V. MUTAGENICITY STUDIES

A. Introduction

The Salmonella/microsome mutagenicity test (Ames, et al. 1975a) has recently gained general acceptance for monitoring the mutagenic activity of chemical agents in the environment. Using this and other short-term tests, airborn mutagens have been found in urban air, cigarette smoke, and in aerosols generated from burning conventional hydrocarbon fuels or cellulosic fuels (Chrisp, et al. 1980a: Section D).

We have employed the Salmonella/microsome test, (The Ames test), using a battery of tester strains to monitor the mutagenic activity of rice straw smoke. Smoke samples were collected from a small scale laboratory incinerator, and then four different burning rice fields were sampled both upwind and downwind. During this time a plume sample from a number of burning fields was collected by aircraft, and samples from a pilot-scale steam generator (incinerator) were collected at the request of the California Energy Commission. Finally, a large scale, well-controlled laboratory burning tower was used to collect smoke from high and low moisture content straws. For more information on particular samples and analyses performed, see Table II-2 and V-1.

To aid the reader in understanding the contents of this chapter, a brief description of the principles upon which the Ames test is based, and the current interpretation of significance of the Ames test will be discussed.

This will be followed by a literature review of the mutagenicity of other combustion products as assayed by the Ames test and factors which affect that mutagenicity. Due to the large number of samples taken during the course of this study some confusion may occur as to what samples were tested in the Ames test; therefore, Section E of this Chapter lists samples tested, their method of collection and method of extraction. In addition, Section E explains terminology used in this Chapter. Finally, the materials and methods used are given followed by results and a discussion of the various smoke samples tested.

B. Basic Principles of the Ames Mutagenicity Test

The Ames mutagenicity test is a bacterial assay designed to detect chemicals causing mutation. There are a number of processes which can cause bacterial mutation. The Ames test detects two of the major processes, i.e., base pair substitution and frame shift mutagenesis.

The test uses a bacterium, Salmonella typhimurium, which requires histidine for cell division. This strain of Salmonella typhimurium can be converted to a nonhistidine requiring state through mutation of the bacteria by the action of chemical mutagens. The bacteria, along with the chemical to be tested, are plated onto a chemically defined agar containing trace quantities of histidine. Histidine allows the bacteria to divide several times and, in turn, to express any mutation which may occur from exposure to the test chemical. After a few divisions, the histidine is consumed and only those bacteria which have mutated to a histidine independent state can continue to divide. After 48 hours, the bacteria which have mutated to

histidine independence (histidine prototrophy) form colonies visible to the unaided eye. These colonies are counted as a measure of the mutagenicity of the tested chemical. In many instances metabolism of a compound is necessary before the chemical becomes mutagenic. To include the element of metabolism in the Ames test, a liver homogenate is added to the agar plate with the bacteria and test compound. This liver homogenate is referred to as S-9, since the liver homogenate is first centrifuged at 9000x gravity and the supernatant fraction is used in the Ames test.

Various strains of Salmonella typhimurium are used in order to determine whether a chemical is a frame shift or a base pair substitution mutagen. Strains TA98, TA1537 and TA1538 are frame shift mutagen detectors while TA100 and TA1535 are base pair substitution mutagen detectors. All five strains are genetically engineered to increase their sensitivity to mutagenic compounds. The four basic changes induced upon their ancestrial wild type phenotype are: 1) histidine requirement; 2) deep rough mutation (resulting in a defective cell wall which allows easier entry of test compound); 3) defects in normal DNA excision repair processes to facilitate expression of DNA damage as a frame shift or base pair substitution mutation; and 4) incorporation of extra DNA (R factor) into TA98 and TA100 strains to enhance error prone repair of DNA and increase sensitivity to mutagenic compounds (Hollstein, et al., 1979). These genetically engineered changes are the result of work by Bruce Ames of UC Berkeley.

This test has many advantages and is currently used world wide to screen for genotoxic compounds. A large body of information has been

accumulated using this test and is available in the literature. For further references, see Section D, Mutagenicity of Combustion Products. In addition, Ames' method papers in Mutation Research, Volumes 31 347-364 and 113 (3-4) 173-215 are good sources of basic information on the Ames test.

C. Significance of the Ames Test

The significance of the Ames Test is based on its demonstrated ability to detect known chemical carcinogens and to predict the carcinogenicity of previously undetected carcinogens (Ames, et al. 1975b). To date, between 80 percent and 90 percent of tested chemical carcinogens have been shown to be positive in the Ames test (McCann, et al. 1975). In addition, a low rate of false positives (13%) and false negatives (10%) occuring in this test has made it an attractive preliminary screening test prior to the expensive and time consuming animal carcinogenesis tests (McCann, et al. 1976). Faced with a high cancer rate (one in five will die of cancer in the U.S.), an increasing lung cancer rate (Murphy, et al. 1981) and a vast number of new and untested chemicals being produced each year, the regulatory agencies are in need of an accurate short-term test to prevent public exposure to new chemical carcinogens. Due to its inexpensiveness and ability to yield rapid results, the Ames test has been used extensively in the screening of potential carcinogens. This, in turn, has triggered a debate over the validity of extrapolating results of the Ames test to carcinogenesis in animals. Currently, it is accepted that the Ames test, together with a battery of other short-term genotoxicity tests, is a suitable method for screening the large number of untested compounds yet to be evaluated (Bandal,

et al. Eds. 1981, The Pesticide Chemist and Modern Toxicology, Chapter 6, pp. 57-87, by Williams Weisbuger and Brusick.). A positive result in the Ames test and in other short-term mutagenicity tests justifies further testing with animals, but direct extrapolation to effects in animals is not possible. The limitations of short-term tests include differences between a whole animal and a prokaryotic cell in absorption, distribution, metabolism and ultimate fate of a chemical carcinogen.

The Ames test has proved very useful in the prescreening of pure compounds or mixtures of compounds of unknown genotoxicity. In complex mixtures such as smoke, the Ames test is used as a bioassay to zero in on mutagenic components for further testing or environmental surveillance. To determine which compounds in a complex mixture are genotoxic, the complex mixture is subjected to a crude chromatographic separation based on acid-base properties, polarity, or molecular weight of the various components in the mixture. Those fractions which are positive in the Ames test are then further fractionated and those fractions are again tested in the Ames test. In this way components showing the greatest mutagenic activity can be isolated. At this point chemical identification of the important components in the mixture can be made. The preliminary steps in this process have been completed with rice straw smoke and are further discussed in this Section and Chapter III (Chemical Analysis).

D. Mutagenicity of Combustion Products

Perhaps the earliest indication that byproducts of combustion could have adverse effects on human health was the observation by Sir Percivall Pott

Table V-1 Mutagenicity of Airborne Particulates Generated by Combustion

Aerosol Source	Mutagenicity Test	Reference
Automobile exhaust	Ames	Wang et al, 1978
Diesel exhaust	Ames	Huisingh et al, 1978
Kerosene soot	Human lymphoblasts Salmonella typ. Forward Mutation Assay	Kaden et al, 1979
Steel welding fumes	Ames, E. coli polymerase	Hedenstedt et al, 1977
Coal fly ash	Ames, E. coli polymerase	Crowley et al, 1979 Crisp et al, 1978 Fisher et al, 1978
Urban air	Ames	Pellazari et al, 1978
Protein Pyrolysate smoke	Ames	Sugimura et al, 1977
Tobacco Smoke	Ames	Kouri et al, in press
Marijuana smoke	Ames	Busch et al, 1979
Joss stick smoke	Ames	Sato et al, 1980
Wood and peat fly ash	Ames	Löfroth, 1978
Rice straw fly ash	Ames	Olsen et al, 1979

in the late eighteenth century that many of his patients with cancer of the scrotum were chimney sweeps (Casarett, et al. 1975). Since that time, investigations on the chemical processes involved in incomplete combustion have revealed that polycyclic aromatic hydrocarbons are formed during pyrolysis (Edward, et al. 1975). A number of these compounds have been shown to be mutagenic, as well as carcinogenic, in both laboratory animals and man (Searle, et al. 1976). Aerosols produced as byproducts of incomplete combustion have been tested for their genetic toxicity using the Ames test and other short-term tests (Table V-1). The sixth international symposium sponsored by Battelle Memorial Institute concerned the physical and biological chemistry of polynuclear aromatic hydrocarbons (Cooke, et al. 1982) and is an interesting reference on recent research on PAH's.

Urban aerosols also listed in Table V-5 were noted to contain industrial and automobile-generated aerosols. All of these aerosols are mutagenic, and based on the chemistry of pyrolysis, other aerosols produced by incomplete combustion would also be expected to be mutagenic. Exposure to aerosols listed in Table V-1 occur in the personal environment (tobacco smoke), occupational environment (steel welding fumes) and ambient environment (automobile exhaust).

Chrisp, et al. 1980a, noted that the mutagenic potency of airborne particles was determined by the following factors, relative to the combustion process, sampling methods and sample handling:

1. Combustion temperature and air-fuel stoichiometry;
2. Size distribution of the aerosols sampled;
3. Mutagenic artifacts created by sampling method;
4. Temperature of sample collection;
5. Meteorology;
6. Aerosol exposure to ultraviolet light;
7. Extraction and fractionation procedures.

Of prime importance in determining combusion emissions is combustion temperature and air fuel stoichiometry (Edwards, et al. 1974). Kubitscheck, et al. 1980, has shown that for coal fly ash changes in these parameters change the mutagenicity of coal ash emitted from a laboratory scale, fluidized-bed combustor. For agricultural burning, fuel moisture and fuel loading (per acre) affect combustion temperature and air fuel stoichiometry (Darley, et al. 1974) and could be expected to alter mutagenicity of particulate matter released from burning.

A number of factors related to sampling have been shown to be of importance in determining the mutagenicity of collected aerosols. Chrisp, et al. 1980, and Commoner, et al. 1978b have demonstrated increased mutagenic activity with decreasing particle size for coal fly ash and urban aerosols. This, coupled with the difference in lung deposition between large (>10 microns) and small particles (<10 microns) (Task Group on Lung Dynamics, 1966 and Mercer, 1973) makes collection of aerodynamically-sized particles desirable. In this study Ames testing was on aerodynamically-sized particles in the respirable range (<3.5 microns). Sampling urban aerosols, Pitts,

et al. 1978 has shown that mutagenic artifacts can be generated by drawing large volumes of air over particles trapped on the face of a glass-fiber filter. Alternative methods of sampling which remove trapped particles from the sampling air stream should help prevent formation of mutagenic artifacts (Chrisp, et al., 1980a).

Mutagenicity of coal fly ash, diesel exhaust, wood smoke, and peat smoke has been shown to be affected by the temperature at which the aerosol is collected (Fisher, et al. [1979]; Lofroth [1978]; Huisingh, et al. [1978]). Mutagenicity of these aerosols increases with lower sampling temperatures and is believed to be due to condensation of vapor phase materials on particulate matter (Natusch, et al. [1978]). Commoner, et al. (1978b) found meteorology to be an important determining factor in mutagenicity of urban aerosols, especially when a point source was emitting large amounts of mutagenic aerosols. Effects of meteorology on sampling was also evident in the present study where particulate matter samples taken upwind from a burning field contained particulate matter from burning fields even further upwind. Effect of ultraviolet light on the mutagenicity of aerosols is currently being studied for urban atmospheres (Pitts, 1983). Fisher, et al. (1979) showed that mutagenicity of coal fly ash was not decreased by exposure to ultraviolet light.

Sample handling after collection has also been documented to affect mutagenic activity. Huisingh, et al. (1978) noted that slight decreases in mutagenic activity as determined by the slope of the dose response curve (Salmonella typhimurium TA1538) occurred after storage of diesel exhaust

particles at refrigerator temperatures. In addition, large decreases in maximal mutagenic response also resulted from storage of diesel exhaust particles at refrigerator temperatures. Cigarette smoke condensate stored at room temperature for 40 days showed a 50% reduction in mutagenic activity (Mizusaki, et al. 1977). Extraction and fractionation of a sample is very important in determining quantites and potency of mutagenic material recovered. Eppler (1980) noted differences between various extraction techniques and lists results for a number of different methods of fractionation, for a number of sample types including: airborne particulates, fly ash, soot, arc welding, automotive emissions and tobacco smoke condensate. Specific references to particular fractionations used in the present study are made in the Materials and Methods Section of this Chapter and Chapter III.

E. Terminology and Samples Tested in the Ames Test

Because terminology is vital to understanding any discussion of results, the following definitions are given to add clarity to subsequent discussions.

Particulate Matter Extract (PME) - The material solublized from particulate matter by solvent extraction, not including the original extracting solvent.

Specific Mutagenic Activity (SMA) - The revertants per plate per milligram particulate matter extract. This number is usually determined from the

Table V-2

SAMPLES TESTED IN THE AMES TEST

Sample	Method of Collection	Size Range	Method of Extraction
Laboratory Rice Straw Smoke	Hivol Air Sampler Glass Fiber Filter	All Sizes	6-Hour Soxhlet w/Acetone
Butte County (12/5/78)			
402 Meters	Hivol Glass Fiber Filter	All Sizes	For Glass Fiber Filter
402 Meters	XAD-4 Resin Behind GFF	Vapors	
422 Meters	XAD-4 Resin Alone	a.	
1609 Meters	Hivol Glass Fiber Filter	All Sizes	
1609 Meters	XAD-4 Resin Behind GFF	Vapors	
Yolo County (11/8/79)			
Field Edge (Upwind)	Two-Stage Respirable Particle Sampler-GFF	50% 3.8 Microns	6-Hour Soxhlet with Acetone
Field Edge (Downwind)	Two-Stage Respirable Particle Sampler-GFF	50% 3.8 Microns	
Sacramento County (11/9/79)			
Field Edge (Upwind)	Two-Stage Respirable Particle Sampler-GFF	50% 3.8 Microns	6-Hour Soxhlet with Acetone
Field Edge (Downwind)	Two-Stage Respirable Particle Sampler-GFF	50% 3.8 Microns	
Sacramento County (11/21/79)			
Field Edge (Upwind)	Two-Stage Respirable Particle Sampler-GFF	50% 3.8 Microns	6-Hour Soxhlet with Acetone
Field Edge (Downwind)	Two-Stage Respirable Particle Sampler-GFF	50% 3.8 Microns	

a. Size distribution of aerosols collected by XAD-4 resin bed is unknown.

Table V-2 (Continued)

Sample	Method of Collection	Size Range	Method of Extraction
Riverside Burning Tower Sample	Hivol Air Sampler-GFF	All Sizes	Benzene/ Methanol Sonication
Fly Ash Samples			
Coal Fly Ash Incinerator	Cyclone-Centripeter	2.2 Micron	DMSO Sonication
Baghouse (EC1) Incinerator	Baghouse	Uncharacterized	DMSO Sonication
Baghouse Exhaust (EC2)	Two-Stage Respirable Particle Sampler-GFF	50% 3.8 Microns	Benzene/ Methanol Sonification
24-Hour Los Angeles Particulate Matter Sample	Hivol Air Sampler-GFF	All Sizes	Benzene/ Methanol Sonification

slope of the linear portion of the dose response curve (using linear regression to determine slope). It is important to note that SMA is a measure of the potency of collected particles. It does not represent a measure of mutagenic activity in a volume of air sampled.

Although the specifics concerning each sample tested in the Ames test are described in materials and methods of this Chapter, Table V-2 is provided as a convenient reference for reading the remainder of the Chapter.

F. Materials and Methods

Ames Test. The Ames test is known to exhibit variability in results between laboratories (Chrisp, et al. (1980b). Ashby, et al. (1978), identified at least 14 different factors which introduce variability into results between laboratories. Therefore, a description of methods used to increase reliability of results is given.

The Ames test was performed as described by Ames, et al. (1975), with the exception of the following refinements to technique. The scoring of Salmonella revertant colonies on the agar plate used a Biotran II automatic colony counter (New Brunswick Scientific, New Brunswick, NJ). The automatic colony counter was calibrated at monthly intervals by comparison between hand-scored and machine-counted plates. Using linear regression, a correction factor for the colony counter was determined for plates between 0 and 500 colonies. For agar plates between 500 and 2000 colonies, multiple linear regression was used to determine the appropriate correction factor and, in

turn, colony number per plate. Ames tester strains (courtesy of Professor Bruce Ames) were maintained as frozen nutrient broth cultures at $-60^{o}C$. Cultures for routine assays were started with innocula from frozen nutrient broth culture. These frozen master cultures were tested before use for various characteristics as described by Ames, et al. (1975). Nutrient broth cultures used in the Ames test were adjusted to a cell density of 1 to 2×10^{9} cells per ml before use to ensure uniform bacterial number per plate between assays. S-9 was prepared as described by Ames, et al. (1975), using Arochlor 1254 as the inducing agent with 250 ug protein per plate used in all assays.

Bottom agar plate volume was accurately controlled (15 ml/plate) to ensure water soluble mutagens were tested at uniform concentration. Pitts, et al. (1980) has found uniform plate volume to significantly improve reproduceability in the Ames test. The test procedure has been briefly described in Section B of this Chapter and provides a summary of the Ames test as described by Ames, et al. (1975).

Sample Collection. The Butte County field (12/5/78) used sampling techniques different from those described for the remaining field samples (Chapter II). These samples were analyzed using only the Ames test, and therefore, sampling methods are described in detail here. Samples of rice straw smoke were collected with a high volume air sampler (Bendix Environmental Science Division, Baltimore, Maryland) with flow rates from 1.0 to 1.3 cubic meters/min. Smoke samples were collected for one hour at 0, 402 and 1609 meters downwind from the burning field. All samplers were equipped

with XAD-4 resin (Rohn Hass, Philadelphia, PA) which required previous cleanup (Herman, et al. 1978). The 402 meter and 1609 meter samplers also were fitted with glass fiber filters (P180-G, Misco Scientific, Berkeley, CA).

Laboratory rice straw smoke samples used in solvent-solvent fractionation were collected on glass fiber filters using high-volume air samplers (1 to 1.3 cubic meters/min). Rice straw was burned in five-pound lots on a 3.5 foot diameter burning platform. Smoke was concentrated by a funneled stack four feet high with a bottom diameter of 3.5 feet and a top diameter of eight inches. The funnel was positioned two feet about the burning platform. Samples were collected from the top of the stack.

Cigarette smoke extract and a 24-hour particle sample from Los Angeles, CA were used to compare mutagenic potency between rice straw smoke and other aerosols. Cigarette smoke was generated from a standard brand of filter cigarette producing 19 milligrams tar/cigarette (Federal Trade Commission, 1974) when smoked. The cigarette smoke was trapped in Nanograde acetone (Mallinckrodt) using a gas washing bottle (Pyrex ASTM 40-60 course fritt) packed in dry ice. Cigarettes were burned for a total of 6.75 minutes at a flow rate of 40 ml/min for a series of 15-second durations, followed by 30-second pauses. The acetone was removed under a gas stream of nitrogen and the residue was redissolved in DMSO (Malinckrodt) and tested immediately. The 24-hour particle samples from Los Angeles, CA were provided by the California Air Resources Board. The sample consisted of particulate matter collected on glass fiber filters over a 24-hour period, using a high-volume air sampler.

