Project # RS-2

CHARACTERIZATION OF HAZARDOUS CONSTITUENTS IN RICE STRAW SMOKE

Project Leaders: D.P.H. Hsieh and J.N. Seiber

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Level of 1981 Funding: $17,500.00

Objectives and Experiments Conducted by Location to Accomplish Objectives:

I. Objectives:

To continue investigations into the biological activity of rice straw smoke collected from open burning, including identification of biologically active constituents in smoke particulate samples. The principal objectives for 1982 were:

1. To identify components in four fractions from smoke particulates collected at the UCR burning tower which had the highest activity in the Ames Mutagencity assay.

2. To compare these four fractions with those from samples collected adjacent to an open field in terms of chemical composition and mutagenicity.

3. To determine changes in composition and mutagenicity when smoke particulates were exposed to air and light under simulated environmental conditions in the laboratory.

4. To extend the environmental analyses to smoke particulate samples collected downwind from an open field burn, from another type of grain stubble and from diesel engines.

II. Experiments

Objective I

The methodology used for sampling, extracting, fractionating, and assaying for mutagenic activity and for component identification of rice straw smoke particulate matter (RSS-PM) samples has been previously described (1981 Rice Research Board Project Report RS-2, Hsieh *et al.* 1981). A brief outline of the methodology is shown in Figure 1.

During 1982 work was continued on identifying the constituents in the HPLC fractions that were mutagenically active in RSS-PM collected from the UCR

burning tower. The relative mutagenic activity and the weight contribution to the whole extract of each fraction are shown in Figure 2.

Polynuclear aromatic hydrocarbons (PAH) were found in fraction II (Table 1). Some PAH's are mutagens, although none of those identified in RSS-PM fraction II are known mutagens. Fraction III (Table II) contains primarily phenolic compounds, none of which have been shown to be mutagenic to date. Fraction IV (Table III) contains various oxygenated compounds, including aldehydes which have demonstrated mutagenic activity in the Ames Assay. Identification of components in fraction V is still in progress.

Objective 2

Rice straw smoke particulate matter was collected adjacent to a burning rice field with the purpose of comparing the mutagenicity of actual samples with those collected at the UCR burning tower. In order to determine the contribution of each fraction to the mutagenic activity of the whole extract, the activity of each fraction was multiplied by the weight percent of that fraction. The results of these calculations are in Figure 3.

Figure 3 data show that the field sample was only about one-third as mutagenic as the UCR tower sample and that the contributions of each fraction to the activity of the whole extract varied significantly between the field and tower samples. The greatest contribution to the mutagenic activity for the burning tower sample was furnished by the more polar fractions (IV,V,VI and VII) while in the field sample fraction III made the greatest contribution.

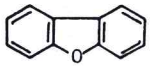
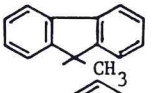
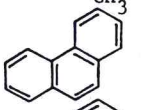
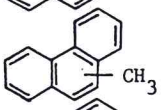
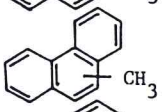
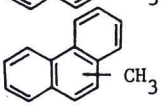
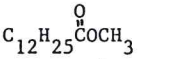
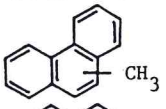
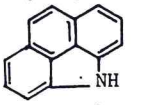
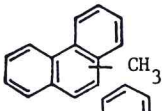
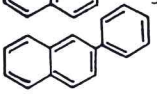
In general, the later fractions (IV-VII) contain the more polar, oxygenated compounds. With this fact in mind the higher activity of the later fractions in the burning tower may be attributed to the formation of more polar, oxygenated compounds under the Riverside atmospheric conditions which existed at the time the burning tower samples were collected, particularly a relatively high oxidant and ozone level. Since the oxidant/ozone level in the Sacramento Valley atmosphere at the time the field samples were taken was much lower, fewer of the more polar oxidized compounds were found in the field-collected RSS-PM.

