analyze chemically as is, a method was developed to separate the mixture into several fractions of simpler composition.

A fractionation scheme using normal phase high pressure liquid chromatography (HPLC) somewhat analogous to that employed by Crowley, et al. to fractionate shale oil, a complex mixture containing similar types of compounds, proved successful in separating rice straw smoke into discrete fractions. The best separation was achieved by using an analytical Bondapak CN HPLC column with a hexane to 90% acetone solvent gradient at 1.0 ml/min. In order to obtain enough of each fraction for the mutagenicity assay the capacity of the system was increased by substituting a semi-preparatory Bondapak CN HPLC column for the analytical column.

The percentage weight and mutagenic activity, with and without rat liver homogenate, of each fraction relative to the whole rice smoke extract is represented graphically in Fig. 4. The percent mutagenic activity was calculated by dividing the number of revertants per fraction by the number of revertants for the whole extract. The number of revertants is equal to the specific mutagenic activity (rev/ug) (Fig. 3 and 5) multipled by the total weight of the fraction (ug). Weight recovery of the whole extract through the HPLC separation was 88%, but the summed recovery for mutagenic activity was 220%. This high recovery is mainly due to the exceptional activity of fraction VII, which may be an anomoloy as the mutagenic activity for this fraction is based on only two dose levels. The Ames assay for this fraction will be repeated. Discarding the value for fraction VII the summed recovery of mutagenic activity for the remaining fractions is 124%.

Fractions IV and VII represent the greatest contribution to mutagenic activity for compounds requiring metabolic activation, (i.e. +S-9, rat liver homogenate). Fraction IV also contains the major portion of direct acting mutagens, compounds that demonstrate mutagenicity in the absence of metabolic activation. It should be noted at this point

that the overall mutagenic activity requiring metabolic activation for the rice straw smoke fractions is quite low compared to that of the benzo(a)pyrene control, Fig. 5, and that the level of activity for direct acting mutagens is even lower (Fig. 5).

The specific mutagenic activity (of each fraction, in the presence of rat liver homogenate (Fig. 3), shows a 4-fold increase over that for the whole extract for fractions IV through VI, and a 26-fold increase for fraction VII. Fraction VII does not appear to contain any direct acting mutagens as indicated by the data in Fig. 5.

Capillary gas chromatographic (GC-MS) data has been collected for HPLC fractions I - VI. The major portions of fraction VII and VIII and a smaller portion of fraction VI are too non-volatile to identify by the use of GC-MS. They will require further separation employing reverse-phase HPLC and solid probe mass spectrometry, experiments now in progress. General compound classes found so far in fractions I - VI are shown in Table 1 and the identification of specific compounds in the remaining fractions can soon be completed with the GC-MS data collected over the past three months. Compounds identified to date are listed in Table II.

Fraction I, which contained primarily saturated hydrocarbons and hydrocarbons with only one site of unsaturation, had insignificant mutagenic activity as is expected for these types of compounds. Fraction II contained primarily polynuclear aromatic hydrocarbons (PAH's), some of which have demonstrated activity in the Ames Assay. The relatively low level of activity in this fraction indicates that those PAH's (i.e. Benzo(a)pyrene) which are highly mutagenic are either absent or are present at extremely low levels. GC-MS of fraction III demonstrated the presence of several phenols and cresols, which, although responsible for the characteristic odor of the smoke, are not known to be mutagenic in themselves.

Fraction IV contains oxygenated compounds such as diones and quinones; some compounds of this type are either active in the Ames Assay directly or are metabolically activated in the presence of rat liver homogenate (S-9). Fraction V contains compounds of increasing polarity, including nitrogen heterocycles and amines. These compound groups include reported mutagens. Preliminary work on fraction VI indicates the presence of compounds similar to those in fraction V, but of higher molecular weight.

Finally, fractions VII - VIII are made up of very polar constituents which are not volatile enough for gas chromatography. As mentioned earlier, different methods of separation and identification are to be used on these fractions.

Studies to accomplish objective three--environmental fate of rice straw smoke and its biologically active constituents--had to await the development of an efficient fractionation method, and characterization of the various fractions as to their level of mutagenicity. Now that method development is essentially complete we are in a position to begin environmental fate studies during 1982.