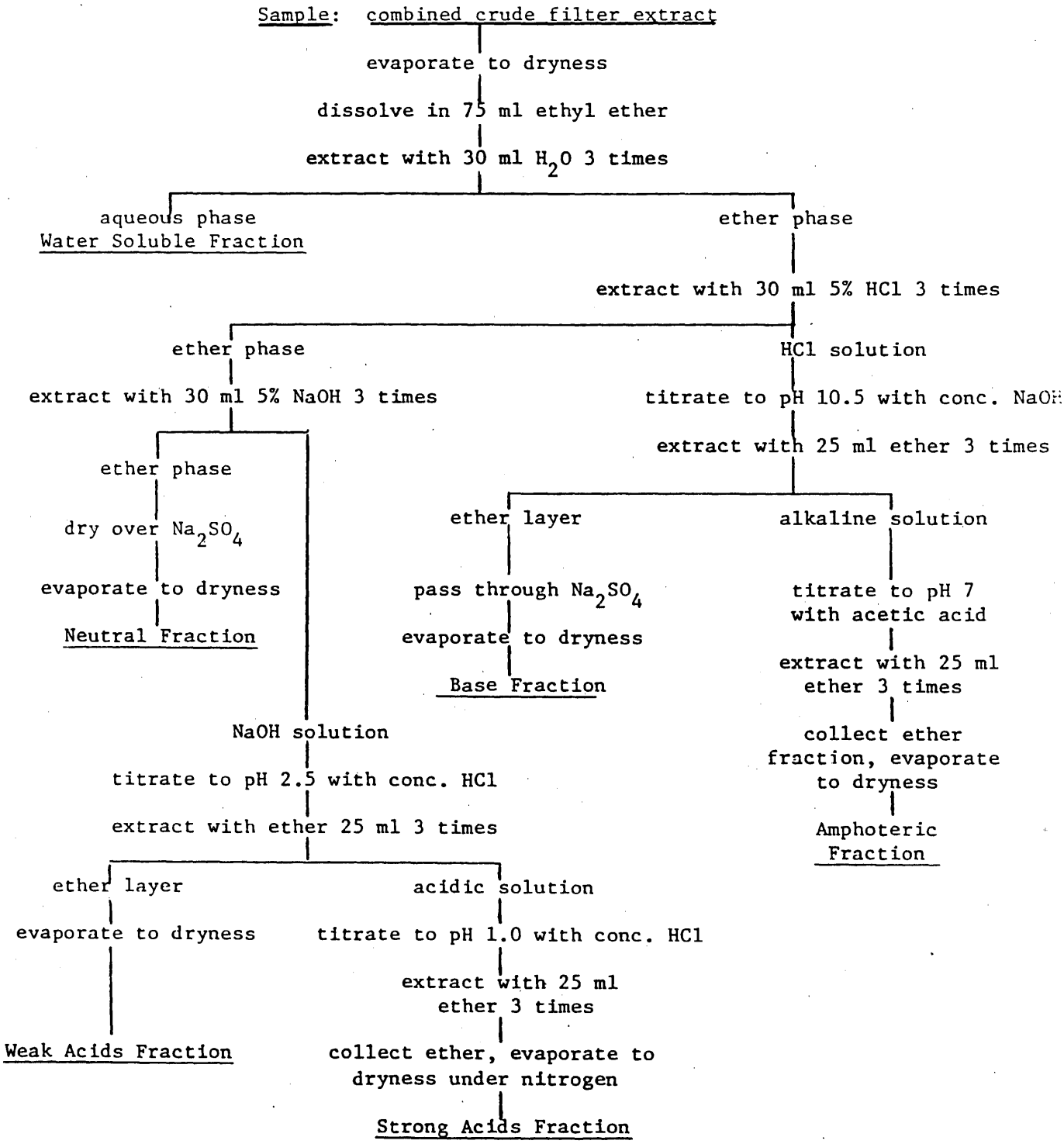
Coal fly ash tested is from a 750 megawatt coal-fired electric power plant. Method of collection and fractionation are described by Fisher, et al. (1978b). Briefly, fly ash was aerodynamically fractionated in situ from the stack breeching, after the electrostatic precipator of a 750 megawatt coal-fired electric power plant burning low sulfur, high ash, and high moisture coal. Of the four size fractions of coal fly ash, cut size four (with a volume median diameter of 2.2 microns) was chosen for the present study since it is most similar in size distribution to rice straw smoke.

Sample Preparation. Both the incinerator baghouse ash sample (see Chapter II, Sampling) and cut #4 of coal fly were extracted with DMSO (Dimethyl Sulfoxide, Mallinckrodt Analytical grade). Ash was weighted into centrifuge tubes and sonicated for one hour in a sonic bath with the appropriate volumes of DMSO. Samples were then centrifuged for 30 mins at 10,000 times gravity and the DMSO removed and stored at $-60^{o}C$ until tested (within 24 hours of extraction). Dosing in the Ames test was based on a volume of DMSO representing a given weight of fly ash; for example, if 50 mg of fly ash was extracted with 2.5 ml DMSO, then 50 microliters of DMSO was considered to equal 1 mg of fly ash.

Extraction of Riverside burning tower samples tested in the Ames test is described in Chapter III, Chemistry, and employed a methanol/benzene mixture and sonication. Remaining filter samples tested were extracted for six hours in a soxhlet extractor (Pyrex 3740) using nanograde acetone (Mallinckrodt). XAD-4 resin (Rohn Hass, Philadelphia, PA) was shaken for one hour with nanograde acetone using a Gyrotory Shaker (New Brunswick

Figure V-1

Solvent-Solvent Fractionation Scheme for Laboratory Smoke Samples



Scientific), then washed three times with fresh solvent. Light was excluded from all samples during sample workup. Samples were then concentrated to 10 ml under reduced pressure and transferred to tared glass vials. A one ml aliquot was reduced to dryness under a stream of nitrogen gas and the dry residue weighted. Appropriate aliquots of sample were then reduced to dryness under a stream of nitrogen and resuspended in DMSO for immediate use in the Ames test.

Fractionation of Laboratory Smoke Extract. The laboratory smoke samples were fractionated using the method of Shriner, et al. (1964). The extraction procedure is outlined in Fig. V-1. Smoke particulate matter extract (PME) was reduced to dryness under nitrogen and redissolved in diethyl ether. Successive extractions of the diethyl ether with water, 5% hydrochloric acid, 5% aqueous sodium hydroxide, yielded water soluble, basic-amphoteric, and neutral-acidic fractions, respectively. The basic amphoteric fraction was further fractionated by titration to pH 10.5, followed by ether extraction (bases) and then titration to pH 7.0, followed by ether extraction (amphoterics). The neutral fraction was contained in the original ether phase while further fractionation of the acidic fraction in the 5% NaOH aqueous solution was accomplished by titration to pH 2.5, extraction with ether (weak acids) and then titration no pH 1 and extraction with ether to recover strong acids.

Calculation of Mutagenic Potency. Determination of mutagenic potency was based on the slope of the steepest linear portion of the dose response curve. Slope was determined by linear regression using response to a minimum of 3 doses.

G. Results and Discussion

1. Field Samples.

To identify major experimental parameters, the Butte County sample (12/5/78) was used as the representative sample for determination of the following: 1) the distribution of mutagenicity between particulate and vaporized materials; 2) the relative sensitivity of the five major Salmonella tester strains; and 3) the presence of direct acting and/or promutagens. Findings from this preliminary study were used for the design of subsequent experiments on the remaining field samples.

Distribution of Mutagenic Potency. One of the key questions pertaining to agricultural burning or any other combustion process is whether the mutagens produced are in the vapor phase where they can remain for extended periods in the atmosphere or in the particulate fraction where environmental fate processes such as impaction, condensation, or sedimentation can help clean the air. Vapor-phase material is subject to chemical reactions in the atmosphere which can either increase or decrease their toxicity (Pitts 1983); however, physical removal is not as readily accomplished as with particulate material. Complicating the detection of vapor-phase mutagens is a lack of established methodologies for collection and subsequent testing of vapor-phase mutagens of low molecular weight and high vapor pressure. The methods used in the present study provide limited information on the distribution of mutagens between the vapor phase and particulate matter. Much pioneering work in vapor-phase mutagen detection remains before definative information on mutagen distribution can be gathered.

Table V-3

Distribution of Mutagenicity between Particulates

and Vaporized Material from Rice Straw Burning

Sample site (meters downwind)	Means of collection[a]	Material collected	Specific mutagenicity[b] (rev/plate/mg ext.)
control	filter	blank	no response
control	resin	blank	no response
402	filter	PM	1227 ± 50
402	resin	vapors	no response
402	resin	PM + vapors	196 ± 50
1609	filter	PM	395 ± 50
1609	resin	vapors	no response

[a] For details see Materials and Methods.

[b] Revertants/plate/mg smoke condensate determined from slope of linear portion of dose-response curve as fitted by linear regression. Each data point represents the average of triplicate plates. Incubation was with S-9 enzyme fraction.

In the present study, the samples collected from Butte County (12/5/78); specifically, the 402 and 1609 meter samples were used in our examination of mutagens in the vapor phase of rice straw smoke. At the 402 and 1609 meter sample sites of the Butte County field, smoke samples were collected by first passing smoke through a glass fiber filter (trapping particulates), and then through an XAD-4 resin bed (trapping vapor phase material). In addition, at the 402 meter sample site, another high volume air sampler was set up which contained only a XAD-4 resin bed (trapping both vapor phase material and particulate matter). These various samples, along with results in the Ames test, are listed in Table V-3.

The present study provided limited information on vapor phase mutagens owing to lack of developed methodologies. Specifically, a method by which a highly volitile compound could be trapped efficiently and then be recovered for testing was not available. Therefore, our methodology aimed at recovering mutagens with relatively low vapor pressures. While not directly quantitated, the workup of the XAD-4 resin (see materials and methods - this Chapter) would produce extracts with compounds of vapor pressures considerably lower than that of the extraction solvent, acetone (vapor pressure at $20^{o}C$ of 184.8 mm Hg). Observation of the acetone extracts from the XAD-4 resin positioned behind the glass fiber filter in the sampling train showed them to be light brown in color indicating some material was trapped by the XAD-4 resin. This material is probably vapor-phase material of low vapor pressure which were present: a) in the smoke in the vapor-phase; or b) condensed on particulate matter and revolitilized as air was drawn over particulate matter trapped on the surface of the filter (in front of the XAD-4 resin). With

these limitations on the completeness of vapor-phase sampling kept in mind, the distribution of mutagenic activity between particulate and vapor-phase materials is summarized in Table V-3. Samples from the 402 and 1609 meter downwind sample sites were mutagenic if collected by glass fiber filters and had an SMA (specific mutagenic activity) between 395 and 1227 revertants/plate/milligram particulate matter extract. Solvent extract of the XAD-4 resin positioned behind the glass fiber filter contained no measureable mutagenic material. It is possible that a larger sample might detect a mutagen if present at low concentrations.

In order to confirm that XAD-4 resin was capable of collecting mutagenic materials, samples were also collected using only the XAD-4 resin bed at the 402 meter sample site (Table V-3). A considerably lower SMA (196 rev/plate/mg smoke PME) was found in the sample collected by XAD-4 resin alone. The low mutagenicity in this sample may be a result of: a) incomplete collection of particles in the resin bed owing to the relatively loose resin bed packing; b) dilution by nonmutagenic material collected by the resin; or c) less efficient extraction of the resin relative to the glass fiber filter.

PMEs of smoke particles collected by glass fiber filter from the 1609 meter (one mile) sample site are less mutagenic than those collected from the 402 meter sample site (at $p = 0.05$ level, t-test), suggesting a definite reduction in mutagenic potency of the particles with time and/or distance from the burning field. Whether the potency continues to decrease with time and/or distance will require testing of samples collected further downwind and

repeated sampling to confirm if this is indeed true. The lack of mutagenicity in the XAD-4 extracts (XAD-4 located behind a glass fiber filter in the sampling train) is consistent for both the 1609 and 402 meter sample sites (see Table V-3).

Relative Sensitivity of Tester Strains. The relative sensitivity of the five tester strains of Salmonella typhimurium to the mutagenicity of rice straw smoke PME is shown in Table V-4. Based on percent increase over background (control), TA1538 shows the greatest sensitivity, followed by TA98, TA1537, and then TA100. TA1535 showed no response to the smoke PME. Mutagenic response is seen for all sensitive tester strains with and without S-9 activation, indicating the presence of both direct acting and promutagens. Greater specific mutagenic activity in the presence of S-9 was observed with TA98, TA1537, and TA1538, all frameshift mutation testers. The mutagenic response of TA100 is reduced in the presence of liver S-9, possibly due to detoxification or nonspecific binding of mutagens to proteins in the S-9, Wang, et al. (1981). These results indicate the presence of frameshift mutagens in the smoke PME (see mutagenicity of field samples for discussion of TA100). Benzo(a)pyrene served as a positive control and was detected by all strains except TA1535. Based on sensitivity and mutagen specificity, TA98 and TA100 were chosen for testing subsequent samples.

Mutagenicity of Field Samples. The mutagenicity of the other field samples, tested with TA98 and TA100 is summarized in Table V-5. For these samples, mutagenicity testing was performed on extracts of particles less than 3.5 microns in size. The large cut of the cyclone samplers (see Chapter II)

Table V-4

Relative Mutagenicity of Rice Straw Smoke Extract

(unfractionated) to Five Strains of Salmonella typhimurium

Tester Strain	Activation System (S-9)[b]	Mutagenicity (revertants/plate/mg extract)[a] Control[c]	Benzo(a)pyrene (20 µg)	(% control)	Smoke[d] Extract (1 mg)	(% control)
TA98	-	12 ± 4	16 ± 3	(133)	142 ± 5	(1183)
	+	28 ± 1	631 ± 84	(2253)	593 ± 84	(2118)
TA100	-	110 ± 7	102 ± 30	(93)	718 ± 74	(653)
	+	108 ± 9	1216 ± 446	(1126)	482 ± 55	(446)
TA1535	-	15 ± 5	10 ± 2	(66)	15 ± 3	(100)
	+	12 ± 3	31 ± 10	(258)	11 ± 3	(92)
TA1537	-	7 ± 3	7 ± 2	(100)	45 ± 10	(643)
	+	7 ± 1	122 ± 11	(1743)	95 ± 17	(1357)
TA1538	-	20 ± 7	10 ± 3	(50)	66 ± 3	(330)
	+	19 ± 3	228 ± 19	(1516)	445 ± 78	(2342)

[a]Average of triplicate plates ± one standard deviation.

[b]The S-9 activation system was prepared from Sprague-Dawley rats pretreated with Aroclor 1254.

[c]Bacteria with smoke extract.

[d]From Butte County sample taken 402 meters downwind from burning field.

Table V-5

Mutagenicity of Rice Straw Smoke Extracts

tested with TA98 and TA100

Sample	Code	Mutagenicity (rev/plate/mg) TA98 +S-9[c]	TA98 -S-9	TA100 +S-9	TA100 -S-9	Notes
Yolo (11-8-79)						
Upwind	F1UA	0	0	0	0	Less than 1 mg material available for testing
Downwind	F1DA	268	14	665	1826	
Sacramento (11-9-79)						
Upwind	F2UA	143	39	373	502	Test run on 0.5 mg material
Downwind	F2DA	442	28	694	458	
Sacramento (11-21-79)						
Upwind	F3UA	217	NT[a]	NT	NT	Enough material to test only TA98 +S-9
Downwind	F3DA	349	36	166	330	
Butte (402 m) 12-5-78)						
Upwind	F4UA	0	0	NT	NT	Less than 1 mg material available for testing
Downwind	F4DA	1227	117	374[b]	608[b]	

[a] NT = not tested

[b] mutagenicity based on single dose data

[c] The S-9 activation system was prepared from Sprague-Dawley rats pretreated with Aroclor 1254.

(and note that all sampling of rice straw smoke with the exception of the Butte County [12/5/78] samples were done with these two-stage cyclone samplers) is greater than 3.5 microns in size and did not provide sufficient material to allow testing in the Ames test. This is in agreement with previous reports that 95% of the smoke particles (by mass) are less than 3.5 microns in diameter (Darley, et al. 1974).

On several of the sampling days the large quantity of rice straw being burned in adjacent fields made it impossible to collect upwind controls for testing the mutagenicity of ambient air not containing smoke. Limited testing showed no mutagenicity (for PME samples of comparable volume) is seen on days of good air quality in this area (Olsen and Hsieh, 1981).

The SMA of upwind controls (143-217 revertants/plate/milligram particulate matter extract) in Table V-5 reflects the fact that these upwind controls are actually downwind samples of other burning fields in the area. The SMA of these samples being less than that of the Butte County sample at 1609 meters (395 revertants/plate/milligram particulate matter extract) is consistent with our suggestion in the foregoing section that there may be a decrease in the mutagenicity of rice straw smoke with distance and/or time. It is important to note that SMA relates to potency of collected particles and not to the quantity of mutagenic material in a given volume of air. The possible reasons for this decrease in specific mutagenic activity with distance may include: a) dilution of the more distant samples with nonmutagenic aerosols; b) degradation of mutagenic components by environmental fate processes such as ultraviolet light; or c) volitilization of mutagenic compounds off the surface of the particulate matter.

Fig. V-2 Typical Dose Response for Field Samples Tested With TA98.

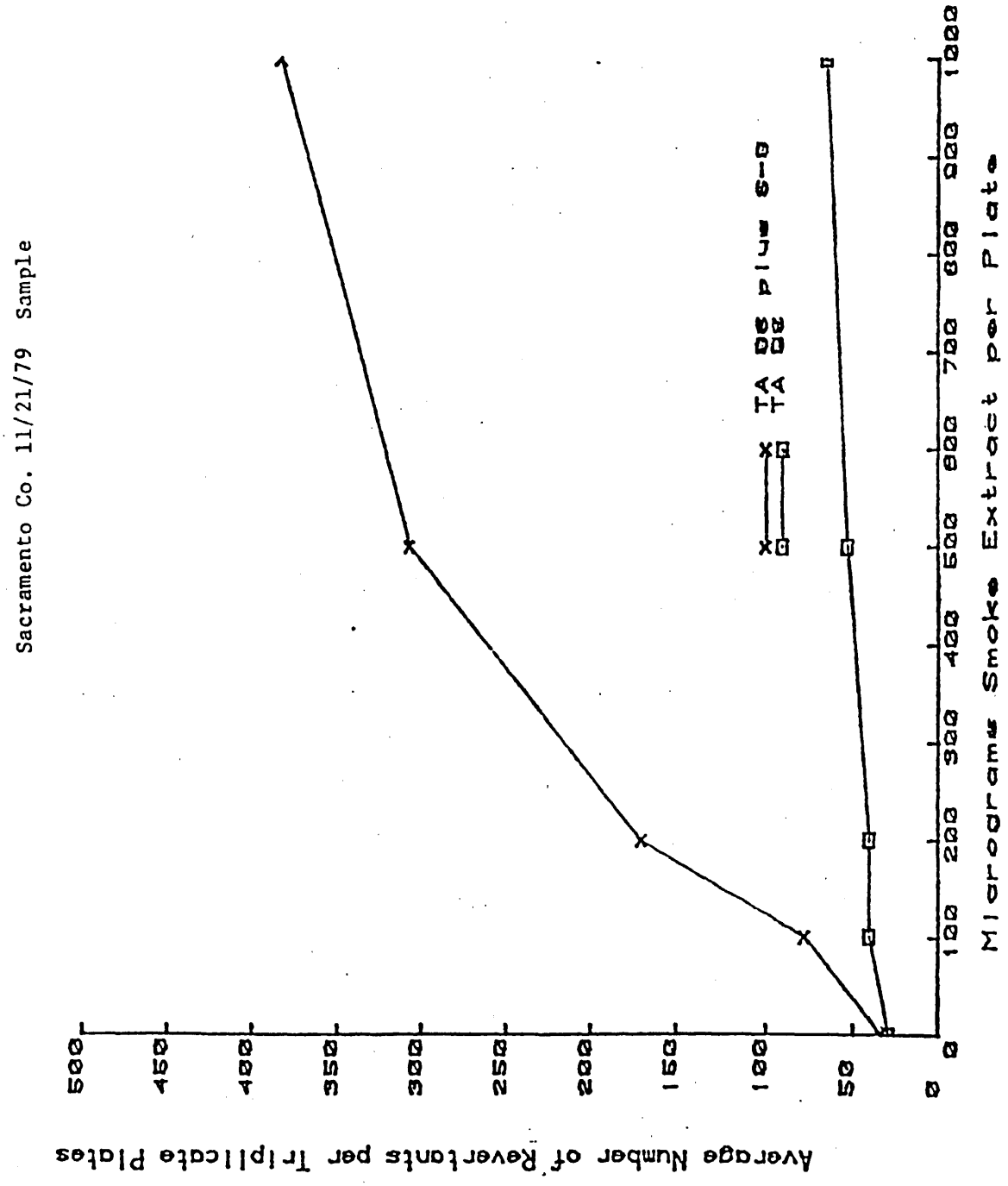
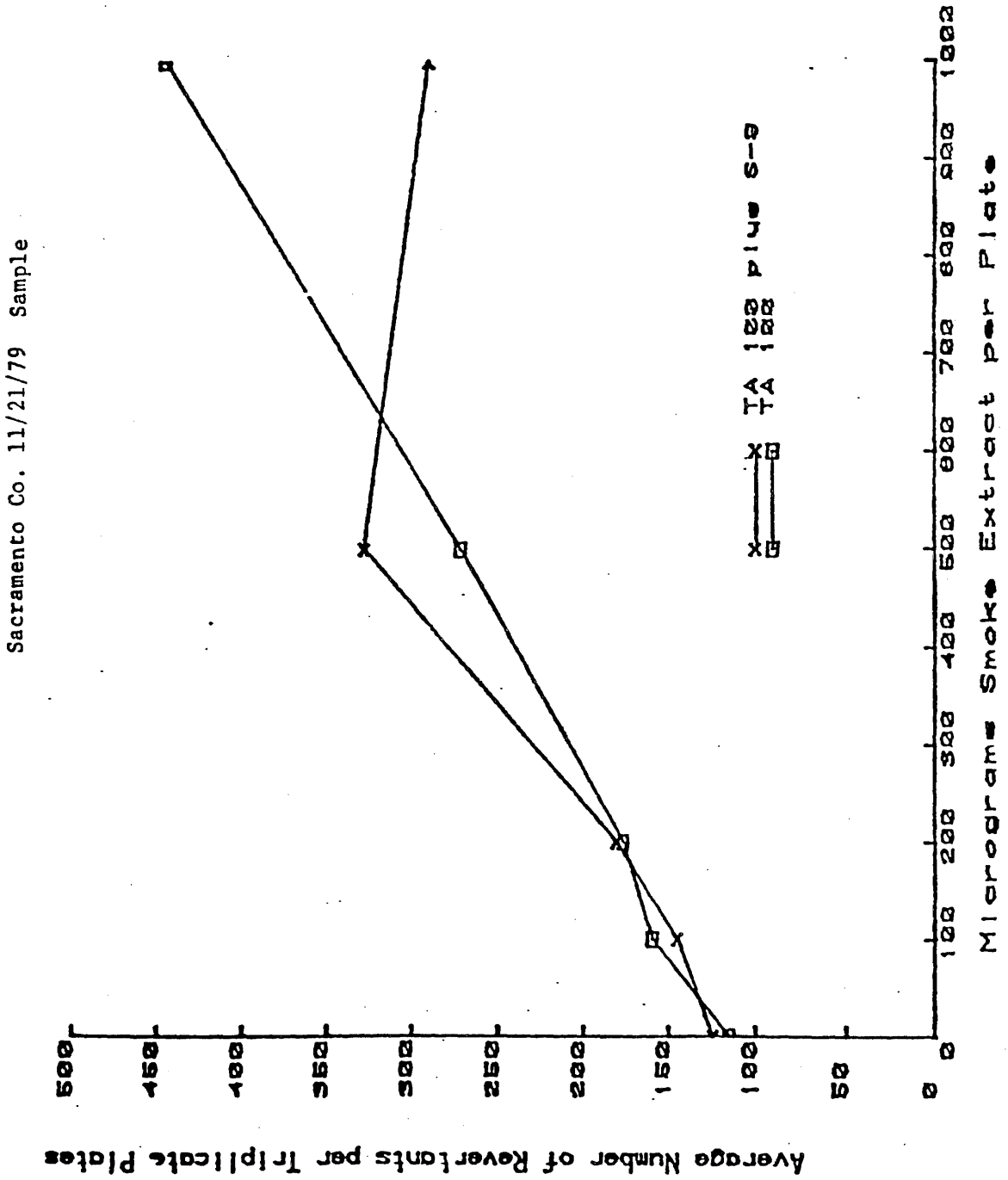