The field sample HPLC fraction II--the fraction containing the PAH's--actually had the highest specific mutagenic activity, but since this fraction represents less than one per cent of the extractable organic matter its contribution to the total mutagenicity is quite low. Although no known mutagens have yet been found in HPLC fraction III the contribution of this fraction to overall mutagenicity may be due to the presence of low levels of high molecular weight PAH or other compounds not yet identified. Additional field sample extracts will be fractionated and analyzed to substantiate and/or clarify the above results.

Objective 3

The exposure of smoke particulate matter to ambient atmospheric conditions such as sunlight and oxygen may have an effect on the chemical composition and, consequently, on the mutagenicity. In order to determine the effects of sunlight on the particulate matter a laboratory experiment simulating exposure

Table I. Compounds Identified in HPLC Fraction II from UCR Burning Tower RSS-PM Sample.

SCAN	MOL FORM	MW	
416	$C_{12}H_{10}$	154	
497	$C_{12}H_8O$	168	
535	$C_{13}H_{10}$	166	
563	----	---	— — —
596	$C_{14}H_{12}$	180	
639	$C_{14}H_{10}$	178	
694	$C_{15}H_{12}$	192	
697	$C_{15}H_{12}$	192	
700	$C_{15}H_{12}$	192	
704	$C_{14}H_{28}O_2$	228	
705	$C_{15}H_{12}$	192	
707	$C_{14}H_9N$	191	
710	$C_{15}H_{12}$	192	
731	$C_{16}H_{12}$	204	

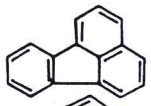
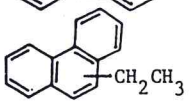
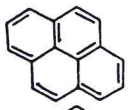
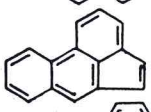
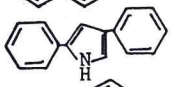
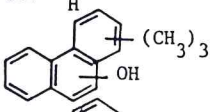
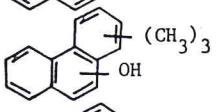
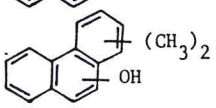
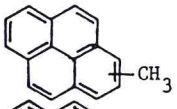
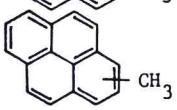
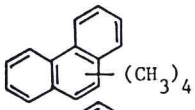
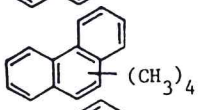
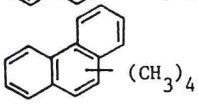
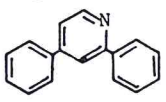
SCAN	MOL FORM	MW	
772	$C_{16}H_{10}$	202	
785	$C_{16}H_{14}$	206	
795	$C_{16}H_{10}$	202	
797	$C_{16}H_{10}$	202	
799	$C_{16}H_{13}N$	219	
808	$C_{17}H_{16}O$	236	
813	$C_{17}H_{16}O$	236	
822	$C_{17}H_{16}O$	236	
827	$C_{17}H_{12}$	216	
838	$C_{17}H_{12}$	216	
846	$C_{18}H_{18}$	234	
850	$C_{18}H_{18}$	234	
857	$C_{18}H_{18}$	234	
884	$C_{17}H_{13}N$	231	

Table II. Compounds Identified in HPLC Fraction III from UCR Burning Tower RSS-PM Sample.