Summary of 1981 Research by Objectives:

1. The Ames Salmonella assay was used to establish the most mutagenically active fractions of rice straw smoke extract obtained from liquid chromatographic separation. Although it was possible to enhance activity of certain fractions relative to that for the original extract, no fraction approached benzo(a)pyrene, in activity.

2. Chemical characterization data has been collected for six fractions by capillary gas chromatography interfaced to a mass spectrometer. Fractions consisting of compounds containing oxygen and/or nitrogen were found to be the most active in the Ames mutagenicity assay.

3. Work on objective three will begin in January, 1982.

References:

1. Hsieh, D.P.H., J.N. Seiber, G.L. Fisher, T.J. Mast, E.H. Olsen, J.E. Woodrow and J.F. Yee. "Potential Health Hazards Associated with Particulate Matter Released from Rice Straw Burning." Final Report, California Air Resources Board, A8-093-31, May 1981.

2. Crowley, R.J., S. Siggia and P.C. Uden, "Class Separation and Characterization of Shale Oil by Liquid Chromatography and Capillary Column Gas Chromatography." Anal. Chem. 52 (1980) 1224-1233.

Publications or Reports:

1. Mast, T.J., J.E. Woodrow, J.N. Seiber. "Analysis of Organic Particulate Matter from Rice Straw Smoke." Paper presented 182nd National Meeting American Chemical Society, New York, N.Y. August 1981.

2. Olsen, H.E., J.Yee, T.J. Mast, J.E. Woodrow, G. Fisher, J.N. Seiber and D.P. Hsieh. "An Evaluation of PAH Content, Mutagenicity, and Cytotoxicity of Rice Straw Smoke." Presented at 5th International Symposium on Polynuclear Aromatic Hydrocarbons, Columbus, OH. October, 1981.*

3. Hsieh, D.P.H., J.N. Seiber, G.L. Fisher, T.J. Mast, E.H. Olsen, J.E. Woodrow and J.F. Yee. "Potential Health Hazards Associated with Particulate Matter Released from Rice Straw Burning." Final Report, California Air Resources Board, A8-093-31, May 1981.*

* 2 and 3 supported by funding from California Air Resources Board.

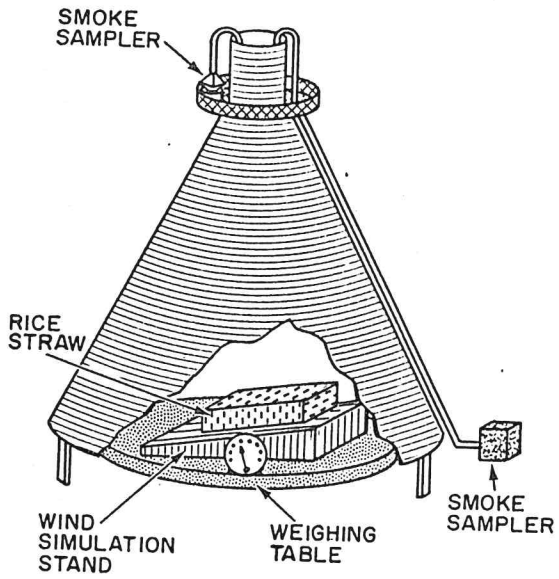


Figure 1. Burning tower, University of California - Riverside

Fractionation Scheme for Rice Straw Smoke

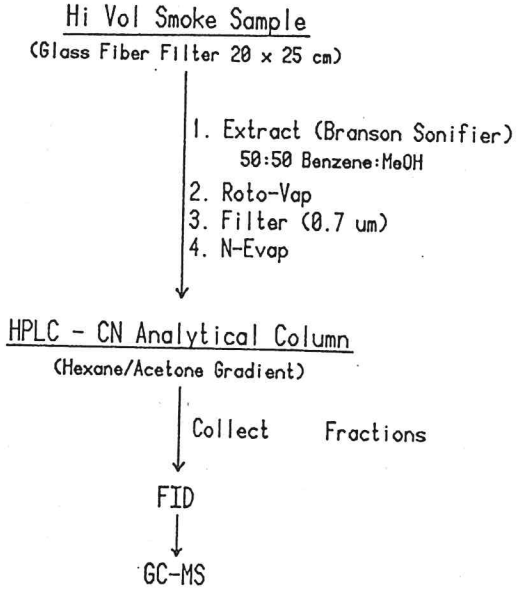


Figure 2.