Fig. V-3 Typical Dose Response for Field Samples Tested With TA100.



Comparing the mutagenic response of TA98 to various field samples (Table V-5, there is a consistent increase in the mutagenicity of smoke PMEs with the addition of liver S-9, indicating the presence of frameshift mutagens in all the field smoke samples. A smaller response is seen in TA98 without S-9 activation which may be attributable to the presence of direct acting frameshift mutagens in the smoke. The mean SMA for TA98 plus S-9 for all field samples is 571.5 revertants/plate/milligram particulate matter extract with a standard deviation of $\pm$443. If the Butte County sample is excluded, then the mean for the remaining samples becomes 353 revertants/plate/milligram particulate matter extract with a standard deviation of $\pm$74.

The significant reduction in variability with respect to SME of different samples to TA98 plus S-9 may reflect differences in sampling (see Chapter II) and distance of sample site from the burning field. Similar analysis of data for TA98 without S-9 shows a similar reduction in variability with omission of the Butte County sample, again indicating this variability may be associated with sampling methodology.

For TA100, positive mutagenic response was observed with all the tested smoke PMEs, with and without metabolic activation, confirming the presence of direct acting and promutagens in the samples. Compared to the linear dose response curves for TA98 (Fig. V-2) using doses between 0 and one milligram per plate, the response in TA100 over the same dose range is generally not linear (Fig. V-3). At low doses, metabolic activation enhanced mutagenicity in TA100, while at high doses, a lower mutagenic response was observed with the addition of liver S-9. The smaller mutagenic response for

high doses of smoke condensate with TA100 and S-9 suggest a number of possibilities including: a) possible toxicities from activated promutagens thus reducing bacterial population available for mutagenesis; and b) nonspecific binding of direct acting mutagens to protein in the S-9 (Wang, et al. (1981). For comparison of mutagenic potencies, the slopes of linear portions of the dose response curve should be used. At lower doses in TA100, where the response is more linear, it is evident that promutagens are more mutagenic than direct-acting mutagens for these samples.

Even though the tester strain TA100 is derived from TA1535 (sensitive to mutagens causing base pair substitutions), the positive response of TA100 but not of TA1535 (Table V-3) makes it uncertain whether there are base pair substitution mutagens in the tested samples. The nonspecificity for base pair substitution mutagens in TA100 is believed to result from an increase in error-prone DNA repair with the addition of plasmid pKM101 (the R factor). Error prone repair of DNA damaged by other than base pair substitution mutagens is presumed to result in mutation and a coincidential loss in strict specificity of TA100 for base pair substitution mutagens.

2. Fractionation of Rice Straw Smoke Particulate Matter Extracts

In order to determine the distribution of mutagenic components by chemical class, rice straw smoke PMEs were fractionated by two methods, solvent-solvent extraction and column chromatography. Briefly, PME of laboratory-generated rice straw smoke was solvent extracted to yield five fractions: basic; neutral; weakly acidic; strongly acidic; and amphoteric

Table V-6

Mutagenic Activity of
Laboratory Rice Straw Smoke Extract
Fractionated by Solvent-Solvent Extraction

Sample	Weight (%)	Mutagenic Activity (rev/plate/mg)[a]	Relative Mutagenic Activity (%)
Whole extract	100.0	185	100.0
Fractions:			
Basic	4.8	706	18.3
Neutral	7.1	352	13.5
Weakly acidic	3.6	244	4.8
Strongly acidic	0.5	no response	
Amphoteric	1.0	245	1.3
Sum of Fractions	17	not tested	37.9
Residue	79	not tested	

[a]Based on slope of dose-response curve as determined by linear regression. All values are from assays using microsomal enzyme activation (S-9).

Table V-7

Mutagenic Activity of Riverside Burning Tower Samples
after Column Chromatography on Sephadex LH-20

Fraction	Weight[b] (%)	Mutagenic Activity[c] (rev/plate/mg)	Relative Mutagenic[d] Activity (% whole extract)
Whole extract	100.0	2169	100.0
1	7.5	0	0
2	1.7	0	0
3	2.0	0	0
4	4.6	318	0.7
5	66.9	2311	71.0
6	21.4	955	9.4
Sum of fractions[e]	104.1	1763	81

[a]See Chemistry, Chapter III for specific details of fractionation

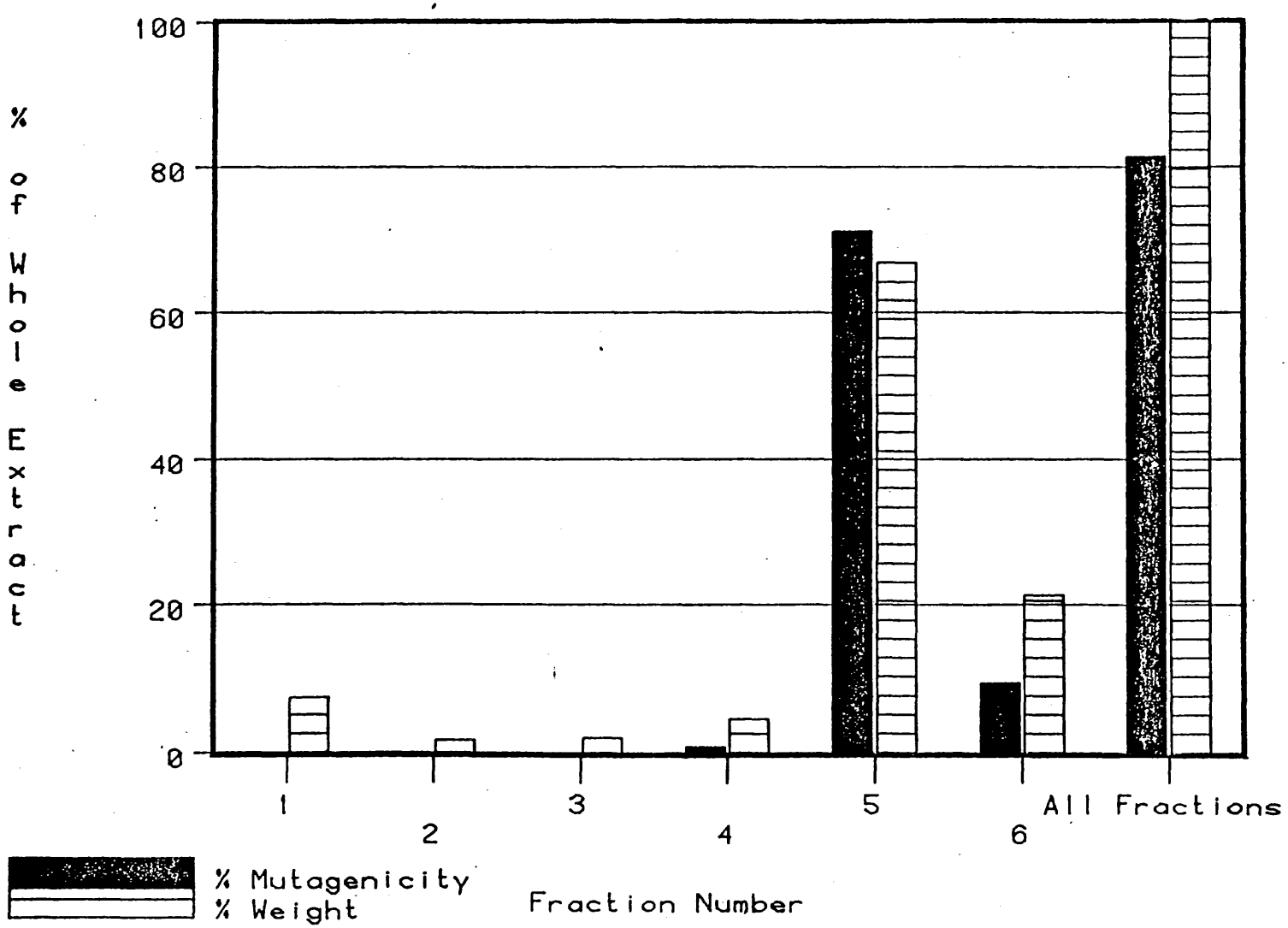
[b]Weight % = Weight of fraction recovered divided by weight of extract fractionated

[c]Slope, determined by linear regression from the steepest portion of the dose-response curve. TA98 +S-9 used for determination

[d]Relative mutagenic activity = (weight % x fraction mutagenic activity)/whole extract mutagenic activity

[e]Sum of fractions is total for each category

Fig. V-4 Mutagenicity of Fractionated Burning Tower Smoke Extract



compounds (see Materials and Methods, this Chapter). Rice straw smoke PME from the Riverside burning tower was fractionated on a Sephadex LH20 column using tetrahydrofuran and methanol to yield six fractions (see Chapter III). Tables V-6, V-7 and Fig V-4 summarize results obtained by these two methods of fractionation.

Solvent-Solvent Fractionation. Table V-6 lists the percent recovery by weight for each fraction, its mutagenicity to TA98, and the percent of whole smoke PME mutagenicity it represents. Extraction with water yielded a preciptate accounting for 79% by weight of the whole PME. Only 38% of the total mutagenic activity of the whole PME was recovered in the five fractions, leaving the remaining mutagenic activity to be accounted for by water extracted material (not tested) or possible synergistic effects of components in the whole PME. The incomplete recovery of mutagenic activity in similar fractionations of aerosol extracts has been reported in the literature (Teranishi, et al. [1978]). The basic and neutral fractions are seen to be most mutagenic based on specific mutagenic activity followed by the weakly acidic and amphoteric fractions. The strong acid fraction showed no mutagenic response.

Previous studies on airborne mutagens suggest that some of the mutagenic components in the rice straw smoke extract may be aromatic amines. Teranishi, et al. (1978) fractionated particulate matter collected in an industrial area of Japan and found the same general order of specific mutagenic activity (revertants/plate/milligram smoke PME) as rice straw smoke, i.e., bases-neutral-acidic fraction. Kier, et al. (1974) tested fractionated

cigarette smoke extract and also found that the basic fraction was most mutagenic based on specific mutagenic activity. Yoshida, et al. (1978) implicated the aromatic amines as being responsible for approximately 50% of the mutagenic activity in cigarette smoke. The basic constituents of petroleum substitutes, especially the aromatic amines, are the major contributors to the mutagenic activity in this complex mixture (Guerin, et al. 1980). In rice straw smoke the aromatic amines (basic fraction) and poly-nuclear aromatic hydrocarbons (neutral fraction) are prime candidates for further research in identification of mutagenic components.

Column Chromatography. Table V-7 summarizes mutagenic activities of whole PME after column chromatography on Sephadex LH20 resin, by the methods described in Chapter III. For fractions 1, 2, and 3, there was insufficient material to allow determination of a dose response curve. For fractions 1 and 3, tests were run on TA98 with and without S-9 at a dose of 500 micrograms/plate, and for fraction 2, 488 micrograms per plate was tested. No mutagenic activities above background were seen in these three fractions. Fraction 5 contained the majority of smoke extract by weight and also the highest specific mutagenic activity. The histogram in Fig. V-4 compares fractions 1 thorough 6 with the whole smoke PME based on percent weight and percent mutagenicity of the whole extract.

Ten polyaromatic hydrocarbons identified in fraction 5 by gas chromatography-mass spectroscopy (Chapter III) are listed in Table V-8 along with previous literature references for aerosols containing these compounds and their mutagenicity. Fraction 5 contains both mutagenic (1, 2

Table V-8

Polycyclic Hydrocarbons Identified in Fraction 5 by GC-MS System
(Comparison with Other Aerosols and Biological Effects)

Compounds Identified in Fraction 5[a]	Aerosol Previously Identified as Containing this Compound	Mutagenic Response in *Salmonella typhimurium*	Reference	
			Mutagenicity	Aerosol Identified as Containing this Compound
phenanthrene	Smoke from leaf burning	negative	McCann *et al.*, 1975	Friedman *et al* , 1977
anthracene	Smoke from leaf burning	negative	McCann *et al.*, 1975	Friedman *et al.*, 1977
pyrene	Smoke from leaf burning	negative	McCann *et al.*, 1975	Friedman *et al.*, 1977
fluorene	Soot	negative	McCann *et al.*, 1975	Kaden *et al.*, 1979
acenaphthene	Soot	positive	Kaden *et al.*, 1979	Kaden *et al.*, 1979
carbazole	Soot	negative	Kaden *et al.*, 1979	Kaden *et al.*, 1979
fluoranthene	Urban aerosol	negative	Tokiwa *et al.*, 1977	Tokiwa *et al.*, 1977
1,2-benzanthracene	Urban aerosol	positive	Tokiwa *et al.*, 1977	Tokiwa *et al.*, 1977
chrysene	Urban aerosol	positive	Tokiwa *et al.*, 1977	Tokiwa *et al.*, 1977
triphenylene	Urban aerosol	positive	Tokiwa *et al.*, 1977	Tokiwa *et al.*, 1977

[a] See Chapter III (Chemistry) for further details.

benzathracene, chrysene, acenaphthene, triphenylene), and nonmutagenic (phenanthrene, anthracene, pyrene, fluorene, carbazole, fluoranthene) compounds. The importance of these compounds in determining mutagenic activity of the entire fraction has not been established, however, acenaphthene is not a potent mutagen (Kaden, et al. 1979). No single chemical species would be expected to account for all of the mutagenicity in complex mixtures of this type. Further work is needed to determine the relative contribution of these compounds to the mutagenicity of the whole extract and to identify additional mutagens present in the mixture.

Three nitrogen-containing compounds were tentatively identified in fraction 5 using gas chromatography-mass spectroscopy (see Chapter III, Chemistry) and would be expected to be recovered in the basic fraction after our solvent-solvent fractionation. The presence of basic compounds in fraction 5 of the Sephadex fractionated material (Fraction 5 had the highest specific mutagenic activity for Sephadex fractionation) is consistent with the basic fraction from the solvent-solvent fractionated material which also had the highest mutagenic activity for its respective fractionation method. This may indicate nitrogenous chemicals account for part of the mutagenic activity of rice straw smoke.

The histogram in Fig. V-4 indicates that the mutagenicity of the six fractions generated by gel-filtration roughly parallels the quantity of material recovered in each fraction. Since fractions 1, 2 and 3 of rice straw smoke PME contained little material, the lack of mutagenicity for fractions 1, 2, and 3 of rice straw smoke extract may be a by-product of the

chromatographic method. Brooks, et al. (1979) fractionated diesel exhaust using the same methods and recovered little or no material in fractions 1 or 2. Future refinements in chromatographic technique should allow better resolution of mutagenic from nonmutagenic components in rice straw smoke extract.

3. Energy Commission Samples.

Two kinds of particulate samples (EC1 and EC2) were obtained from a small scale incinerator burning rice straw as a fuel (see Chapter II for a more detailed description of the incinerator). EC1 consisted of an ash sample from the baghouse of the incinerator. After effluent from the incinerator passed through the baghouse it was vented to the atmosphere. EC2 was collected from the baghouse exhaust (otherwise vented directly to the atmosphere) using a two-stage respirable particle sampler. Samples were collected over a four-hour period after the incinerator had been running for one hour and had reached standard operating conditions.

EC1 consisted of a finely divided ash-like material. A portion of this ample was extracted initially with three solvents, benzene, methanol and dimethylsulfoxide (DMSO), in order to optimize extraction efficiency for mutagenic compounds. DMSO was found to be most efficient in this case. Similar results have been observed for coal fly ash (Chrisp, et al. 1980b). Extraction with DMSO was therefore performed on EC1 and coal fly ash (see Materials and Methods) based on extraction efficiency and to facilitate comparison of mutagenic response between the two samples. Dosing in the Ames

Table V-9

Mutagenicity of DMSO Extracts from Incinerator Baghouse Straw Ash and Coal Fly Ash[a]

Sample	Tester strain	Rev/plate/mg[b]	R^{2c}
EC1	TA98	34	.97
EC1	TA98 + S-9	12	.63[d]
Coal fly ash	TA98	64	.94
Coal fly ash	TA98 + S-9	35	.97

[a]Mutagenic components from these samples were extracted with DMSO (see materials and methods).

[b]Mutagenicity (rev/plate/mg) was determined as the slope of a dose-response curve obtained by linear regression on three data points per dose in a dose range of 0 to 2 mg per plate.

[c]Coefficient of determination for linear regression.

[d]Dose-response relationship was non-linear with addition of S-9.

Table V-10

Mutagenicity of Solvent Extracts of Particulate Matter

Collected from Incinerator Baghouse Exhaust[a]

Tester strain	Revertants/plate/mg[b]	R^2[c]
TA98	164	.86
TA98 + S-9	164	.95
TA100	118	.91
TA100 + S-9	127	.95

[a]Mutagenicity components extracted with benzene-methanol with sonication.