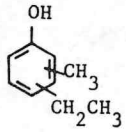
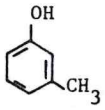
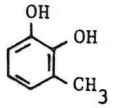
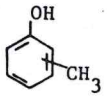
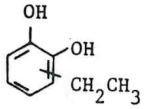
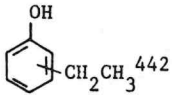
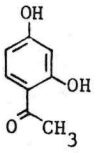
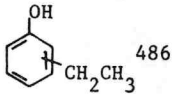
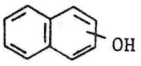
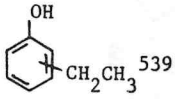
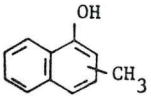
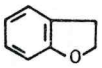
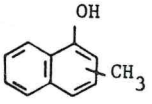
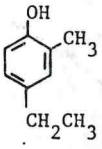
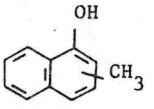
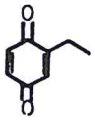
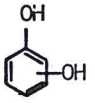
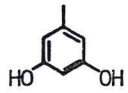
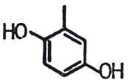
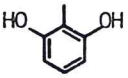
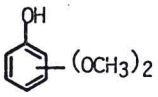
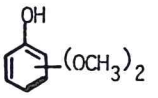
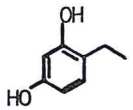
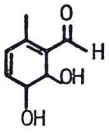
SCAN	MOL FORM	MW		SCAN	MOL FORM	MW	
143	C_6H_6O	94		324	$C_9H_{12}O$	136	
188	C_7H_8O	108		326	$C_7H_8O_2$	124	
202	C_7H_8O	108		406	$C_8H_{10}O_2$	138	
250	$C_8H_{10}O$	122		442	$C_8H_8O_3$	152	
262	$C_8H_{10}O$	122		486	$C_{10}H_8O$	144	
279	$C_8H_{10}O$	122		539	$C_{11}H_{10}O$	158	
297	C_8H_8O	120		553	$C_{11}H_{10}O$	158	
310	$C_9H_{12}O$	136		600	$C_{12}H_{12}O$	172	

Table III. Compounds Identified in HPLC Fraction IV from UCR Burning Tower RSS-PM Sample.

SCAN	MOL FORM	MW	
68	$C_6H_{12}O_2$	116	
147	$C_8H_{10}O$	122	
190	C_8H_8O	136	
247	$C_6H_6O_2$	110	
252	C_8H_8O	120	
260	$C_6H_6O_2$	110	
280	$C_7H_8O_2$	124	
282	$C_7H_8O_2$	124	
292	$C_7H_8O_2$	124	
308	$C_7H_8O_2$	124	
330	$C_8H_{10}O_3$	154	
340	$C_8H_{10}O_3$	154	
361	$C_8H_{10}O_2$	138	
368	$C_8H_8O_3$	152	

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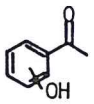
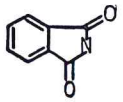
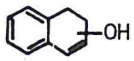
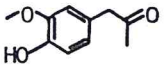
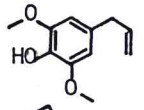
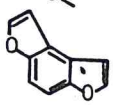
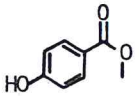
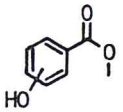
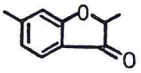
376	$C_8H_8O_2$	136	
414	$C_8H_5NO_2$	147	
446	$C_{10}H_8O$	144	
455	$C_{10}H_{12}O_3$	180	
504	$C_{11}H_{14}O_3$	194	
513	$C_{10}H_6O_2$	158	
521	$C_9H_{10}O_3$	166	
523	$C_9H_{10}O_3$	166	
560	$C_{10}H_{10}O_2$	162	
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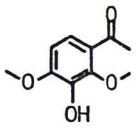
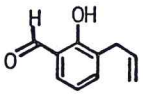
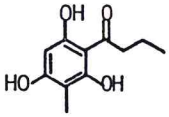
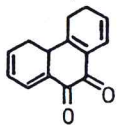
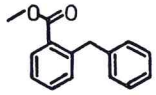
630	$C_{10}H_{12}O_4$	196	
659	$C_{10}H_{10}O_2$	162	
670	$C_{11}H_{14}O_4$	210	
744	$C_{14}H_8O_2$	208	
795	$C_{15}H_{14}O_2$	226	

of the smoke particulate matter to sunlight and oxygen was performed. A filter containing RSS-PM collected from a burning rice field was placed in a closed, air-filled chamber and exposed to a Xenon lamp for 24 hours (light exposed); another filter, collected at the same time, was placed in an identical chamber but light was excluded (dark control). Both filters were then extracted, fractionated and their whole extracts and fractions assayed for mutagenic activity. The results of this experiment are shown graphically in Figure 4.