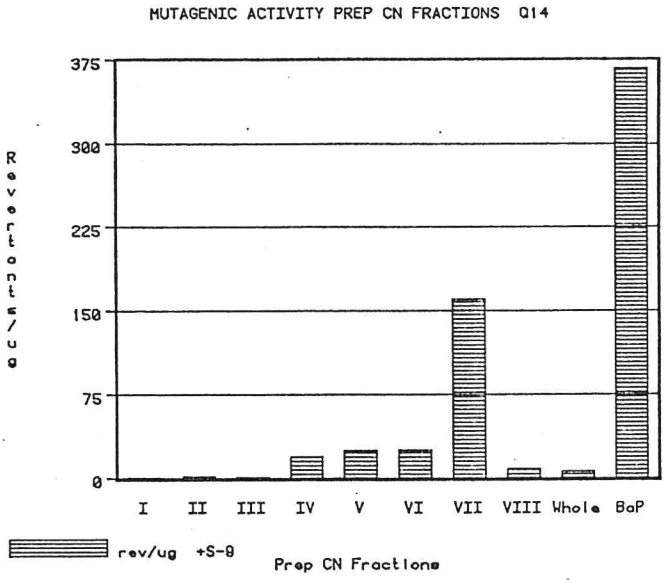


Figure 3. Mutagenic activity rice straw smoke HPLC fractions in the presence of rat liver homogenate.

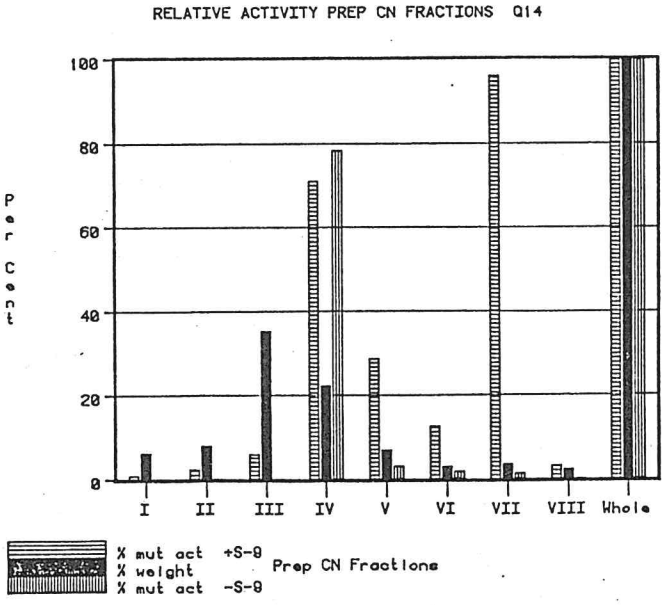


Figure 4. Percent weight and percent mutagenic activity of rice straw smoke HPLC fractions relative to the whole extract.

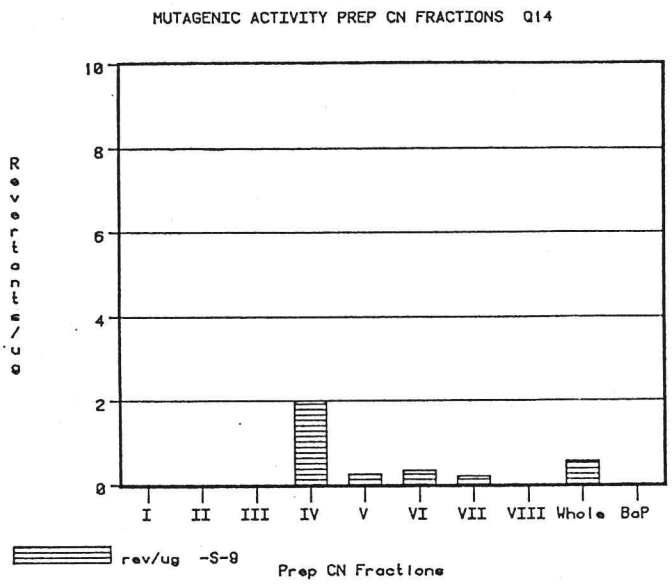


Figure 5. Mutagenic activity rice straw smoke HPLC fractions in the absence of rat liver homogenate.