[b]Mutagenicity calculated from the slope of a dose-response curve obtained by linear regression.

[c]Coefficient of linear regression.

test was based on a volume of DMSO representing (x) milligrams of extracted fly ash, i.e., if 50 milligrams of fly ash is extracted with 2.5 ml DMSO, then 50 microliters of DMSO represents one milligram of fly ash. The coal fly ash sample was collected downstream from an electrostatic precipator in a modern coal-fired power plant and had a volume median diameter of 2.2 microns (Fisher, et al., 1978b). The mutagenicity of the extracts from EC1 and coal fly ash to TA98 is shown in Table V-9. The mutagenicity was measured as revertants/plate/milligram extracted sample as calculated from the slope of a dose response curve determined by linear regression.

The SME of EC1 is less than that of coal fly ash regardless of metabolic activation. In the presence of the S-9 metabolism system, a lower response was seen for TA98 for both EC1 and coal fly ash. This lower mutagenic response may be due to detoxification by the S-9 system, possible toxicity of activated components to the bacteria, or nonspecific binding of mutagenic compounds to protein in the S-9 (Wang, et al. (1981).

The mutagenic components of EC2 were extracted from glass fiber filters with benzene/methanol (1/1) using sonication, as described in Chapter III (Chemical Analysis). The mutagenicity of PMEs to TA98 and TA100 is shown in Table V-10. The test results indicate that the sample is mutagenic to both tester strains, and that metabolic activation does not influence the mutagenicity. Since the particulate matter escaping the baghouse (Sample EC2) was: collected in a manner different from the baghouse sample (EC1), was composed of a different size fraction of aerosol, and extracted with a different solvent system, comparison between the two samples is not possible.

The most that can be concluded is that mutagens are incompletely collected by the baghouse.

Table V-11

SPECIFIC MUTAGENIC ACTIVITY OF RICE STRAW SMOKE, CIGARETTE SMOKE, AND PARTICULATE MATTER COLLECTED FROM LOS ANGELES AIR (TA98 + S-9)

Sample	Specific Mutagenic Activity (revertants/plate/mg PME)
Rice Straw Smoke at Downwind Edge of Field[a]	268-442
Rice Straw Smoke One Mile Downwind from Field[b]	395
Rice Straw Smoke from Incinerator Baghouse Exhaust	164
Particulate Matter Collected in Downtown Los Angeles[c]	1,124
Particulate Matter Collected at Cal State Los Angeles[d]	1,286
Experimental Cigarette Smoke[e]	786

[a]Data from field samples Yolo (11-8-79); and Sacramento (11-9-79) and 11-21-79)

[b]Data from Butte (12-5-78)

[c]A 24-hour particulate sample provided by the California Air Resources Board

[d]Data from Pitts, et al. 1980

[e]See materials and methods (this chapter)

4. Comparison of Specific Mutagenic Activity between Various Smoke Samples

Comparison of specific mutagenic activity was made between different rice straw smoke samples, cigarette smoke, and particulate matter samples collected at two sites in Los Angeles (Table V-11). This comparison provides a rough ranking of the mutagenicity of rice straw smoke relative to other common mutagenic aerosols. Specific mutagenic activity spans an approximate 10-fold range with rice straw smoke samples occupying the low to mid-portion of this range. It is important to remember that this comparison is of mutagenicity of collected particles and not of mutagenic material per unit volume of air. These results indicate that rice straw smoke is not an unusually mutagenic aerosol. Since the Ames test is not infallible, more *in vitro* testing using other short-term tests for genotoxicity is in order to confirm results in the Ames test.

Ames test assay conditions used in this comparison are also important in affecting the specific mutagenic activity measured for the various samples. The protocol we employed was designed to minimize assay variation, however, we used one concentration of S-9 enzyme preparation rather than optimizing S-9 concentration for individual samples. Optimization for individual samples was not carried out due to small sample size in some cases and our desire to compare samples under the same assay conditions. Optimization of individual samples affects response in the assay and could alter the comparisons made here. S-9 optimization and other variables in the assay are another reason why results in the Ames test can be misleading if they are extrapolated to the *in vivo* situation.

5. Significance of Ames Test Results for Rice Straw Smoke

Because of the expensive, difficult, and lengthy testing required to assess the toxicity of an air pollutant it is important to gain as much information as possible from short-term bioassays such as the Ames test.

Because the Ames test has definite limitations any extrapolations of data from the Ames test to effects (carcinogenicity) in man cannot be made. This is well illustrated by the ongoing studies of the South Coast Air Basin of California (Los Angeles area). The air pollution of the South Coast Air Basin is internationally known and has received intensive study (Pitts, et al. 1981). The aerosols produced in this area have been well characterized in terms of their significant mutagenic activity in the Ames test (Pitts, et al. 1980). Based on results in the Ames test it would be expected that populations living in the South Coast Air Basin would have elevated incidences of cancer related to exposure to air pollutants. Yet, an epidemiological association between exposure to these air pollutants and lung cancer has not been established (Pike, et al. 1979). It is difficult, if not impossible, to detect and then link cancer in human populations with atmospheric mutagens. Epidemiological studies of cancer rates are very costly to perform and their findings are easily complicated by numerous unrelated environmental and lifestyle factors which contribute to cancer causation. Despite the Ames test's limitations it does have utility as a preliminary indicator of potentially carcinogenic chemicals. When a large number of chemicals are compared in the Ames test with data from animal carcinogenesis studies good agreement between mutagenic and carcinogenic effects are seen (Heddle, 1982). Many investigators feel testing in a battery of short-term tests helps to screen out false positive and false negative results which may occur in the Ames test. When compounds of widely different carcinogenic potency are compared, their relative mutagenic potency may often correlate with their carcinogenic potency (Meselson, et al. 1977). There are many exceptions and there is disagreement as to whether this concept is completely valid (Heddle,

1982). This means that the Ames test is often able to discriminate between carcinogens and non-carcinogens and possibly provide a rough rank order of relative potency.

6. Issues to be Resolved - Mutagenicity

When viewed as a whole, the results of our Ames testing of the smoke particles points to several specific issues that might be addressed in future studies. Since rice straw smoke does not appear to be unusually mutagenic compared to other aerosols we tested (Table V-11), other questions concerning the health implications of exposure of human populations become important. Specifically, what happens to the chemical composition and mutagenicity of the smoke during transport in the atmosphere; what are the doses of smoke-derived particulate matter populations are exposed to; and, does genotoxicity represent a concern? Confirmation of rice straw smoke mutagenicity in other genotoxicity assays could justify studies of rice straw smoke carcinogenicity.

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VI. PULMONARY ALVEOLAR MACROPHAGE TESTING

A. Introduction

Evaluation of potential health hazards associated with the disposal of rice straw via burning in the field can be facilitated by studies into the biological nature of the by-products generated by the burning. Short-term *in vitro* assays such as the Ames mutagen assay or the pulmonary alveolar macrophage (PAM) functional assay can be used to screen materials that are potentially hazardous. The Ames mutagen assay is used to predict effects on genetic material and the PAM functional assay allows an assessment of cytotoxic effects. The PAM assay has been utilized in studies on the biological effects of combustion byproducts such as coal fly ash, toxic chemicals (metals), and bacterial agents (Waters, *et al.*, 1975; Davis-Scibienski and Beaman, 1980; Fisher, *et al.*, 1980). This assay is particularly useful in the study of particles in the inhalable size range (aerodynamic diameter 0.2 - 5.0 μm). An understanding of the applicability of the short-term PAM functional assay as a screening test for potentially hazardous material of the inhalable size range can be obtained by a discussion of respiratory tract and lung physiology and specifically the role of the PAM in the lung defense system.

The physics and chemistry of aerosols, the anatomy of the respiratory tract, and airflow patterns in the lung airways ultimately determine the respiratory tract deposition of inhaled particles (Yeh, *et al.*, 1976). The major physical factors affecting lung deposition of inhaled particles are the aerodynamic properties and the chemical reactivity of the aerosol in the lung airways. Lung deposition is generally discussed in terms of particle deposition in three regions: the nasopharyngeal, tracheobronchial,

and pulmonary regions (Task Group on Lung Dynamics, 1966). The nasopharyngeal region is composed of the nose and throat, extending to the larynx; the tracheobronchial region consists of the trachea and bronchial tree, including the terminal bronchioles; and the pulmonary region consists of the respiratory bronchioles and the alveolar structures. Particles greater than 10 μm (aerodynamic diameter) are effectively collected in the nasopharyngeal region via the phenomena of impaction (Stern, et al., 1973). Tracheobronchial and pulmonary deposition generally increase with decreasing particle size. Particles, 2-10 μm, settle or impinge upon the walls of the trachea, bronchi, and bronchioles, while particles approximating 0.1 to 2 μm may reach the alveolar sacs. In consideration of the nature of particle deposition, aerodynamically size-fractionated aerosols (50% efficiency, <3.8 μm aerodynamic diameter) from rice straw burning were collected for this study.

The rate of clearance of deposited particles from the respiratory tract will be determined, in part, by their chemical behavior in the lung microenvironment of the particles. Hygroscopic particles deposited in the respiratory tract will be rapidly cleared by dissolution and subsequent passage into the blood stream. Less soluble particles deposited on the mucociliary escalator of the tracheobronchial region and on the nasopharyngeal region will be rapidly cleared with half-times on the order of one day and a few minutes, respectively (Task Group on Lung Dynamics, 1966). Relatively insoluble particles, such as those expected from rice straw burning, deposited in the pulmonary region can be phagocytized by the pulmonary alveolar macrophage or transported within pulmonary alveolar macrophage to the mucocilliary escalator. The biological half-time of material in the pulmonary region is very much a function of particle chemical composition; half-times

of hundreds of days are possible. However, the dissolution of surface-associated chemical components of particles need not be a requisite for their interaction with the biological system. Direct particle surface-cell interaction is demonstrated when macrophages phagocytize inhaled particles. An example of "insoluble" particle-cell interaction may be made with asbestos (McLemore, et al., 1979).

Inhaled particles, deposited in the pulmonary region of the respiratory tract, will be phagocytized by the PAM. Exposure of these vitally important immunologically effector cells to deposited inhaled particles may give rise to manifestations of toxicological and pathological responses in the lung. For example, it is well documented that PAM play a major role in protecting the lung against invasion by inhaled bacteria (Truit and Mackaness, 1971; Goldstein, et al., 1974; Davis-Scibienski and Beaman, 1980). Also, the phagocytosis and clearance of all inhaled particles is effected by the PAM. Recent data indicate that the PAM is involved with rejection of metastasizing cancer cells and in communication and activation of other immunological effector cells including lymphocytes (Keller, 1976; Zuckerman and Douglas, 1979). Damage to PAM may also result in release of lysosomal enzymes which may react with lung tissue to result in either fibrotic or emphysematous damage (Grant, et al., 1979). Therefore, it is evident that the potential biological hazard of inhaled particles may be manifested by interaction with the pulmonary alveolar macrophage.

The awareness of the important role of PAM in the immune defense system has generated much interest in the development of PAM functional assays. PAM functional assays utilizing various species and measures of functions have been used extensively for the screening of toxic metals

and their salts, particles from industrial situations, and interaction studies of bacteria and PAM.

A rabbit PAM functional assay developed at the Environmental Research Center at Research Triangle Park, N.C., was used to rank cytotoxicities of a series of metallic chlorides (Waters, et al., 1974b; Waters, et al., 1975). The relative cytotoxicities of a series of size-fractionated industrial particles have been compared using a modification of this rabbit PAM assay (Campbell, et al.) In general, these screening tests measure effects on viability and enzyme content. This type of assay has also allowed an assessment of the contribution of interactions of toxic trace elements in the toxicity of environmental pollutants. Cytotoxicity of metallic compounds has been shown to be directly related to solubility (Waters, et al., 1974a).

Results from a rat PAM functional assay suggested that there was a direct relationship between cytotoxicity of particles, for example asbestos, to cultured PAM and fibrogenicity in the living animal (Conning, et al., 1970). This relationship was independent of particle shape, size, concentration, or of the phagocytic potential.

The cytotoxic effects of silica on mouse peritoneal or rabbit alveolar macrophage have been shown by a variety of methods which include failure to exclude trypan blue or other dyes (Keusch and Rüttner, 1978), as well as release of lysosomal and cytoplasmic enzymes into the medium (Kessel, et al., 1963). Silica particles have two types of cytotoxic effects on macrophage (Allison, 1975). Rapid cytotoxicity occurred when relatively large amounts of silica were added to macrophage in serum-free medium. When moderate amounts of silica were added to macrophage in serum containing medium, delayed cytotoxicity was observed.

The Laboratory of Energy-Related Health Research (LEHR) at the University of California, Davis, has developed an in vitro murine PAM technique that allows simultaneous quantitation of PAM phagocytic ability, attachment, adherence, and viability (Fisher, et al., 1978). In this system, phagocytic ability was represented by the number of carbonized latex microspheres ingested by attached PAM. Attachment was quantified by determining the fraction of the viable cells plated that initially attach to glass; adherence was measured by determining the fraction of seeded cells that remained attached to glass coverslips after initial attachment. Viability of the PAM was determined by trypan blue exclusion. This technique has been successfully modified for dog, rat, bovine, and rabbit PAM. The bovine PAM assay was developed with these points in mind: 1) the large quantities of PAM obtainable, 2) the ease in obtaining the PAM and 3) PAM from a mammalian system. However, the bovine used are outbreed, nonlaboratory reared animals and a large variation in "control" exposure response must be expected. In modifying this bovine PAM functional assay for this Rice Straw Smoke Study, consideration of the effects of particle concentration and the temporal kinetics of phagocytosis on measured PAM functions was necessary (Fisher, et al., 1978).

B. Materials and Methods

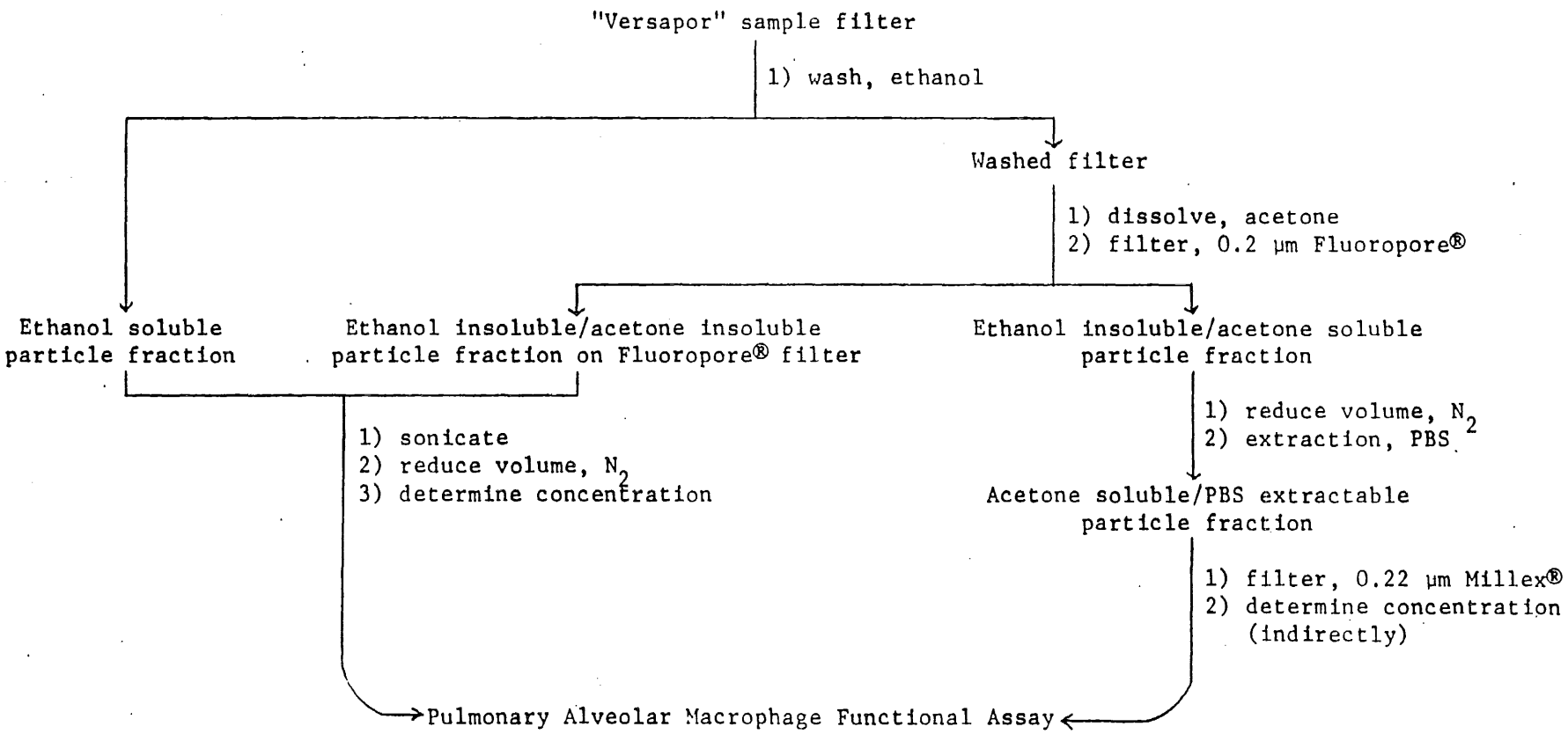
Sample Preparation (Figure VI-1). Air sampler filters (frozen in acid-washed jars) were allowed to thaw and equilibrate at room temperature. These sample filters (Versapor A, 8" x 11", Gelman) and appropriate control filters were weighed on a Mettler analytical balance (to ± 0.1 mg). One half of each sample filter was reserved for trace element analysis, while the other half of the filter was cut into small pieces (≃1-1/2" square) and weighed on the Mettler balance. The pieces of filter were placed two at a time in a 400 ml beaker containing 25-50 ml filtered (0.2 μm pore size) absolute ethanol and mechanically shaken to wash off all ethanol soluble material of the rice smoke particles from the filter. This ethanol solution was retained.

The ethanol washed filter pieces were then placed in a 400 ml beaker with ≃50 ml of reagent grade acetone and sonicated until the filter material ("Versapor") was completed dissolved in order to release the deeply imbedded insoluble component of the smoke particles. The nylon backbone of the filter was rinsed with acetone and discarded, while the acetone rinse was allowed to drain back into the beaker. The "Versapor"/acetone solution was diluted further with acetone and filtered through a Millipore filtration apparatus containing a 0.2 μm pore size Fluoropore® membrane filter (Millipore, 47 mm). The eluate containing acetone soluble material from the rice smoke particles was retained.