The weight of each fraction, as a per cent of the whole extract, was not significantly affected by exposure to simulated sunlight over a 24 hour period. However, examination of Figure 4 shows a decrease in mutagenic activiy upon exposure to light for the whole extract as well as for each of the HPLC fractions of the light exposed sample. In both cases fraction III made the largest contribution to the mutagenicity of the whole extract; it is the activity of this fraction which is most significantly decreased upon exposure to simulated sunlight. As stated earlier, no known mutagens have been identified in fraction III, but further identification work is currently being performed.

It should be noted that the RSS-PM collected from the field burn had no direct-acting mutagenic activity, that is, activity requiring oxidation by enzymes in rat liver homogenate extract (S9). The samples collected from the UCR burning tower had both direct and indirect mutagenic activity, once again perhaps due to the high oxidant and ozone levels present in the atmosphere at the time the UCR samples were collected.

Objective 4

RSS-PM was collected ca. 400 yards downwind from a burning rice field as well as directly adjacent to the field for purposes of comparing their mutagenic activities. Smoke particulate matter was also collected adjacent to a burning barley field, and a sample of diesel soot from an Oldsmobile engine was obtained (courtesy of the California Air Resources Board). The mutagenic activity of their whole extracts were compared to the activity of whole extracts of RSS-PM collected adjacent to the burning field and from the UCR burning tower. The results of these experiments are shown in Table IV.

The downwind field sample was slightly less mutagenic than the sample collected adjacent to the field, apparently confirming the lower activity which resulted when RSS-PM was exposed to light in the lab. The smoke from the barley straw had somewhat higher activity than that from the field-collected rice straw. At this time it can not be said whether these differences are significant due to the biological variability associated with the Ames Assay. None of the field samples demonstrated direct acting mutagenicity as was found for both the UCR burning tower samples and for the diesel soot particulate matter.

Table IV. Mutagenic Activity of Various Smoke Paticulate Matter Extracts.

	Indirect Mutagenicity +S9[a]	Direct Mutagenicity -S9[b]
Rice Field-Adjacent	0.44	-
Rice Field-Downwind	0.35	-
Rice Straw-UCR	1.11	0.26
Barley Straw	0.56	-
Diesel Soot	0.17	0.24

a. Presented as per cent of the activity of benzo[a]pyrene control.
b. Presented as per cent of the activity of 2-nitrofluorene control.

Summary

Characterization of the mutagenic constituents in rice straw smoke particulate matter (RSS-PM) was continued during 1982. RSS-PM samples were collected adjacent to burning rice field and compared with RSS-PM samples collected downwind from the same field, RSS-PM samples collected from the UCR burning tower, barley straw smoke particulate matter, and diesel soot. Two filters containing RSS-PM collected adjacent to a burning rice field were used in an experiment designed to determine the effects of sunlight of RSS-PM. One of these filters was exposed to simulated sunlight for a 24 hour period while the other was not exposed to light. Some results and conclusions are:

1. The capability of the analytical methodology to separate the whole extract into compound classes has been demonstrated, and is reproducible. Over 75 compounds have been identified to date.

2. RSS-PM samples collected from the UCR burning tower had a higher level of mutagenic activity than field collected RSS-PM samples, perhaps due to the high ozone and oxidant present in the Riverside atmosphere at the time the straw was burned.

3. No direct-acting mutagenic activity was found in either the RSS-PM field samples or in the barley straw smoke particulate matter samples. However, both the UCR burning tower samples and the diesel soot contained direct-acting mutagens.

4. The mutagenic activity of barley straw smoke was slightly greater than that of rice smoke although the difference may not be statistically significant.

5. RSS-PM samples taken downwind from a burning rice field showed an apparent decrease in mutagenic activity as compared to samples taken adjacent to the field. Further samples should be taken to substantiate these results.