Table I.

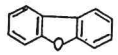
COMPOUND CLASSES IN PREP CN HPLC FRACTIONS

FXN I	FXN II	FXN III
Alkanes Alkenes	PAH (<248 mw) Methylated PAH	PAH (>248 mw) Subst. Phenols Long Chain Alcohols
FXN IV	**FXN V**	**FXN VI**
Oxygenated PAH Oxygen Heterocycles Furans Aldehydes	Nitrogen Heterocycles	Nitrogen Heterocycles

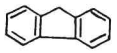
Table II. COMPOUNDS IDENTIFIED IN HPLC FRACTION II

$C_{12}H_{10}$
154

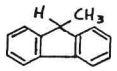
$C_{12}H_8O$
168



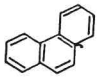
$C_{13}H_{10}$
166



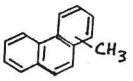
$C_{14}H_{12}$
180



$C_{14}H_{10}$
178

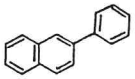


$C_{15}H_{12}$
192 (5 isomers)



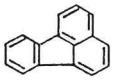
$C_{14}H_9N$
191

$C_{16}H_{12}$
204

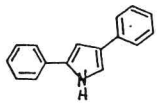


$C_{16}H_{10}$
202

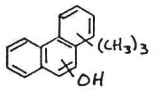
$C_{16}H_{10}$
202 (3 isomers)



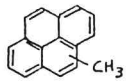
$C_{16}H_{13}N$
219



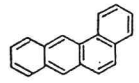
$C_{17}H_{16}O$
236
3 isomers



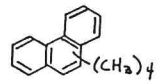
$C_{17}H_{12}$
216
2 isomers



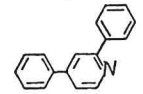
$C_{18}H_{22}$
228
(or isomer)



$C_{18}H_{18}$
234
3 isomers



$C_{17}H_{13}N$
231
2 isomers



$C_{20}H_{12}$
252

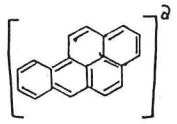
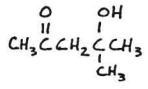
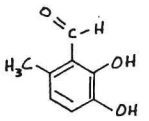
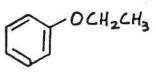
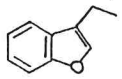
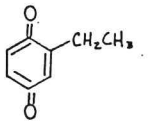
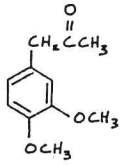
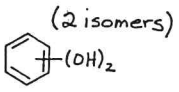
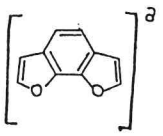
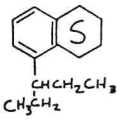
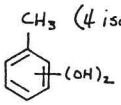
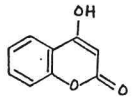
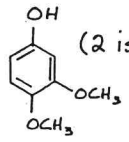
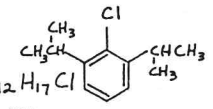
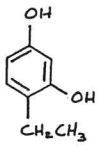
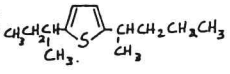


Table II. (cont.) COMPOUNDS IDENTIFIED IN HPLC FRACTION IV

$C_6H_{12}O_2$ 116		$C_8H_8O_3$ 152	
$C_8H_{10}O$ 122		$C_{10}H_8O$ 144	
$C_9H_8O_2$ 136		$C_{10}H_{12}O_3$ 180	
$C_6H_6O_2$ 110	(2 isomers) 	$C_{10}H_6O_2$ 158	
C_8H_8O 120		$C_{15}H_{22}$ 202	
$C_7H_8O_2$ 124	CH_3 (4 isomers) 	$C_9H_6O_3$ 162	
$C_8H_{10}O_3$ 154	 (2 isomers)	$C_{12}H_{17}Cl$ 196	
$C_8H_{10}O_2$ 138		$C_{13}H_{22}S$ 210	

a) compounds in brackets tentatively identified.