The Fluoropore® filter was then sonicated in the ethanol wash retained previously. At this stage, the ethanol soluble material and the acetone insoluble material were combined. The volume of this ethanol soluble and acetone insoluble material mix was reduced under N_2 to less than 1 ml and

Figure VI-1

Schematic of Extraction of Rice Straw Smoke Sample
from "Versapor" Filters



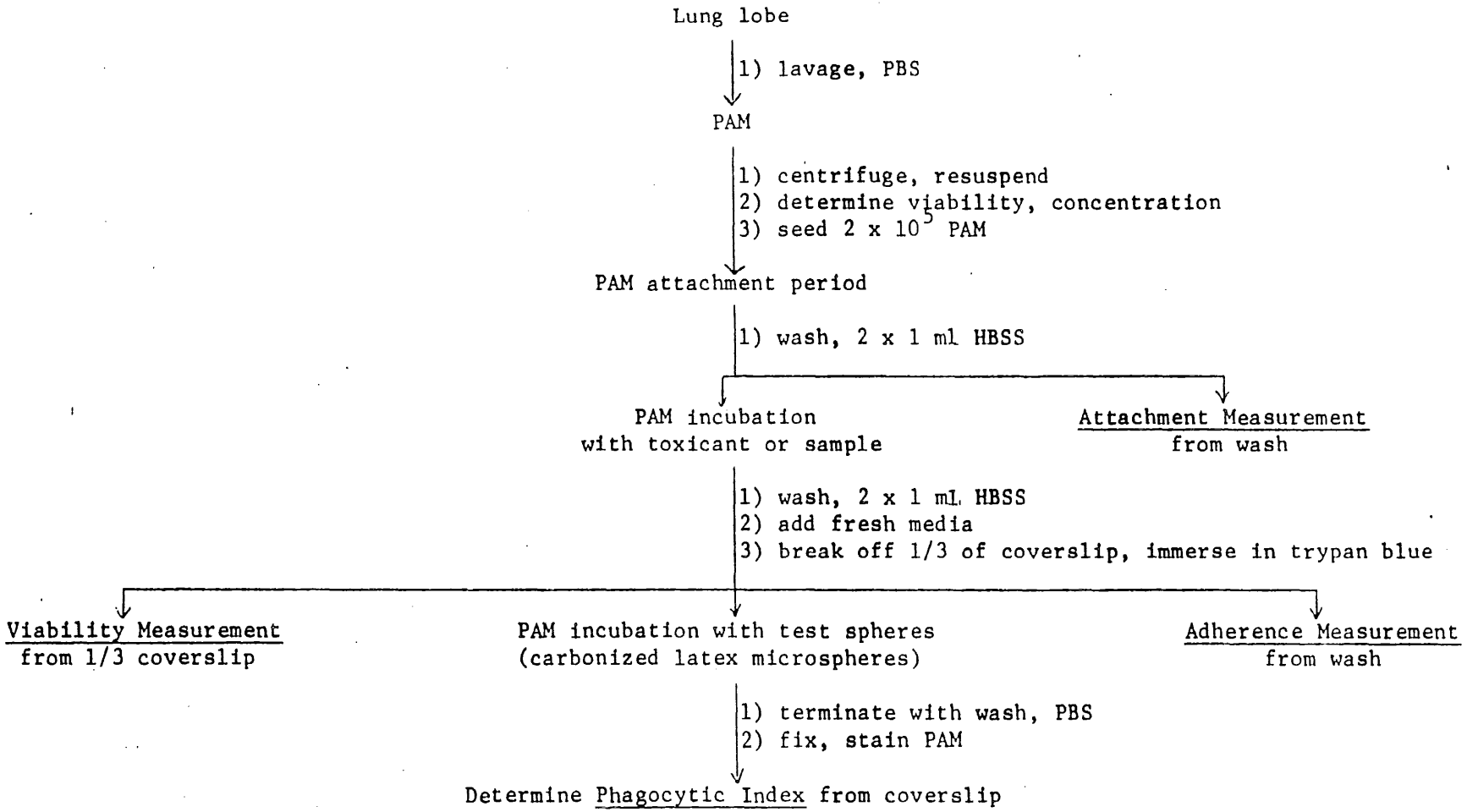
this sample was stored in an acid washed glass centrifuge tube with a teflon lined screw cap. The volume of acetone eluate was reduced to 25 ml and stored in a 40 ml acid washed glass centrifuge tube with a teflon lined screw cap.

The concentration of the ethanol soluble and acetone insoluble mix was determined by evaporating off a 20 ul aliquot in a tantalum weigh boat and weighing the residue on a microbalance (Perkin-Elmer Autobalance Microbalance Model AD-2Z) to ± 10 ug. Total sample retrieved from the sample filter and the efficiency of sample extration methods were calculated at this time (see Table VI-1). Also, one part of the concentrated acetone eluate was placed with four parts of phosphate buffered solution (PBS, GIBCO) in a 60°C waterbath until the acetone had evaporated off. The remaining aqueous phase was filtered through a 0.22 um pore size Millex® filter (Millipore) with a syringe to remove "Versapor" residues. This filtered PBS containing some of the acetone soluble component of the sample was immediately used in conjunction with the ethanol fraction of rice straw smoke for study in the macrophage functional assay. Upwind samples (ambient controls) and blank filter controls were treated in similar fashion.

Macrophage Assay (Figure VI-2). The right middle lobe of bovine lungs were collected from local slaughter-houses. The lobe was clamped off with a hemostat and removed sterilely with scissors, then placed in a plastic bag and transported on ice back to the laboratory. The lung lobe was prepared for lavage with the insertion of a blunt tip 16 gauge needle into the bronchus and clamping it down with a hemostat. The lung lobe was initially instilled with 60 ml of cold (4°C) Ca- and Mg-free phosphate buffered solution (PBS) via a 60 ml sterile sy-

Figure VI-2

Schematic of Pulmonary Alveolar Macrophage (PAM) Functional Assay



until 500 ml of lavage fluid had been withdrawn. The lung lobe was gently massaged after each instillation of PBS. The lavage fluid was pooled in 250 ml conical plastic centrifuge tubes and held on ice.

The lavage fluid was centrifuged for 30 minutes at 1100 rpm (International, 18 cm radius), the supernatant was decanted and the cellular pellet resuspended with approximately 2 ml of a complete media consisting of 79% M-MEM (with 1% antibiotic-antimycotic), 1% L-glutamine, and 20% fetal calf serum (GIBCO) (Fisher et al., 1978). An aliquot of the cell suspension was diluted with a 0.4% trypan blue-PBS (1:5) solution and a hemacytometer was used to determine the concentration of macrophage. An initial viability assessment of the macrophage was made at the same time using the trypan blue exclusion viability test. Smears of the cell pellet were also made to provide differential cell counts. The cell concentration was adjusted to a live macrophage concentration of 2×10^6 macrophages per ml. Total cell yields ranged from 1.5×10^6 to 1×10^8 macrophages and initial viabilities were from 90% to 99%.

PAM (2×10^5) were seeded into glass Leighton tubes containing a 35 mm x 11.5 mm glass coverslip covered with 1 ml complete media. The PAM were allowed to attach to the coverslip for 1/2 to 1 hour at 37°C. After the initial attachment period, the medium was decanted and the Leighton tubes were washed two times with Hank's Balanced Salt Solution (HBSS, GIBCO) warmed to 37°C. One ml of fresh complete media at 37°C containing the sample material at various concentrations, or control media, was added to the Leighton tubes. The nonattached cells collected by the washing procedure were pooled in a 50 ml centrifuge and counted later.

The PAM were incubated at 37°C with the sample material at two time intervals (2 hours and/or 21 hours). At the end of the incubation period the coverslips were washed two times with 1 ml of HBSS. One ml of fresh complete media (without sample material) was added. Viabilities were taken at this time by breaking off approximately one-third of the coverslip and immersing it in 0.4% trypan blue-PBS. Two hundred cells were scanned for viability as measured by the trypan blue exclusion viability test.

The phagocytic ability of the PAM was measured by the addition of carbonized latex microspheres (3-5 u diameter) (3M) at a particle-to-cell ration of 20:1 for 30 minutes at 37°C. Termination of phagocytosis was accomplished by washing the coverslips of attached PAM with non-sterile PBS and fixing the PAM, by forced air drying. The coverslips were stained with a Leishman-Wright stain (Harleco) for 3 minutes and buffer at pH 6.8 was added for 2 minutes. The coverslips were mounted with mounting media (Protexx,® Scientific Products) on microscope slides for reading on a Leitz Ortholux microscope at magnification of 400X. PAM were judged to have phagocytized if at least one sphere was more than one-half ingested into the cytoplasm of the PAM. Indirect measurement of the adherence ability of PAM was made by counting PAM found in the wash generated in termination of phagocytosis.

C. Results

A total of eleven rice straw smoke samples, and available controls were tested in the PAM functional assay. These samples consist of three field burns, three low moisture straw controlled burns, three high moisture straw controlled burns, a California Energy Commission incinerator burn, and a composite of plume samples taken by EPA aircraft. Table VI-2 lists the sample designations, type, and available controls. Specific sampling parameters of each sample are found in Chapter II Table II-2 through Table II-6.

The effect of these samples on PAM adherence, viability, and phagocytosis was assessed in the bovine PAM assay. The PAM were exposed to rice straw smoke particle samples (50% efficiency <3.8 μm aerodynamic diameter) at 0.03, 0.01 and 0.003 mg/ml levels for an incubation time of 21 hours. A few samples were studied at a higher level of 0.1 mg/ml and some were incubated for only 2 hours. Experiments were also performed to assess the effects of various experimental parameters. These parameters include the effects of ethanol, glassbeads (nontoxic particle effect), silica (positive toxic particle effect) and the effect of PBS (with acetone evaporated off and "Versapor" residues filtered out) on PAM function. Previous studies at LEHR on the effects of coal fly ash on PAM function are useful for evaluating the relative toxicity of rice straw smoke.

In general, control studies with ethanol and PBS (with acetone evaporated off and "Versapor" residues filtered out) showed that they would have no toxic effects on PAM phagocytic ability at levels used routinely in the assay. PAM exposed to higher levels of ethanol, 3% and 10%, did have decreases in viability (Table VI-3). A 3% level of ethanol was the

Table VI-2

Rice Straw Smoke Samples Studied in the PAM Assay

Sample designation Sample date	Type	"Versapor" filters collected		Sample exposure groups utilized in PAM assay			
		upwind	downwind	control	blank	upwind	downwind
Yolo Co. (11-8-79)	Field burn	X	X	X	X	X	X
Sac. Co. (11-9-79)	Field burn	X	X	X	X		X
Sac. Co. (11-21-79)	Field burn	X	X	X	X		X
Aircraft (1979)	Aerial plume sample		X[a]	X	X		X
CEC incinerator (2-28-80)	Incinerator baghouse exhaust sample	X	X		X	X	X
JY11 (7-23-80)	Low moisture straw controlled burn	X	X	X		X	X
JY13 (7-23-80)	Low moisture straw controlled burn	X	X	X		X	X
JY15 (7-23-80)	Low moisture straw controlled burn	X	X	X		X	X
JY17 (7-24-80)	High moisture straw controlled burn	X	X	X		X	X
JY19 (7-24-80)	High moisture straw controlled burn	X	X	X		X	X
JY20 (7-24-80)	High moisture straw controlled burn	X	X	X		X	X

[a]This sample is a composite of aerially collected plume samples.

Table VI-3

Control Experiments PAM Assay Data: 21 Hour Incubation Time

Sample Expt. Date	Conc. µl/ml	Phagocytosis[b] n	$\bar{X}$(%) SD	% Control	Viability[c] %	Adherence[d] %
Ethanol (8-20-80)	Control	4	73.9 ± 3.8	100.0	98.5	97.7
	1	4	74.2 ± 3.8	100.4	96.2	96.7
	3	4	83.1[a] ± 2.3	112.4	97.8	97.5
	10	4	80.9 ± 5.7	109.5	96.8	98.0
	30	4	87.1[a] ± 2.7	117.7	74.5	98.0
Ethanol (8-27-80)	Control	4	71.6 ± 3.3	100.0	97.5	95.3
	3	4	72.5 ± 3.7	101.3	95.2	96.6
	10	4	71.6 ± 7.6	100.0	97.0	98.0
	30	4	65.6[a] ± 3.7	91.6	86.2	97.6
	100	4	1.0[a] ± 1.1	1.4	0.2	96.1
PBS/"Versapore" Acetone	Control	4	78.4 ± 8.2	100.0	94.0	90.7
	50	4	75.1 ± 4.0	95.8	93.0	95.0
	100	4	81.8 ± 5.2	104.3	94.8	92.4
	200	4	79.9 ± 2.2	101.9	93.8	91.1
	400	4	72.5 ±10.5	92.5	96.2	94.0

[a] Significantly ($p \leq 0.05$) different from control

[b] $PI = \frac{\text{Number of cells containing test spheres}}{\text{200 cells counted}} \times 100$

[c] n = 2, viability = $\frac{\text{Number of cells alive}}{\text{200 cells counted}} \times 100$, based on trypan blue exclusion test

[d] n = 4, adherence = $\frac{2 \times 10^5 \text{ PAM seeded} - \text{nonattaching cells} - \text{nonadhering cells}}{2 \times 10^5 \text{ PAM seeded}} \times 100$

highest recommended addition into the PAM assay. Glassbeads were used to assess the effect of a nontoxic particle on PAM function. At levels of exposure thought to be typical of the rice straw smoke samples, the glassbeads (2-4 μm diameter) had no significant effects on PAM function (Table VI-4). Previous studies at LEHR show that at higher dose levels, similar to those used in coal fly ash studies, (up to 1 mg/ml) significant ($p<0.05$) decreases were found in the PAM ability to phagocytize and at the 1 mg/ml level cell lysis was widespread. Cell lysis cannot be quantified with this PAM test, therefore, the occurrence of cell lysis can only be noted by observation of cell membrane integrity and cell coverage at the time the glass coverslips are utilized to measure viability and phagocytic ability. Since adherence measurements (measured by subtracting out cells washout from the total seeded) are calculated indirectly, cell lysis is not likely to be reflected in this measurement.

Silica particles, an extensively studied cytotoxic agent, had typical effects on the PAM (Table VI-4). After 21 hours of incubation in serum containing media, extensive cell lysis occurred at dose levels ranging from 0.03 mg/ml to 0.3 mg/ml. This lysis was not reflected in the adherence measurement and only to a limited extent in the viability measurement. PAM coverage on the glass coverslip was nil; therefore, no phagocytic measurement was made. At the lower dose level of 0.01 mg/ml, a significant ($p<0.05$) decrease in phagocytosis was present. No decrease in viability was noted.

The coal fly ash studies with bovine PAM show a dose-response relationship for the phagocytic function at levels ranging from 0.03 mg/ml to 1 mg/ml (Table VI-4). The phagocytic index (PI) were significantly ($p<0.05$)

Table VI-4

Particles PAM Assay Data: 21 Hour Incubation Time

Sample Expt. Date	Conc. ml/ml	Phagocytic Index[b] n	$\bar{X}$(%) SD	% Control	Viability[c] %	Adherence[d] %
Glass beads (10-21-80)	Control	4	69.1 ±11.7	100.0	97.0	98.8
	0.003	3	65.2 ± 5.5	94.3	97.2	98.9
	0.01	4	65.2 ± 5.1	94.3	88.5	98.8
	0.03	4	66.4 ± 6.2	96.0	98.8	98.2
	0.10	3	61.2 ± 6.9	88.5	98.2	97.5
Silica[g] (8-12-80)	Control	4	71.4 ± 4.1	100.0	95.2	97.5
	0.01	4	47.4[a] ± 2.4	66.4	94.0	95.5
	0.03[e]	4	-	-	81.0	94.2
	0.1[e]	4	-	-	88.2	94.0
	0.3[e]	4	-	-	45.0	92.4
Cut 4 Fly Ash[f] (9-25-79)	Control	4	40.8 ± 3.2	100.0	99.0	98.7
	0.03	4	28.5 ± 9.2	69.9	97.7	97.5
	0.1	4	23.5[a] ± 7.1	57.6	89.5	95.9
	0.3	4	9.1[a] ± 3.9	22.3	85.7	86.7
	1.0	4	-	-	62.5	69.0

[a] Significantly ($p \leq 0.05$) different from control

[b] $PI = \frac{\text{Number of cells containing test spheres}}{\text{200 cells counted}} \times 100$

[c] n = 2, viability = $\frac{\text{Number of cells alive}}{\text{200 cells counted}} \times 100$, based on trypan blue exclusion test

[d] n = 4, adherence = $\frac{2 \times 10^5 \text{ PAM seeded} - \text{nonattaching cells} - \text{nonadhering cells}}{2 \times 10^5 \text{ PAM seeded}} \times 100$

[e] This dose lysed PAM. There was sparse coverage at the time of viability measurement.

[f] 24 hour incubation time, 2.2 µm ± 1.8 µm volume median diameter (McFarland, et al., 1977)

[g] 216 Silica-MIN-U-SIL (Whitaker, Clark and Daniels, Inc.) 98% (by weight) less than 5 µm.

decreased at all levels. The decrease in the phagocytic index at the very high levels of 0.3 mg/ml and 1 mg/ml may be due to some extent to the particle effect that can be observed with a "non-toxic" particle, i.e., glassbeads. Trends of decreasing viability and adherence measurements with increasing dose levels of coal fly ash were also evident. In general, it can be seen that coal fly ash and silica particles exert markedly different cytotoxic effects on PAM at comparable dose levels. There is also a strong temporal effect present in the coal fly ash study.

The rice straw smoke particle samples were tested in a fashion similar to the coal fly ash studies. In general, the phagocytic index was the function of choice in evaluating the relative toxicities of the various rice straw samples. PAM viability and adherence measurement did not generally vary from control values. A few exceptions occurred occasionally at the highest dose levels.

Tables VI-3 to VI-8 give the mean phagocytic indices, viabilities and adherence measurements of all the studies on rice straw smoke particle samples, experimental control parameters, and coal fly ash. Phagocytic indices (PI) (% of 200 PAM counted that have ingested carbonized latex microspheres) within each sample study are examined using a student's t-test. Less emphasis has been made in presenting information on the effects of the samples on viability and adherence measurements since they do not appear to be as sensitive as phagocytic indices to the toxic effects of rice straw smoke particles on PAM function.

The low moisture straw controlled burn samples, JY11, JY13, JY15 all showed significant ($p<0.05$) tendency to reduce phagocytosis at the 0.01 mg/ml and 0.03 mg/ml levels (Table VI-6). In addition, for sample JY15 the lowest level, 0.003 mg/ml, was also significantly ($p<0.05$) active. The

Table VI-5

Field Burn PAM Assay Data: 21 Hours Incubation Time

Sample Sample Date Expt. Date	Conc. mg/ml	Phagocytic Index[b] n	$\bar{X}$(%) SD	% Control	Viability[c] %	Adherence[d] %
Sac. Co. (11-9-79) (11-13-80)	Control	4	78.5 ± 6.8	100.0	95.0	94.8
	0.003	4	76.5 ± 4.3	97.5	93.2	92.8
	0.01	4	74.6 ± 7.4	95.0	99.8	95.8
	0.03	4	60.1[a] ± 2.9	76.6	96.8	94.8
	blank	4	86.5 ± 1.7	110.2	94.0	93.6
Sac. Co. (11-21-79) (11-6-80)	Control	4	80.0 ± 3.4	100.0	97.2	96.4
	0.003	4	75.4 ± 4.1	94.2	96.2	95.6
	0.01	3	73.2 ± 5.2	91.5	95.5	93.8
	0.03	4	60.4[a] ± 5.5	75.5	97.5	94.8
	blank	4	85.4[a] ± 2.4	106.8	98.0	95.2
Sac. Co. (11-21-79) (11-20-80)	Control	4	35.4 ± 4.6	100.0	97.5	97.6
	0.03	4	12.1[a] ± 5.3	34.2	98.5	96.8
	0.1	4	4.2[a] ± 1.3	11.9	87.2	97.3
	0.3[e]	4	-	-	14.8	94.9
	blank	2	33.0 ± 2.8	93.2	98.0	97.3
Yolo Co. (11-8-79) (10-1-80)	Control	4	50.2 ± 8.6	100.0	98.5	94.3
	0.003	3	55.5 ±11.1	110.6	95.0	88.9
	0.01	4	43.4 ± 5.5	86.5	97.5	94.1
	0.03	4	35.1[a] ± 5.4	69.9	91.0	95.1
	blank	4	58.3 ± 8.3	116.1	94.8	96.1
Yolo Co. (11-8-79) (11-20-80)	Control	4	35.4 ± 4.6	100.0	97.5	97.6
	upwind	4	35.1 ± 3.7	99.3	96.2	94.9

[a] significantly ($p<0.05$) different from control

[b] $PI = \frac{\text{Number of cells containing test spheres}}{\text{200 cells counted}} \times 100$

[c] n = 2, viability = $\frac{\text{Number of cells alive}}{\text{200 cells counted}} \times 100$, based on trypan blue exclusion test

[d] n = 4, adherence = $\frac{2 \times 10^5 \text{ PAM seeded} - \text{nonattaching cells} - \text{nonadhering cells}}{2 \times 10^5 \text{ PAM seeded}} \times 100$