6. The level of mutagenic activity of a RSS-PM field sample exposed to sunlight for 24 hours was reduced when compared with a dark control. This result and that in (5) above indicate that "weathering" of RSS-PM in the atmosphere may reduce mutagenic activity. Again, these results await substantiation.

7. The highest level of mutagenic activity per unit weight for the field RSS-PM samples was found in HPLC fraction II (polynuclear aromatic hydrocarbon fraction) but fraction III (phenolic fraction) made the larger contribution to total mutagenicity owing to its much larger weight when compared with fraction II.

Studies which remain to be done include confirmation of the results obtained in the "weathering" experiments, further identification work on the HPLC fractions of the field samples, and comparison of RSS-PM with smoke particulate matter from tobacco and wood smoke.

References:

Hsieh, D.P.H., J.N. Seiber, T.J. Mast, H.E. Olsen and J.E. Woodrow. "Characterization of Hazardous Constituents in Rice Straw Smoke." Rice Research Board Annual Report, Dec. 1981 (Project #RS-2)

Mast, T.J., H.E. Olsen, D.P.H. Hsieh, and J.N. Seiber "Characterization of the Mutagenic Fractions of Organic Constituents in Rice Straw Smoke" presented at 184 th American Chemical Society Annual Meeting, September 12-17, 1982 Kansas City, MO.

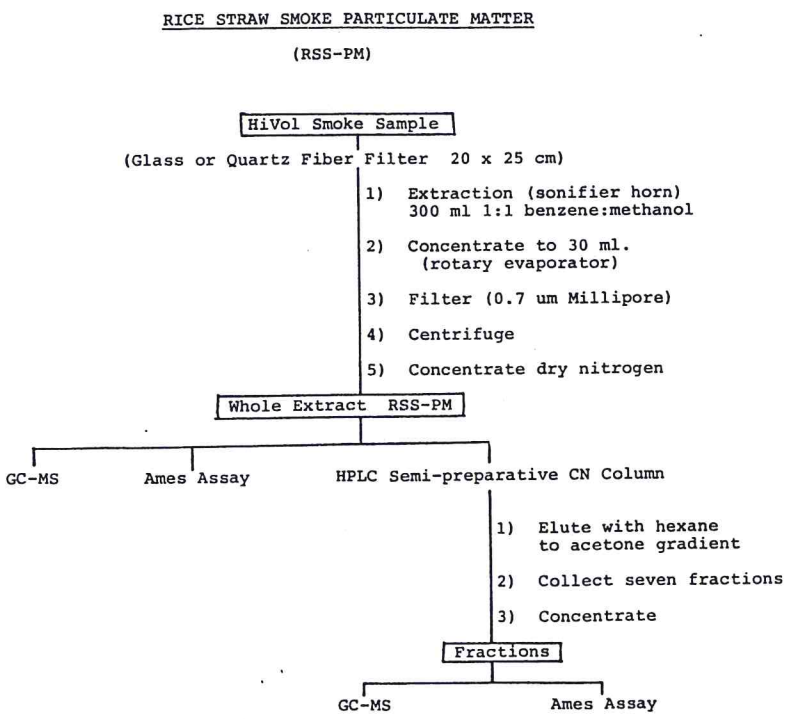


Figure 1. Analytical scheme for rice straw smoke particulate matter.