[e] This dose lysed the PAM

Table VI-6

Low-moisture Straw Controlled Burn PAM Assay Data: 21 Hour Incubation Time

Sample Sample Date Expt. Date	Conc. mg/ml	Phagocytic Index[b] n	$\bar{X}$(%) ± SD	% Control	Viability[c] %	Adherence[d] %
JY11	Control	4	43.6 ± 5.0	100.0	97.8	91.1
(7-23-80)	0.003	4	41.8 ± 5.9	95.9	94.8	89.2
(10-16-80)	0.01	4	34.8[a] ± 5.0	79.8	94.0	89.7
	0.03	3	16.8[a] ± 2.5	38.5	92.5	93.0
	upwind	4	53.6[a] ± 2.6	122.9	96.2	93.8
JY13	Control	4	83.1 ± 8.6	100.0	92.2	99.0
(7-23-80)	0.003	4	80.8 ± 5.6	97.2	96.2	97.7
(10-30-80)	0.01	4	65.8[a] ± 6.4	79.2	93.2	96.4
	0.03	4	35.1[a] ± 4.4	42.2	77.2	95.4
	upwind	4	86.2 ± 3.1	103.7	97.8	96.4
JY15	Control	4	67.0 ± 8.6	100.0	95.2	97.0
(7-23-80)	0.003	4	48.1[a] ± 4.4	71.8	94.0	96.7
(10-30-80	0.01	4	41.4[a] ± 2.9	61.3	94.8	97.5
	0.03	4	13.8[a] ± 1.0	20.6	81.2	96.5
	upwind	4	74.0 ± 4.8	110.4	90.2	96.0

[a] Significantly ($p \leq 0.05$) different from control

[b] $PI = \frac{\text{Number of cells containing test spheres}}{\text{200 cells counted}} \times 100$

[c] $n = 2$, viability $= \frac{\text{Number of cells alive}}{\text{200 cells counted}} \times 100$, based on trypan blue exclusion test

[d] $n = 4$, adherence $= \frac{2 \times 10^5 \text{ PAM seeded} - \text{nonattaching cells} - \text{nonadhering cells}}{2 \times 10^5 \text{ PAM seeded}} \times 100$

Table VI-7

High-moisture Straw Controlled Burn PAM Assay Data: 21 Hour Incubation Time

Sample Sample Date Expt. Date	Conc. mg/ml	Phagocytic Index[b] n	$\bar{X}$(%)	SD	% Control	Viability[c] %	Adherence[d] %
JY17	Control	4	69.2	7.3	100.0	93.8	94.1
(7-24-80)	0.003	4	69.2	12.0	101.0	96.2	93.6
(10-9-80	0.01	4	54.6[a]	4.9	78.9	93.0	95.1
	0.03	4	37.8[a]	3.6	54.6	94.0	92.8
	upwind	4	76.6	9.1	110.7	97.0	94.6
JY19	Control	4	73.9	15.11	100.0	94.2	96.4
(7-24-80)	0.003	3	61.0[a]	5.2	82.5	97.0	96.9
(10-9-80)	0.01	4	64.6	6.1	87.4	92.8	95.7
	0.03	4	44.4[a]	9.5	60.1	89.2	96.2
	upwind	4	76.5	3.2	103.5	90.2	95.1
JY20[e]	Control	4	69.9	3.8	100.0	96.2	93.5
(7-24-80)	0.003	4	66.5	2.2	95.1	95.8	93.2
(10-9-80)	0.01	4	56.0[a]	5.4	80.1	99.0	92.5
	0.03	4	13.1[a]	3.8	18.7	95.8	93.2
	upwind	4	69.1	2.9	98.9	97.8	95.7

[a] Significantly ($p < 0.05$) different from control

[b] $PI = \frac{\text{Number of cells containing test spheres}}{\text{200 cells counted}} \times 100$

[c] $n = 2$; viability $= \frac{\text{Number of cells alive}}{\text{200 cells counted}} \times 100$, based on trypan blue exclusion test

[d] n = adherence

$$= \frac{2 \times 10^5 \text{ PAM seeded} - \text{nonattaching cells} - \text{nonadhering cells}}{2 \times 10^5 \text{ PAM seeded}} \times 100$$

[e] Doses have 4 times the amount of PBS extracted material utilized in experiments with samples JY17 and JY 19.

Table VI-8

Aircraft Plume Sample and CEC Incinerator Burn PAM Assay Data: 21 Hour Incubation

Sample Sample Date Expt. Date	Conc. mg/ml	n	Phagocytic Index[b] $\bar{X}$(%) SD	% Control[g]	Viability[c] %	Adherence[d] %
Aircraft	Control	NA[h]	NA	NA	NA	NA
(1979)	0.003	4	31.5 ± 6.0	99.6	86.0	92.9
(1-12-81)	0.01	4	25.0 ± 4.1	79.1	91.2	93.1
	0.03	4	10.9[a] ± 2.0	34.5	90.0	92.0
	0.1[e]	4	-	-	99.0	96.3
	blank	4	31.6 ±10.3	100.0	84.5	94.7
Aircraft	Control	4	69.2 ± 4.7	100.0	97.2	96.5
(1979)	0.003	4	63.4 ±10.8	91.6	94.5	96.7
(9-25-80)	0.01	4	53.0 ± 6.1	76.6	97.2	95.5
	0.03	4	21.6[a] ± 2.3	31.2	98.2	97.5
	blank	4	74.8 ± 3.3	108.1	96.2	96.7
CEC incinerator						
(2-28-80)	Control	NA	NA	NA	NA	NA
(1-8-80)	0.003	3	39.5 ± 7.1	NA	87.8	95.3
	0.01	2	41.0 ± 7.8	NA	88.8	97.4
	0.03	4	42.2 ± 3.9	NA	86.2	96.0
	0.1[f]	4	-	-	80	98.5
	blank[f]	4	-	-	74.8	98.2
	upwind[f]	4	-	-	-	94.2

[a] Significantly ($p \leq 0.05$) different from control

[b] $PI = \frac{\text{Number of cells containing test spheres}}{\text{200 cells counted}} \times 100$

[c] $n = 2$, viability = $\frac{\text{Number of cells alive}}{\text{200 cells counted}} \times 100$, based on trypan blue exclusion test

[d] $n = 4$, adherence = $\frac{2 \times 10^5 \text{ PAM seeded} - \text{nonattaching cells} - \text{nonadhering cells}}{2 \times 10^5 \text{ PAM seeded}} \times 100$

[e] This dose lysed the PAM

[f] Cell lysis due to excess (>3%) ethanol

[g] Percent of control or blank

[h] NA - Not available, % of control value actually % of blank

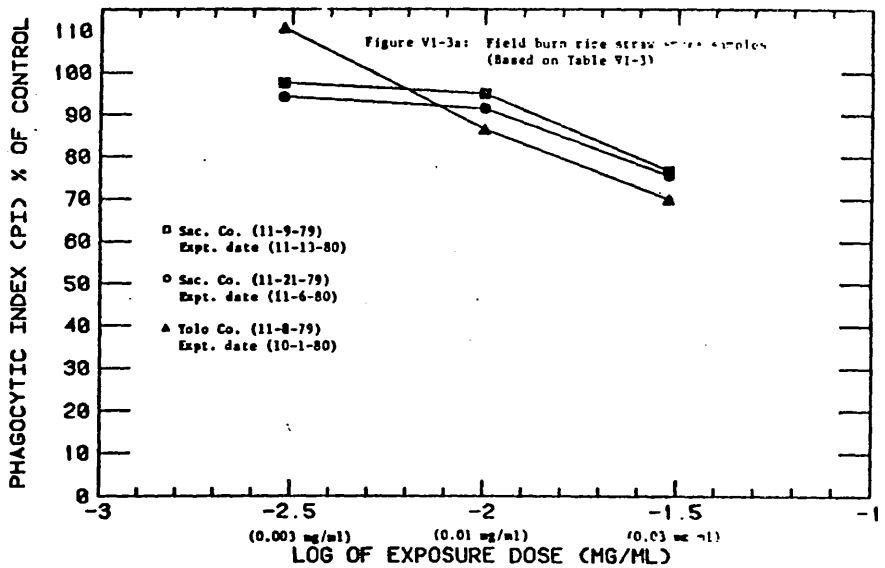
relative lack of toxicity in an upwind control for JY11 is evident from a significantly ($p<0.05$) increased PI. In general, there was no effect on adherence. A decrease in viability was observed at the 0.03 mg/ml for two samples, JY13 and JY15. The high moisture straw controlled burn samples JY17 and JY19 caused significant ($p<0.05$) decreases in PI at the 0.01 mg/ml and 0.03 mg/ml levels (Table VI-7). High moisture straw controlled burn sample JY19 caused significant ($p<0.05$) decrease in PI at 0.003 mg/ml and 0.03 mg/ml, but not at the 0.01 mg/ml level. The samples from the field burns, Sac. Co. (11/9/79), Sac. Co. (11/21/79) and Yolo Co. (11/8/79) caused significant decreases in PI at the 0.03 mg/ml levels (Table VI-5). Additional dose levels of 0.1 mg/ml and 0.3 mg/ml of sample Sac. Co. (11/21/79) were tested. At 0.1 mg/ml level, a significant ($p<0.05$) decrease in PI was observed, while at 0.3 mg/ml level, an extensive lytic effect on PAM was evident. This lytic action was reflected in the very low viability measurement for this exposure level.

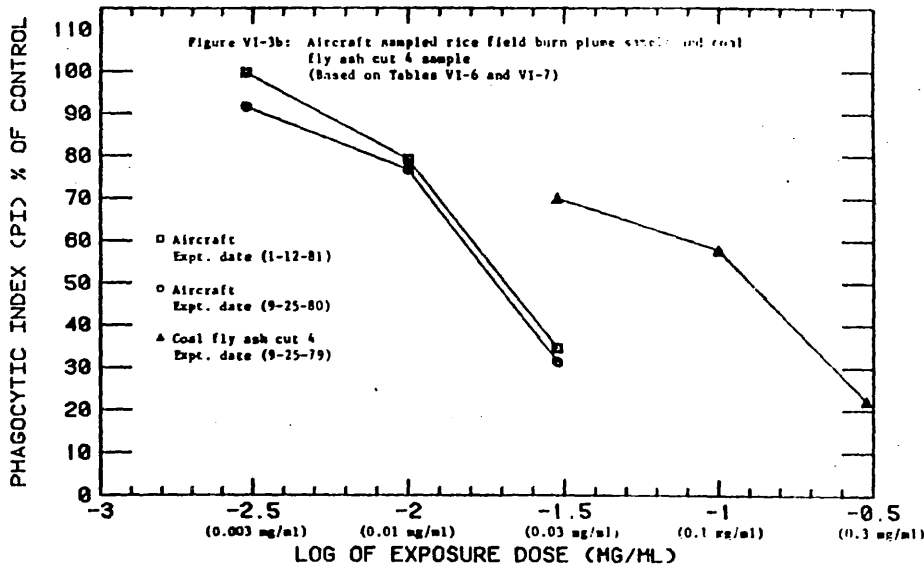
D. Discussion

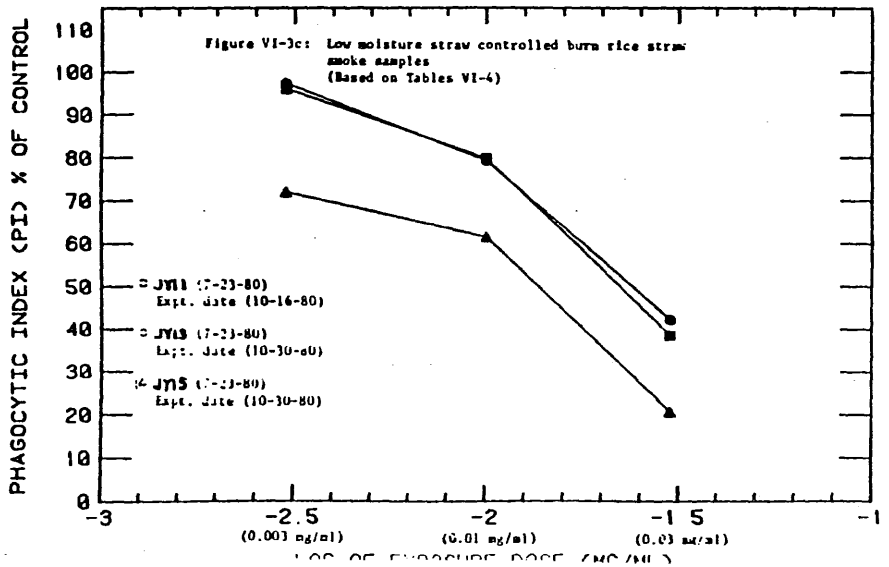
There has been frequent utilization of PAM functional assays for study of inhalable hazardous materials (Aranyi, et al, 1979; McLemore, et al, 1979; Waters, et al, 1975), however much of the emphasis was on PAM viability and/or physiological measurements such as oxygen consumption and lysozyme and enzyme releases. The PAM functional assay utilized for this study on rice straw smoke particles allowed for possible simultaneous evaluation of phagocytic ability, attachment, adherence, and viability. Results of the studies on the effect of rice straw smoke particles on bovine PAM showed that the phagocytic ability was the most sensitive measurement of toxic action.

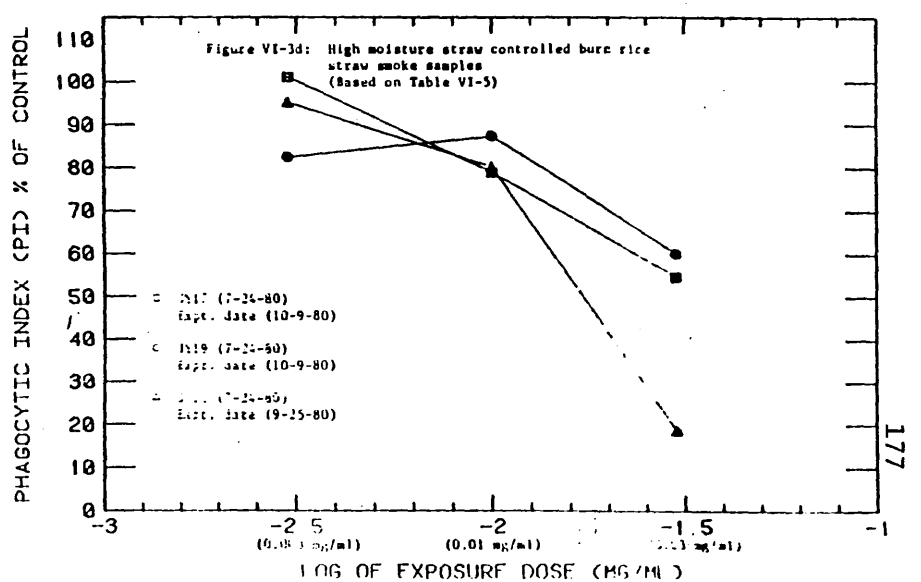
Tables VI-5 through VI-7 amd Figure VI-3 summarize the measured effects of all the rice straw smoke samples on PAM function. All three types of rice straw burn samples, i.e., field, low moisture straw, and high moisture straw had significant ($p<0.05$) toxic effects on PAM phagocytic ability at the 0.03 mg/ml dose level. However, the samples from controlled burns of low and high moisture straw were in addition significantly ($p<0.05$) toxic at the lower 0.01 mg/ml dose. This difference in relative toxicity of the controlled and field burn samples may be due in part to the possibly more homogeneous nature of the controlled burn samples. Possibly, the controlled burn samples have a more uniform particle size. A decrease in PAM viability can be seen at the 0.03 mg/ml dose level of the low moisture controlled burn samples JY13 and JY15. Decreases in viability are not seen at this dose level for the following samples: 1) high moisture straw controlled burns, 2) field burn, and 3) aircraft plume samples. The viability measurement at the range of dose levels

Figure VI-3 PAM Functional Assay Rice Straw Smoke Samples and Coal Fly Ash Cut 4 Sample Phagocytic Index as % of Control Vs. Log of the Exposure Dose (Each point is a average of 3 or 4 replicates).









used in this study was not as sensitive an indicator of cytotoxicity as the phagocytic index. However, dose levels in the 0.1 - 0.3 mg/ml range do have an increased lytic effect and a large decrease in viability was observed for field burn sample, Sac. Co. (11-21-79) experiment date 11-20-80 (Table VI-5).

One aspect of the data that needs to be addressed is the occurrence of a significant ($p<0.05$) increase in phagocytic index for some blank controls (Tables VI-5 and VI-6). This increase in phagocytic index may be due in part to a slightly longer time of incubation with the carbonized latex microspheres. The blank control usually is the last group to be fed microspheres and the last group to be terminated. Therefore when time required to terminate the other exposure groups becomes extended, the blank group is incubated with microspheres for a longer time than the other groups. Also, this blank group is utilized as a control for toxic effects of ethanol and PBS additions in the media and the increase in PI would not reflect a toxic action.

An explanation of the control groups and the results of experiments concerned with their control groups would be appropriate to this discussion. The group labeled control is only incubated with complete media. The blank control group is incubated with ethanol and PBS at levels comparable to those in the highest sample dose level groups (usually the 0.03 mg/ml group). Maximum levels of ethanol and PBS that could be added to the assay were determined by dose-response experiments. Studies have shown that ethanol has rapid onset, transient loss of adherence and decreased phagocytosis (Rimland and Hand, 1980). It was shown that these measured functions approach control values after 180 minutes of exposure at levels of 0.125%

to 1.25% ethanol. Upon evaluation of the data (Table VI-3), it was seen that PBS up to a 40% by volume level had no significant effect on macrophage function at a 21 hour incubation period. PAM incubated with up to 30 µl/ml, or 3%, ethanol had no decrease in function. At the 3% level there were indications of cytotoxic effects as reflected in the viability measurement. A 10% dose level caused a significant ($p<0.05$) decrease in phagocytic index and viability measurements. Therefore, a maximum level of 3% ethanol and 20% PBS was suggested.

The upwind control samples were available for study in the low and high moisture straw controlled burn samples and in the field burn sample Yolo Co. (11-8-79). While upwind samples were taken of the Sac. Co. (11-9-79) and Sac. Co. (11-21-79) burns, the amount of sample collected and the observable smoky environment around the air samplers indicated that these upwind samples were not appropriate "ambient" controls for the PAM assay. The dose level of upwind control samples was determined by calculating the amount of material found in the volume of air equal to the volume of air associated with the highest dose level of the downwind sample. That is, the 0.03 mg/ml level of sample represents a certain volume of air sampled. The amount of material in an equivalent volume of air of the upwind sample was the dose level utilized in the PAM assay. The ethanol and PBS levels were adjusted to be equivalent to those in the downwind samples. In most cases it is seen that the upwind samples had no statistically significant ($p<0.05$) toxic effect on PAM function.

Experimental results clearly indicate that the rice straw smoke samples from the field burns, controlled burns, and aircraft collected plumes have toxic effects on bovine PAM function, in particular phago-

cytic ability. In addition, although the one PAM assay performed on the CEC incinerator baghouse exhaust sample generated unusable data, it is reasonable to assume that this type of rice straw smoke sample would have similar toxic effects on PAM function. The different types of sample were obtained by varying the rice straw combustion conditions. Differences in combustion parameters may effect the degree of response of the samples in the PAM assay, but should not appreciably change the type of response. Mutagenic activity of the samples, rather the cytotoxic activity, would probably be more sensitive to such variations. While clarification of the mechanism of toxic activity of the rice straw smoke particles on PAM function is beyond the scope of this study, it is evident from test results that rice straw smoke particles may contain components that possess toxicity to PAM intermediate to that of silica and coal fly ash tested at LEHR.

The results of the tests performed at LEHR on silica and coal fly ash are shown in Table VI-4. Silica (MIN-U-SIL®, 5 μm, Whitaker, Clark and Daniels, Inc.) had a highly lytic effect on PAM function at 0.03 mg/ml - 0.3 mg/ml dose levels, while a notable difference in the toxic action of coal fly ash (2.2 μm ± 1.8 μm volume median diameter, McFarland, et al., 1977) is that it depressed phagocytosis in PAM but did not lyse the cells except at very high dose levels (1 mg/ml). The rice smoke sample Sac. Co. (11-21-79) did not cause cell lysis until it reached an intermediate dose level of 0.3 mg/ml (Table VI-5). In addition, as Figure VI-3b shows, coal fly ash, cut 4, had a relatively linear dose effect on PI at the exposure range studied (0.3 - 0.3 mg/ml). PI values could not be obtained for silica because it had a highly lytic action at the levels studied. In general, the rice straw samples did not have a strong dose effect on PI at the levels studied (0.003 - 0.03 mg/ml).

Relating experimental PAM functional assay data to potential human health impact of rice straw burning in the field is difficult and probably cannot be realistically made with the minimal information available. However, the calculation below shows that an individual would need to stand downwind and adjacent to a burning field of rice straw for a minimum of 169 days to receive an exposure of rice straw smoke particles equivalent to the 0.03 mg/ml dose that was found to have significant acute toxic activity in bovine PAM *in vitro*. This calculation is as follows:

$$\frac{(30) \times (6 \times 10^6) \times (24)}{(2 \times 10^5) \times (0.615) \times 8.64} = 4065 \text{ hours} = 169 \text{ days}$$

where: acute toxic dose of rice straw smoke particles in bovine PAM assay $= \frac{0.03 \text{ mg}}{2 \times 10^5 \text{ PAM}} = \frac{30 \text{ ug}}{2 \times 10^5 \text{ PAM}}$

Average of field burn rice straw smoke particle concentration $= \frac{0.615 \text{ ug}}{\text{m}^3}$

Minimum number of PAM lavaged from human lung lobe (Territo and Golde, 1979) $= 6 \times 10^6$ PAM

Volume of air inhaled by a normal person $= \frac{8.64 \text{ m}^3}{24 \text{ hr}}$

Certainly, this calculation does not consider many real life influences such as species differences, particle impaction in the respiratory tract, chemical changes of the particles in the environment, or toxic effects of other components of rice straw smoke (for example, CO). However, a very rough estimate of the exposure to rice straw smoke particles necessary to elicit an acute toxic response (reduction in phagocytic ability) in pulmonary alveolar macrophage is provided.

E. Conclusion

The use of a short-term *in vitro* PAM functional assay has contributed to our attempts to evaluate the potential health implications of rice straw burning. This assay, suitable for studying inhalable particles, has been used to screen the toxicity of the particulate matter in rice straw smoke and it has allowed us to estimate the relative toxicity of rice straw smoke particles in relation to two extensively studied environmentally important particles, silica and coal fly ash. The relative toxicity of the three is in the decreasing order: silica>rice straw smoke>coal fly ash. This order of relative toxicity was based on their effects on phagocytic index and a qualitative comparison of their effect on cell lysis in the bovine PAM functional assay. In relating this bovine PAM functional data with potential human health impact, it was calculated that a minimum of 169 days of exposure to concentrated rice straw smoke particles in the field would be equivalent to the dose level of rice straw smoke particles found to have significant acute toxicity on bovine PAM function *in vitro*.

The scope of this study has been limited by time and material. These constraints did not allow for optimization of sampling conditions for the rice straw smoke particles, and as a result, the PAM assay was not performed under optimum conditions. Simplification of sample preparation and fewer experimental parameters to control for in the PAM assay would be desirable.

Some suggestions for future studies of rice straw smoke particles, or any other particles from combustion, would include (1) more dose-response and temporal studies, (2) study of the effects of these particles on the PAM of other animal species (e.g., mouse, rat, rabbit, dog), (3) identification

and isolation of the toxic components of the rice straw smoke sample, and testing them in the PAM assay, and (4) spiking the rice straw smoke sample with known cytotoxic agents such as polyaromatic hydrocarbons.

In conclusion, the PAM assay is a useful short-term *in vitro* biological test that allows one to evaluate the potential importance of some environmental insults, such as inhalable particles from combustion sources (e.g., agricultural waste removal and energy production), to human health. Specifically, this concept was applied to the rice straw smoke samples collected in this study and the experimental results generated by this research group to assign a relative toxicity to rice straw smoke particles.

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VII. CONCLUSIONS

The potential health hazards associated with particulate matter released from rice straw burning were assessed by two short-term *in vitro* bioassays: the Ames *Salmonella*/mammalian-microsome mutagenicity test (Ames test) and the pulmonary alveolar macrophage (PAM) cytotoxicity test. Rice straw smoke samples were also chemically analyzed for elemental composition, polyaromatic hydrocarbons, and other organic components. In addition, pesticide residues in unburned rice straw were analyzed to assess their potential presence in the smoke.

Chemical Analysis

An analytical method for rice straw smoke particulate matter extract (PME) was developed involving extraction by sonification, liquid chromatographic fraction by gel permeation chromatography (GPC) and computerized gas chromatography/mass spectroscopy (GC/MS). GPC of PME yielded six fractions of which two (fractions 5 and 6) had the largest quantity of mutagenic materials. Fluorescence spectra of fractions 5 and 6 indicated these fractions were a complex mixture of highly fluorescent compounds typical of polyaromatic hydrocarbons (PAHs). About 10 to 15 percent of observed GC peaks in fraction 5 were tentatively identified by GC/MS. Compounds identified included mutagenic and nonmutagenic PAHs and heterocyclic compounds.

Elemental analyses showed that the material trapped on the air filters consisted almost entirely of plant-related materials as indicated by the potassium to calcium ratio. This was interpreted as evidence that there was little or no contamination of smoke samples with soil particulate matter.

Pesticide Analysis

Analysis of the unburned straw showed that the residues of two chemicals applied to the rice early in the season (before July 1), were below the analytical detection limit (0.04 ppm for MCPA herbicide and 0.04 ppm for molinate (Ordram) herbicide) at the time of burning. 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.

Mutagenicity Studies

Particulate matter extracts of rice straw smoke samples were mutagenic in Salmonella typhimurium strains TA98, TA100, TA1537, and TA 1538, with no or without metabolic activation. Mutagenic activity was associated with particulate matter. Determination of the presence or absence of vapor-phase mutagens in the smoke was not made. Comparison of rice straw smoke with two other mutagenic aerosols (cigarette smoke and particulate matter collected in downtown Los Angeles) revealed rice straw smoke was not an unusually potent mutagen.

Pulmonary Alveolar Macrophage (PAM) Testing

A bovine PAM *in vitro* test developed on this campus allowed simultaneous measurement of the effect on PAM phagocytic ability, attachment, adherence, and viability. The PAM were exposed to rice straw smoke particle samples in the size range (<3.8μ aerodynamic diameter). A dose level of 30 μgm/ml culture media, produced significant toxic effects on the phagocytic ability of PAM, but effects on adherence and viability were not evident. Comparison of rice straw smoke particles with coal fly ash and silica of similar aerodynamic size showed rice straw smoke particles to be intermediate in toxicity to coal fly ash and silica.

Recommendations for Future Work

Based on our findings in the Ames test, PAM cytotoxicity assay and chemical analyses rice straw smoke warrants further investigation. Several areas of uncertainty exist in regard to exposure to rice straw smoke. The actual doses of smoke populations are exposed to needs to be quantitated. In addition, our study concentrated on characterization of the smoke as it is released from the source, however, populations are exposed to smoke which has been transported over some distance. Atmospheric reactions during transport may change the composition of the smoke and should be investigated. Finally, *in vivo* testing (in sensitive human populations, if possible) would be useful to determine potential accute responses of human populations.

APPENDIX I

Data collected by ARB source Van during test burns at UCR.

U.C.R Rice Straw emissions
tested 7-22 & 23, 1980

Project No. C80-040

TABLE

Summary of Results

Pollutant \ Test No.	1	2	3	Average*
SO_2 (lbs/ton)	67.7	22.1	31.2	26.7
HC (lbs/ton)	53.8	18.4	23.9	21.2
NO_X (lbs/ton)	12.6	3.6	3.9	3.8
CO (lbs/ton)	25.9	97.6	88.9	93.3
Particulate Matter (lbs/ton)	29.7	13.3	14.7	14.0

*Results from Test #1 were not used to determine average emissions.

(lbs/ton) is the pounds of pollutant per ton of dry rice straw

ate	Time	Bail wt.	Ash	Flow scfm	SO_2 ppm	SO_2 lb/ton	HC ppm	HC lb/ton	NO_X ppm	NO_X lb/ton	CO ppm	CO lb/ton
22	16:40			3740	-		74		38		450	
	16:45 16:50			3190	-		56		4		650	
		20	14			-		59.4		13.3		269
22	17:17			3600	-		50		30		400	
	17:27			2580	-		70		5		610	
		20	14			-		45.4		10.4		214
22	17:40			3420	81.2		63.8		30		528	
	17:45 17:50			2970	71.6		62.2		Ø		596	
		20	14			62		51.4		8.9		257
22	18:07			4440	70.5		54.2		43		450	
	18:12 18:17			3410	78.7		65		5		630	
		20	14			73.4		59.0		17.8		297
23	9:20			3300	70.7		54.6		29.5		590	
	9:25 9:30			2550	29		24		4		557	
		50	34			15.8		12.6		3.6		96
23	10:10			2970	64.3		50.9		26.5		492	
	10:15 10:20			3230	57.1		48.6		6.1		532	
	10:20 10:24			1550	17.3		17.8		3		327	
		49	34¼			20.2		17.0		3.4		10?

	Time	Bail wt.	Ash	Flow scfm	SO_2 ppm	SO_2 lb/ton	HC ppm	HC lb/ton	NO_X ppm	NO_X lb/ton	CO ppm	CO lb/to
23	10:45			2980	92.4		77.1		30.7		627	
	10:50			2380	81.2		66.5		7.3		609	
	10:55 11:00			1910	24		14		Ø		250	
		50	36½			25.6		21.0		3.6		107
23	12:09			2470	92.4		78.3		30.9		536	
	12:14			2400	92.9		78.4		10.0		465	
	12:19 12:24			1650	48		40.9		6.8		266	
		50	34½			29.9		23.1		3.8		84.3
23	14:42			2380	107		79		37		575	
	14:47			1950	66		55		6.5		400	
	14:56			1850	27		23		3.9		256	
		49½	34½			21.7		17.0		3.6		73.2
23	15:21			2750	132		98		34		594	
	15:26			3220	140		105		11		540	
	15:31 15:36			3090	49		36		4.5		300	
		50¼	35¼			47.4		35.8		4.7		121
23	16:05			2900	138		100		36		600	
	16:10			2400	94		71		8.0		434	
	16:14 16:18			2350	31		26		4.9		282	
		50	35			32.8		24.5		4.3		85

				SO_2		HC		NO_X		CO	
e Time	Bail wt.	Ash	Flow scfm	ppm	lb/ton	ppm	lb/ton	ppm	lb/ton	ppm	lb/to
16:57			2650	111		86		28		610	
17:02			1880	91		73		7.0		560	
17:07 17:08			1440	33		25		3.8		330	
	50	35			23.0		18.2		2.80		76.3

File No. C80-040

State of California
AIR RESOURCES BOARD
Stationary Source Control Division
Engineering Evaluation Branch

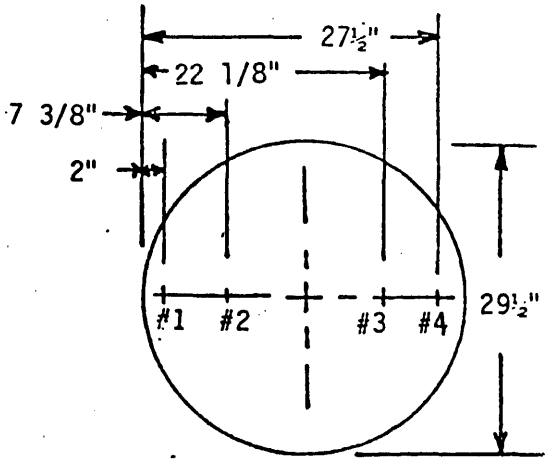
VELOCITY TRAVERSE DATA

Sampling Point	#1		#2		#3		#4	
Time (min.)	$\sqrt{\Delta\rho}$	Ts	$\sqrt{\Delta\rho}$	Ts	$\sqrt{\Delta\rho}$	Ts	$\sqrt{\Delta\rho}$	Ts
0-1	0.176	163	0.254	349	0.219	298	0.203	284
1-2	0.189	185	0.320	370	0.295	326	0.240	370
2-3	0.199	168	0.336	332	0.323	360	0.217	351
3-4	0.205	142	0.309	285	0.316	265	0.179	278
4-5	0.169	129	0.234	191	0.276	203	0.172	194
5-6	0.161	126	0.208	166	0.248	171	0.161	177
6-7	0.147	120	0.190	158	0.241	157	0.138	163
7-8	0.147	119	0.190	148	0.235	147	0.138	151
8-9	0.147	116	0.190	146	0.216	143	0.138	143
9-10	0.147	114	0.182	143	0.212	139	0.138	139
10-11	0.147	113	0.172	138	0.212	138	0.137	140
11-12	0.147	111	0.172	134	0.212	137	0.121	136
Average	0.165	134	0.230	213	0.250	203	0.165	210

Project: Emissions from burning rice straw.

Location: University of California at Riverside.

Remarks: Velocity head, $\Delta\rho$, and stack temperature, Ts, are averages from three EPA Method 5 tests. Velocity head is in inches of water, stack temperature is degrees fahrenheit.



PARTICULATE EMISSIONS TEST SUMMARY AND RESULTS

ARL-SSCL-Engr. Eval. Branch

File No. C-80-040

Project Name: _____

Remarks: test #1

SUMMARY OF TEST DATA

Metered Sample Volume	25.29 cubic feet
Meter Temperature:	60 deg.F
Nozzle Diameter:	0.375 inches
Pitot Tube C-Factor:	0.830
Sampling Time:	48.80 minutes
Avg. delta H Orifice Pressure:	0.70 inches H2O
Avg. √(delta P Pitot Pressure):	0.24 √(inches H2O)
H2O in Impingers and Silica Gel:	10.5 milliliters
Particulate Catch:	78.4 milligrams
Stack Diameter:	29.500 inches
Stack Area:	4.746 square feet
Stack Temperature:	227 deg.F
Barometric Pressure:	28.740 inches Hg
O2 In Stack:	20.30 percent
CO2 In Stack:	0.70 percent
CO In Stack:	0.00 percent

CALCULATED RESULTS:

Isokinetic Ratio:	98.7 percent
Corrected Sample Volume:	24.72 DSCF(68 deg.F)
Particulate Concentration:	0.04695 grain/DSCF(68 deg.F)
Particulate Emissions:	1.33 lb/hr
Stack Flow:	3176 SCFM(dry,68 deg.F)
Stack Velocity:	15.4 feet/second
H2O Vapor In Stack:	2.0 percent
(H2O In Stack is BELOW Saturation)	
Stack Gas Mole Weight(dry):	28.94
Stack Gas Mole Weight(wet):	28.73

Verified by: _____

PARTICULATE EMISSIONS TEST SUMMARY AND RESULTS

ARB-SSCD-Engr. Eval. Branch

File No. C-80-040

Project Name: _____

Remarks: TEST #2

SUMMARY OF TEST DATA

Metered Sample Volume	22.72 cubic feet
Meter Temperature:	60 deg.F
Nozzle Diameter:	0.375 inches
Pitot Tube C-Factor:	0.830
Sampling Time:	55.10 minutes
Avg. delta H Orifice Pressure:	0.60 inches H2O
Avg. √(delta P Pitot Pressure):	0.19 √(inches H2O)
H2O in Impingers and Silica Gel:	6.1 milliliters
Particulate Catch:	86.5 milligrams
Stack Diameter:	29.500 inches
Stack Area:	4.746 square feet
Stack Temperature:	212 deg.F
Barometric Pressure:	28.780 inches Hg
O2 In Stack:	20.30 percent
CO2 In Stack:	0.00 percent
CO In Stack:	0.00 percent

CALCULATED RESULTS:

Isokinetic Ratio:	97.8 percent
Corrected Sample Volume:	22.23 DSCF(68 deg.F)
Particulate Concentration:	0.06004 grain/DSCF(68 deg.F)
Particulate Emissions:	1.31 lb/hr
Stack Flow:	2554 SCFM(dry,68 deg.F)
Stack Velocity:	12.0 feet/second
H2O Vapor In Stack:	1.3 percent
(H2O In Stack is BELOW Saturation)	
Stack Gas Mole Weight(dry):	28.92
Stack Gas Mole Weight(wet):	28.78

Verified by: _____

DRAFT

PARTICULATE EMISSIONS TEST SUMMARY AND RESULTS

ARB-SSCD-Engr. Eval. Branch

File No. C- 80-40

Project Name: _____

Remarks: TEST #3

SUMMARY OF TEST DATA

Metered Sample Volume	19.96 cubic feet
Meter Temperature:	60 deg.F
Nozzle Diameter:	0.375 inches
Pitot Tube C-Factor:	0.830
Sampling Time:	49.30 minutes
Avg. delta H Orifice Pressure:	0.70 inches H2O
Avg. √(delta P Pitot Pressure):	0.21 √(inches H2O)
H2O in Impingers and Silica Gel:	4.7 milliliters
Particulate Catch:	85.8 milligrams
Stack Diameter:	29.500 inches
Stack Area:	4.746 square feet
Stack Temperature:	216 deg.F
Barometric Pressure:	28.720 inches Hg
O2 In Stack:	20.40 percent
CO2 In Stack:	0.60 percent
CO In Stack:	0.00 percent

CALCULATED RESULTS:

Isokinetic Ratio:	87.3 percent
Corrected Sample Volume:	19.49 DSCF(68 deg.F)
Particulate Concentration:	0.06792 grain/DSCF(68 deg.F)
Particulate Emissions:	1.63 lb/hr
Stack Flow:	2804 SCFM(dry,68 deg.F)
Stack Velocity:	13.3 feet/second
H2O Vapor In Stack:	1.1 percent
(H2O In Stack is BELOW Saturation)	
Stack Gas Mole Weight(dry):	28.93
Stack Gas Mole Weight(wet):	28.81

Verified by: _____

DRAFT

[illegible]

(hydrocarbons expressed as : propane)

Oxygen	Carbon Dioxide	Carbon Monoxide	Hydro-carbons	Nitrogen Oxides	Sulfur Dioxide	Temperature
percent	percent	PPMV	PPMV	PPMV	PPMV	Deg. C
from 07/23(0920:43hrs to 07/23(0925:40hrs :						
19.6	1.07	551	54.6	21.5	76.7	0.0
from 07/23(0925:40hrs to 07/23(0930:31hrs :						
20.7	0.301	557	24.6	3.67	29.1	0.0
(pause to span instruments: 07/23(0930:31hrs to 07/23(1010:12hrs)						
from 07/23(1010:12hrs to 07/23(1015:09hrs :						
19.6	0.956	492	50.9	20.5	64.3	0.0
from 07/23(1015:09hrs to 07/23(1020:09hrs :						
20.5	0.357	532	46.6	6.11	57.1	0.0
from 07/23(1020:09hrs to 07/23(1022:39hrs :						
20.7	0.303	327	17.6	2.72	17.3	0.0
(pause to span instruments: 07/23(1022:39hrs to 07/23(1045:43hrs)						
from 07/23(1045:43hrs to 07/23(1050:40hrs :						
19.6	1.16	627	77.1	30.7	92.4	0.0
from 07/23(1050:40hrs to 07/23(1055:40hrs :						
[illegible]	[illegible]	609	[illegible]	7.28	81.2	0.0
from 07/23(1055:40hrs to 07/23(1058:28hrs :						
20.7	0.405	315	17.2	3.75	24.2	0.0
(pause to span instruments: 07/23(1058:28hrs to 07/23(1208:49hrs)						
from 07/23(1208:49hrs to 07/23(1213:46hrs :						
20.0	0.873	536	78.3	30.9	92.4	0.0
from 07/23(1213:46hrs to 07/23(1218:46hrs :						
20.7	0.190	405	78.4	9.67	92.9	0.0
from 07/23(1218:46hrs to 07/23(1223:46hrs :						
20.8	0.123	266	46.9	6.77	46.0	0.0
from 07/23(1223:59hrs to 07/23(1224:05hrs :						
20.8	0.0652	143	19.3	5.96	25.5	0.0
(pause to span instruments: 07/23(1224:05hrs to 07/23(1322:57hrs)						
percent	percent	PPMV	PPMV	PPMV	PPMV	Deg. C

(continued next page)

[illegible]

(percentages expressed as : propane)