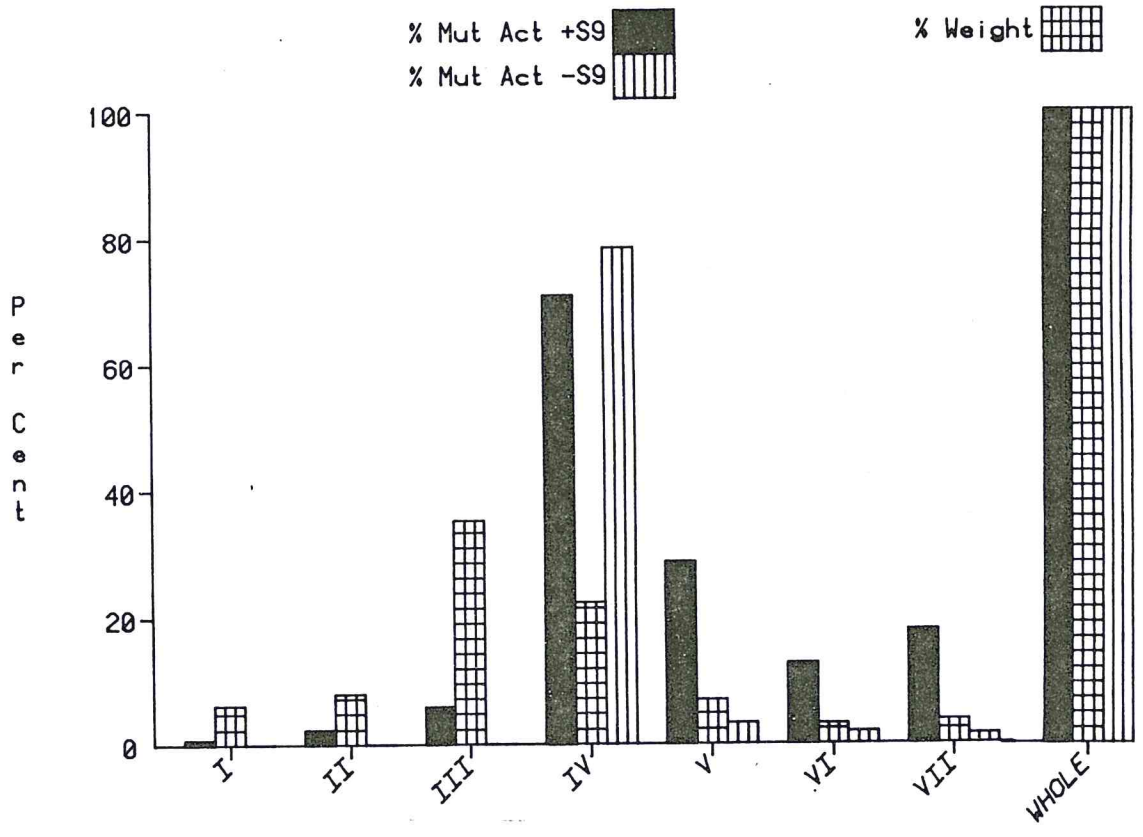


Figure 2. Mutagenic activity and weight distribution of the HPLC fraction from rice straw smoke particulate matter. Collected at the UCR burning tower.

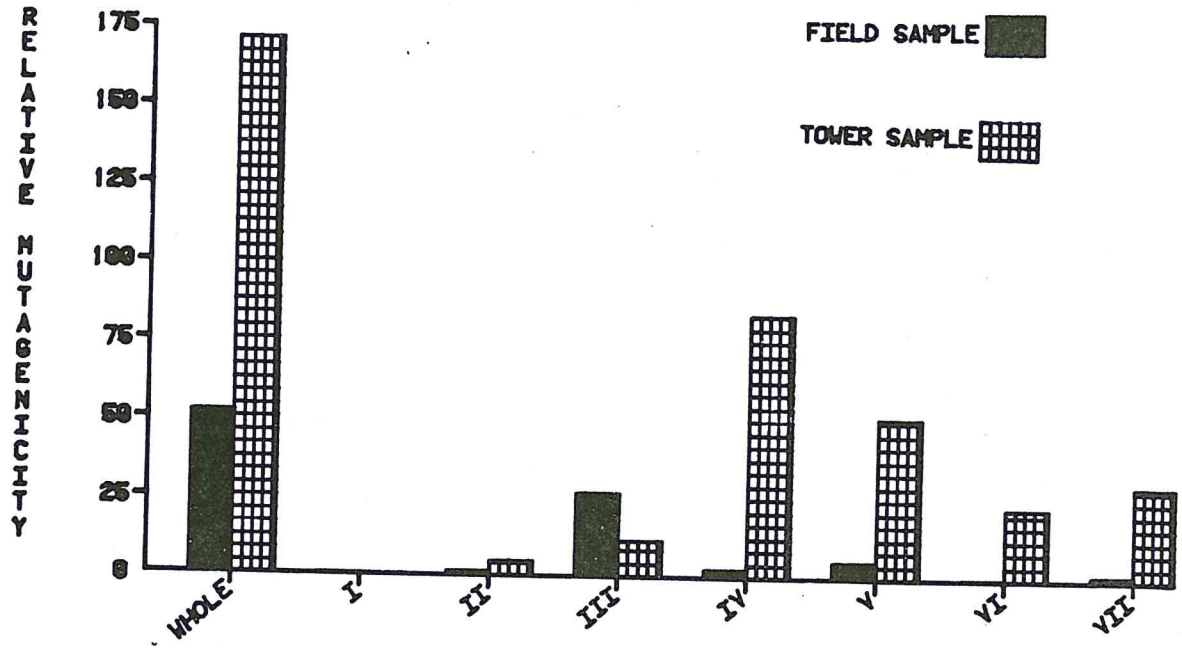


Figure 3. Relative contribution of HPLC fractions of field and UCR burning tower RSS-PM samples to the mutagenicity of the whole extract. The relative contribution was calculated by multiplying the per cent weight of a fraction times its mutagenic activity which is presented as the per cent of the benzo[a]pyrene control.

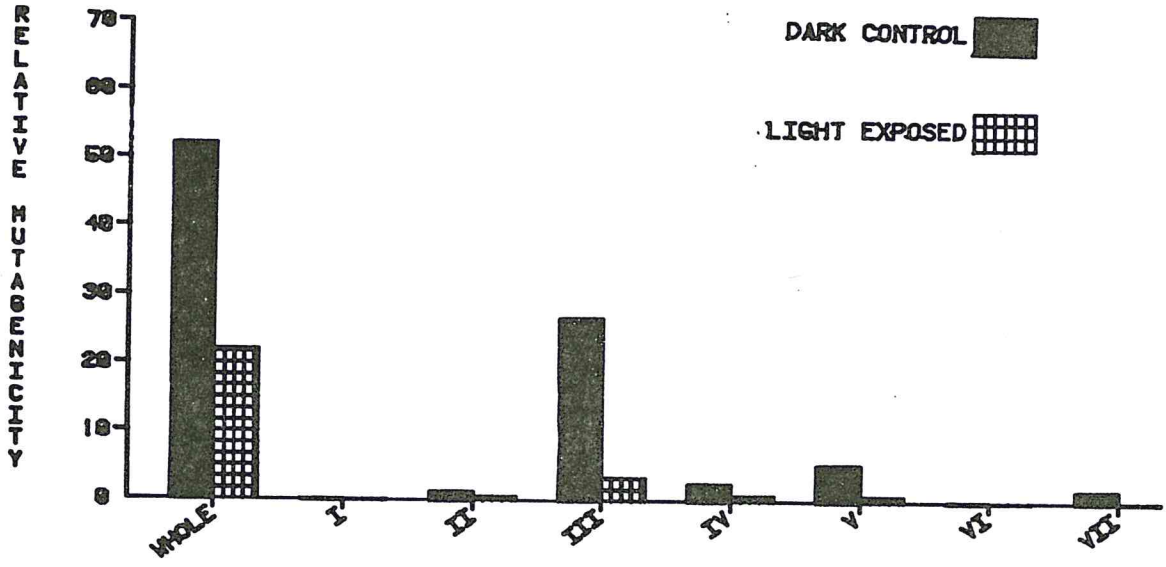


Figure 4. Relative contribution of the HPLC fractions to the mutagenic activity of the whole extract for a RSS-PM field sample exposed to simulated sunlight vs. those from a dark control. (Calculations were done as in Figure 3.)