Argon	Carbon Dioxide	Carbon Monoxide	Hydrocarbons	Nitrogen Oxides	Sulfur Dioxide	Temperature
percent	percent	PPMV	PPMV	PPMV	PPMV	Deg. C
from 07/22@1707:46hrs to 07/22@1708:06hrs :						
21.0	0.070	55.7	-0.222	-7.41	-0.300	0.00584
(pause to span instruments: 07/22@1708:06hrs to 07/22@1720:41hrs)						
from 07/22@1720:41hrs to 07/22@1725:38hrs :						
19.8	1.20	451	37.3	20.4	22.2	0.0647
from 07/22@1725:38hrs to 07/22@1728:56hrs :						
20.7	0.340	625	46.9	-3.62	48.5	0.0213
(pause to span instruments: 07/22@1728:56hrs to 07/22@1740:24hrs)						
from 07/22@1740:24hrs to 07/22@1745:21hrs :						
19.9	1.78	528	63.8	18.8	81.2	0.0589
from 07/22@1745:21hrs to 07/22@1750:21hrs :						
20.7	1.03	590	62.2	-3.90	71.0	0.0215
from 07/22@1750:21hrs to 07/22@1751:57hrs :						
20.8	1.04	347	19.8	-6.88	24.9	0.0102
(pause to span instruments: 07/22@1751:57hrs to 07/22@1807:29hrs)						
from 07/22@1807:29hrs to 07/22@1812:26hrs :						
19.9	2.11	450	54.2	22.9	70.5	0.0688
from 07/22@1812:26hrs to 07/22@1817:26hrs :						
20.7	1.12	629	78.7	-2.55	88.8	0.0236
from 07/22@1817:26hrs to 07/22@1819:32hrs :						
20.8	1.01	410	21.8	-7.02	23.7	0.0181
(pause to span instruments: 07/22@1819:32hrs to 07/22@1840:02hrs)						
from 07/22@1840:02hrs to 07/22@1840:11hrs :						
21.0	-0.029	-4.38	-0.113	2.66	50.6	-0.000243

WATER VAPOR [illegible] TIME:						
20.3	1.202	510.35	52.740	7.28	99.4	0.0460015
percent	percent	PPMV	PPMV	PPMV	PPMV	Deg. C

AFB/SSCD - DATA ACQUISITION SYSTEM PRINTOUT
UCI Tree Straw Burner
DATA:

(hydrocarbons expressed as : propane)

Oxygen	Carbon Dioxide	Carbon Monoxide	Hydro-carbons	Nitrogen Oxides	Sulfur Dioxide	Temperature
Percent	Percent	ppmv	ppmv	ppmv	ppmv	Deg. C
from 07/23@1700:33hrs to 07/23@1700:30hrs :						
20.6	0.453	291	16.9	3.36	23.6	0.0
AVERAGE VALUES DURING TEST:						
19.6	0.718	495	73.4	17.90	96.6	0.0
Percent	Percent	ppmv	ppmv	ppmv	ppmv	Deg. C

file #0

[illegible] - [illegible]

[illegible]:

(Hydrocarbons corrected as : propane)

Oxygen	Carbon Dioxide	Carbon Monoxide	Hydro-carbons	Nitrogen Oxides	Sulfur Dioxide	Temperature
Percent	Percent	PPMV	PPMV	PPMV	PPMV	Deg. C
from 07/23@1736:54hrs to 07/23@1741:51hrs :						
19.6	1.46	661	75.8	28.4	101	0
from 07/23@1741:51hrs to 07/23@1746:48hrs :						
19.9	0.547	928	66.8	9.17	70.3	0.0
(pause to span instruments: 07/23@1746:48hrs to 07/23@1757:50hrs)						
from 07/23@1757:51hrs to 07/23@1802:48hrs :						
19.2	1.30	534	68.6	24.8	96.5	0.0
from 07/23@1802:48hrs to 07/23@1807:48hrs :						
19.9	0.540	550	67.2	4.91	89.6	0.0
from 07/23@1807:48hrs to 07/23@1812:15hrs :						
20.1	0.429	304	22.7	1.89	31.6	0.0
(pause to span instruments: 07/23@1812:15hrs to 07/23@1827:07hrs)						
from 07/23@1827:07hrs to 07/23@1827:10hrs :						
21.0	-0.0690	-0.497	-2.61	1.81	53.7	0.0
AVERAGE VALUES DURING TEST:						
19.6	0.6789	506.555	59.66	13.22	79.9	0.0
Percent	Percent	PPMV	PPMV	PPMV	PPMV	Deg. C

FIELD DATA

Run No. #1

Location UCR

Date 7/02

Operator

Stack Dia. No.

Meter Box No.

VERY IMPORTANT - FILL IN ALL BLANKS

Read and record at the start of each test point.

Leak Test = Before [15" Hg] After

Pitot Tube Factor 0.830

Ambient Temp °F 103 (1545)

Bar. Press. "Hg 28.74 (1545)

Assumed Moisture % 10

Heater Box Setting, °F

Probe Tip Dia., In. 3/8"

Probe Length

Probe Heater Setting

Point	Clock Time	Dry Gas Meter, CF	Pitot In. H_2O ΔP	Orifice ΔH in H_2O Desired	Orifice ΔH in H_2O Actual	Dry Gas Temp. °F Inlet	Dry Gas Temp. °F Outlet	Pump Vacuum In. Hg Gauge	Impinger Temp °F	Stack Press In. Hg	Stack Temp °F
	0000	139.166									
4	01		.06		.86				70	BK	280
	02	139.253	.07		1.0				70		320
1641											32
#4	0000	139.253	.06		.86				65		320
	01	139.30									L
	02	139.370	.08		1.12				69		350
	04	139.40	.05		.70						340
	05	139.51	.04		.57		FLAME OUT		59		205
	06		.04		.57						
	07	139.60	.03		.43				65		170
	08	139.63	.03		.43				60		160
	09	139.67	.03		.43				62		148
	10	139.70	.03		.43						145
	11	139.73	.03		.43				58		140
	13	139.81	.02		.28				61		133
	14	139.842	.02		.28				61		130
8 1750											
#3	0000	139.842									
	01	139.90	.1		1.4				67		340
	02	139.98	.14		1.85				67		340
	03	140.04	.15		2.1				65		345
	04	140.10	.10		1.4				64		240
			.07		1.0		FLAME OUT		64		187
	06	140.25			.70						158
	07	140.30	.05								

FIELD DATA

Run No. #1

Location

Date 7/02/80

Operator

Sample Box No.

Meter Box No.

VERY IMPORTANT - FILL IN ALL BLANKS

Read and record at the start of each test point.

Leak Test = Before / After

Pitot Tube Factor

Ambient Temp °F 97 (1735)

Bar. Press. "Hg 28.74 (1725)

Assumed Moisture %

Heater Box Setting, °F

Probe Tip Dia., In. 7/16

Probe Length

Probe Heater Setting

Point	Clock Time	Dry Gas Meter, CF	Pitot in. H_2O Δ P	Orifice ΔH in H_2O		Dry Gas Temp. °F		Pump Vacuum In. Hg Gauge	Impinger Temp °F	Stack Press In. Hg	Stack Temp F
				Desired	Actual	Inlet	Outlet				
#3	08	140.33	.05		.70				65		148
	09	140.395	.05		.70				65		145
#2	0000	140.395									
	01	140.44	.10		1.4				68		320
	02	140.52	.13		1.85				64		355
	03	140.58	.12		1.7				66		355
	04	140.67	.12		1.7						312
	05	140.72	.08		.70		FLAME OUT				166
	06	140.77	.05		.70				63		168
	07	140.82	.05		.70				65		156
	08	140.87	.05		.70				65		148
	09	140.90	.05		.70				63		148
	10	140.96	.04		.57				63		143
	11	141.00	.04		.57				63		140
1757	12	141.045	.04		.57				62		139
#1	0000	141.045									
	01	141.10	.06		.87				67		150
	02	141.15	.08		1.1				66		170
	03	141.20	.08		1.1						180
	04	141.27	.09		1.27				68		165
	05	141.32	.05		.70		FLAME out		65		131
	06	141.37	.04		.57				64		134
	07	141.415	.04		.57				65		124
	08	141.455	.04		.57				64		124
	09	141.50	.04		.57				63		123
	10	141.545	.04		.57				63		122
	11	141.59	.04		.57				65		113

ΔP Δ' : Δ' = ΔH
.07 = .26 .04 = 5.7 .08 = 1.13
.03 = .43 .05 = .61 .09 = 1.27
.06 = .85 .10 = 1.4
.07 = 1.0 .12 = 1.7

C80-040

FIELD DATA

2016/Ed Rico Straw ≈ 5% H_2O

84 (0910)

Ambient Temp °F 75 (0800)

28.78 (0910)

Bar. Press."Hg 28.79 (0800)

Run No. 2

Location UCR

Date 7/23/80

Operator

Sample Box No.

Meter Box No.

VERY IMPORTANT - FILL IN ALL BLANKS

Read and record at the start of each test point.

Leak Test = Before 15"Hg / After

Pitot Tube Factor 0.830

Assumed Moisture % 10

Heater Box Setting, °F

Probe Tip Dia., In. 3/8"

Probe Length

Probe Heater Setting

Point	Clock Time	Dry Gas Meter, CF	Pitot in. H_2O ΔP	Orifice ΔH in H_2O Desired	Orifice ΔH in H_2O Actual	Dry Gas Temp. °F Inlet	Dry Gas Temp. °F Outlet	Pump Vacuum In. Hg Gauge	Impinger Temp °F	Stack Press In. Hg	Stack Temp °F
Start → #4	0920 0000	141.668	—								
	01	141.71	.05		.61				54		227
	02	141.76	.07		1.0						355
	03	141.80	.05		.61				55		356
	04	141.84	.03		.43		FLAME Out				274
	05	141.89	.03		.43						187
	06	141.92	.02		.28				60		164
	07	141.95	.02		.28				60		158
	08	141.98	.02		.28				60		147
	09	142.00	.02		.28				58		140
End 0930	10	142.04	.02		.28				59		132
Start #3	0000	142.04	—								—
	01	142.08	.04		.57						210
	02	142.10	.07		1.0				64		272
	03	142.00	.08		1.13				60		296
	04	142.25	.11		1.55				60		325
	05	142.31	.07		1.0				59		214
	06	142.336	.04		.57				59		179
	07	142.140	.04		.57				63		163
	08	142.45	.04		.57		FLAME Out @ 8		63		152
	09	142.49	.02		.28				64		139
	10	142.53	.02		.28				66		133
	11	142.55	.02		.28				67		131
	12	142.58	.02		.28				67		128
End	1230	142.603	.02		.28				67		128

-150 12/73

FIELD DATA

Run No. 2

Location UCR

Date 7/23/80

Operator

Sample Box No.

Meter Box No.

VERY IMPORTANT - FILL IN ALL BLANKS

Read and record at the start of each test point.

Leak Test = Before / After

Pitot Tube Factor

Ambient Temp °F 95 (1025)

Bar. Press. "Hg 28.77 (1025)

Assumed Moisture %

Heater Box Setting, °F

Probe Tip Dia., In. 3/4

Probe Length

Probe Heater Setting

Point	Clock Time	Dry Gas Meter, CF	Pitot In. H_2O ΔP	Orifice ΔH In H_2O Desired	Orifice ΔH In H_2O Actual	Dry Gas Temp. °F Inlet	Dry Gas Temp. °F Outlet	Pump Vacuum In. Hg Gauge	Impinger Temp °F	Stack Press In. Hg	Stack Temp °F
Start	1045										
#2	0000	142.603									
	01	142.64	.05		.61				69		272
	02	142.70	.09		1.27				69		310
	03	142.77	.10		1.4				65		363
	04	142.83	.10		1.4				65		319
	05	142.88	.05		.61				67		210
	06	142.90	.04		.57		Flame Out		66		171
	07	142.96	.03		.43				69		162
	08	143.00	.03		.43				67		154
	09	143.04	.03		.43				67		149
	10	143.08	.03		.43				71		149
	11	143.12	.02		.28				69		137
END 1057	1135	143.122	.02		.28				69		135
Start	1125										
#1	0000	143.122									
	01	143.15	.02		.28				84		123
	02	143.19	.02		.28				84		133
	03	143.23	.04		.57				78		138
	04				1.4						
	05										
	06.9	143.44									

3/3

.7

FIELD DATA

Ambient Temp °F 102 (1203)

Run No. #2

1.3

Location UCR

Bar. Press. "Hg 28.76 (1203)

Date 7/23/80

1.3

Assumed Moisture % _____

Operator _____

.7

1

Heater Box Setting, °F _____

Sample Box No. _____

Leak Test - Before After

Probe Tip Dia., In. _____

(.115" Hg)

Meter Box No. _____

Pitot Tube Factor 0.830

Probe Length _____

Probe Heater Setting _____

VERY IMPORTANT - FILL IN ALL BLANKS

Read and record at the start of each test point.

Point	Clock Time	Dry Gas Meter, CF	Pitot in. H_2O ΔP	Orifice ΔH in H_2O Desired	Orifice ΔH in H_2O Actual	Dry Gas Temp. °F Inlet	Dry Gas Temp. °F Outlet	Pump Vacuum In. Hg Gauge	Impinger Temp °F	Stack Press in. Hg	Stack Temp °F
Start	1209	143.440	—								
#2	0000										
1	01	143.48	.02		.28				77		240
2	02	143.52	.04		.57				78		240
3	03	143.56	.02		.28						
4	04	143.60	.02		.28	Ecane	Out		80		152
5	05	143.64	.02		.28				80		140
6	06	143.70	.02		.28				82		137
7	07	143.73	.02		.28				82		131
8	08	143.764	.02		.28				83		128
9	09	143.795	.02		.28				82		129
10	10	143.827	.02		.28				82		121
11	11	143.855	.02		.28				83		120
12	12	143.89	.02		.28				84		119
13	13	143.912	.01		.14				83		118
14	14	143.936	.01		.14				85		116
END	1224	143.94	.01		.14						
AVG					.28		60		82		119
TOTAL	14.2	.500					60				

207

.02 — .24
.03 — .43
.04 — .57
.05 — .61
.07 — 1.0
.08 — 1.13
.09 — 1.27
.10 — 1.4
.12 — 1.7

1/2

FIELD DATA

Run No. 3

Location UCR

Date 7/23/88

Operator

Sample Box No.

Meter Box No.

2014d Rec Straw

VERY IMPORTANT - FILL IN ALL BLANKS

Read and record at the start of each test point.

Leak Test = Before 1.5"Hg / After

Pitot Tube Factor 0.830

Ambient Temp °F 103 (1424)

Bar. Press. "Hg 28.73 (1424)

Assumed Moisture % 10

Heater Box Setting, °F

Probe Tip Dia., In. 3/8

Probe Length

Probe Heater Setting

Point	Clock Time	Dry Gas Meter, CF	Pitot in. H_2O ΔP	Orifice ΔH in H_2O Desired	Orifice ΔH in H_2O Actual	Dry Gas Temp. °F Inlet	Dry Gas Temp. °F Outlet	Pump Vacuum In. Hg Gauge	Impinger Temp °F	Stack Press In. Hg	Stack Temp °F
Start	1443										
#4	0000	143.975									
	01	144.60	.02		.28				61		304
	02	144.04	.03		.43				70		404
	03	144.082	.03		.43				67		356
	04	144.118	.02		.28				71		220
	05	144.15	.02		.28			FLARE OUT 5.4	71		190
	06	144.18	.02		.28				71		182
	07	144.20	.01		.14				72		167
	08	144.23	.01		.14				76		148
	09	144.255	.01		.14				79		142
	10	144.278	.01		.14				79		140
	11	144.30	.01		.14				79		139
	12	144.322	.01		.14				78		135
Stack #3	1521 12.4	144.334	.01		.14				78		132
#3	0000	179.934									
	01	144.36	.02		.28				68		345
	02		.04		.85				67		365
	03	144.47	.09		1.27				64		290
	04	144.53	.09		1.27				68		230
	05	144.59	.09		1.27				67		211
	06	144.66	.09		1.27				63		175
	07	144.721	.09		1.27				67		175
	08	144.785	.08		1.13				66		140
	09	144.84	.08		1.13				66		144
	10	144.905	.08		1.13				67		144
	11	144.96	.08		1.13				64		138
	12	145.02	.08		1.13				67		144
	13.5	145.091	.08		1.13						

2/2

FIELD DATA

Run No. 3

Location UCR

Date

Operator

Sample Box No.

Meter Box No.

VERY IMPORTANT - FILL IN ALL BLANKS

Read and record at the start of each test point.

Leak Test = Before / After 15" Hg

Pitot Tube Factor 0.830

Ambient Temp °F 96 (1712)

Bar. Press. "Hg 28.72 (1712)

Assumed Moisture %

Heater Box Setting, °F

Probe Tip Dia., In. 7/16

Probe Length

Probe Heater Setting

Point	Clock Time	Dry Gas Meter, CF	Pitot in. H_2O ΔP	Orifice ΔH in H_2O Desired	Orifice ΔH in H_2O Actual	Dry Gas Temp. °F Inlet	Dry Gas Temp. °F Outlet	Pump Vacuum In. Hg Gauge	Impinger Temp °F	Stack Press In. Hg	Stack Temp °F
Start 16		145.054									
#2	0000	145.054									
	01	145.09	.03 .05		.43						457
	02	145.14	.09		1.17				73		445
	03		.12		1.7				70		429
	04	145.28	.07		1.10				6		
	05	145.32	.05		1.61		Exhaust Out		69		167
	06	145.38	.04		1.57				68		159
	07	145.42	.03		1.43				74		152
	08	145.46	.03		1.43				72		141
	09	145.505	.02		.43				72		141
	10	145.52	.03		1.43				73		148
	11	145.565	.03		43				72		136
	12	1456.1	.03								
Start 1456	13	1456.48	.03		1.43						
#1	0000	1456.48									
	01	1456.78	.02		.28				76		100
	02	1457.1	.02		.28				74		145
			.03		1.43						
	04	1457.75	.03		43		Exhaust Out		68		110
	05	1458.0	.02		.28				70		115
	06	1458.46	.02		.28				67		106
	07	1458.6	.01		.14				73		100
	08	1458.96	.01		.14				69 75		100
	09	1459.18	.01		.14				75		101
	10	1459.40	.01		.14				76		97
	11	1459.61	.01		.14				78		96
	11.4	1459.71	.01		.14				77		96

FIELD DATA

100	.66
200	.48
300	.36
400	.27

Ambient Temp °F 91 (1.7.12)

Bar. Press. "Hg 28.72 (1.7.12)

Assumed Moisture % 2%

Heater Box Setting, °F

Probe Tip Dia., In. 5/16

Probe Length

Probe Heater Setting

Run No. 1A (ANDERSON)

Location UCR

Date 7/23/80

Operator B. Muchlan

Sample Box No.

Meter Box No.

Pitot Tube Factor .846

VERY IMPORTANT - FILL IN ALL BLANKS

Read and record at the start of each test point.

Leak Test - Before / After

ΔH@ = 2.02

Point	Clock Time	Dry Gas Meter, CF	Pitot in. H_2O ΔP	Orifice ΔH in H_2O Desired	Orifice ΔH in H_2O Actual	Dry Gas Temp. °F Inlet	Dry Gas Temp. °F Outlet	Pump Vacuum In. Hg Gauge	Impinger Temp °F	Stack Press In. Hg	Stack Temp °F
#3	0000	1460.32					60°F				390
	0002	1461.05			.27						350
	3	1461.40			.28						300
	4										
	5	1462.13		forecast	.53						170
	6	1462.50			.56				68		158
	7	1463.00			.56						148
	8	1463.40			.58				67		146
	9	1463.84			.58				70		140
	10	1464.26			.58				70		135
Qm		3.94									194.4
											200
#2	0000	1464.26			.36						200
	1	1464.40			.36						310
	2	4.92			.29						380
	3	5.26			.34						310
	4	5.52		forecast	.48						200
	5	6.00			.56						160
	6	6.38			.56						155
	7	6.82			.56				68		150
	8	7.24			.58				71		140
	9	7.66			.60				69		135
	10	8.13			.60				70		135
	11	8.55			.60						132
	12	9.00			.62				68		130
	13	9.45			.62						127

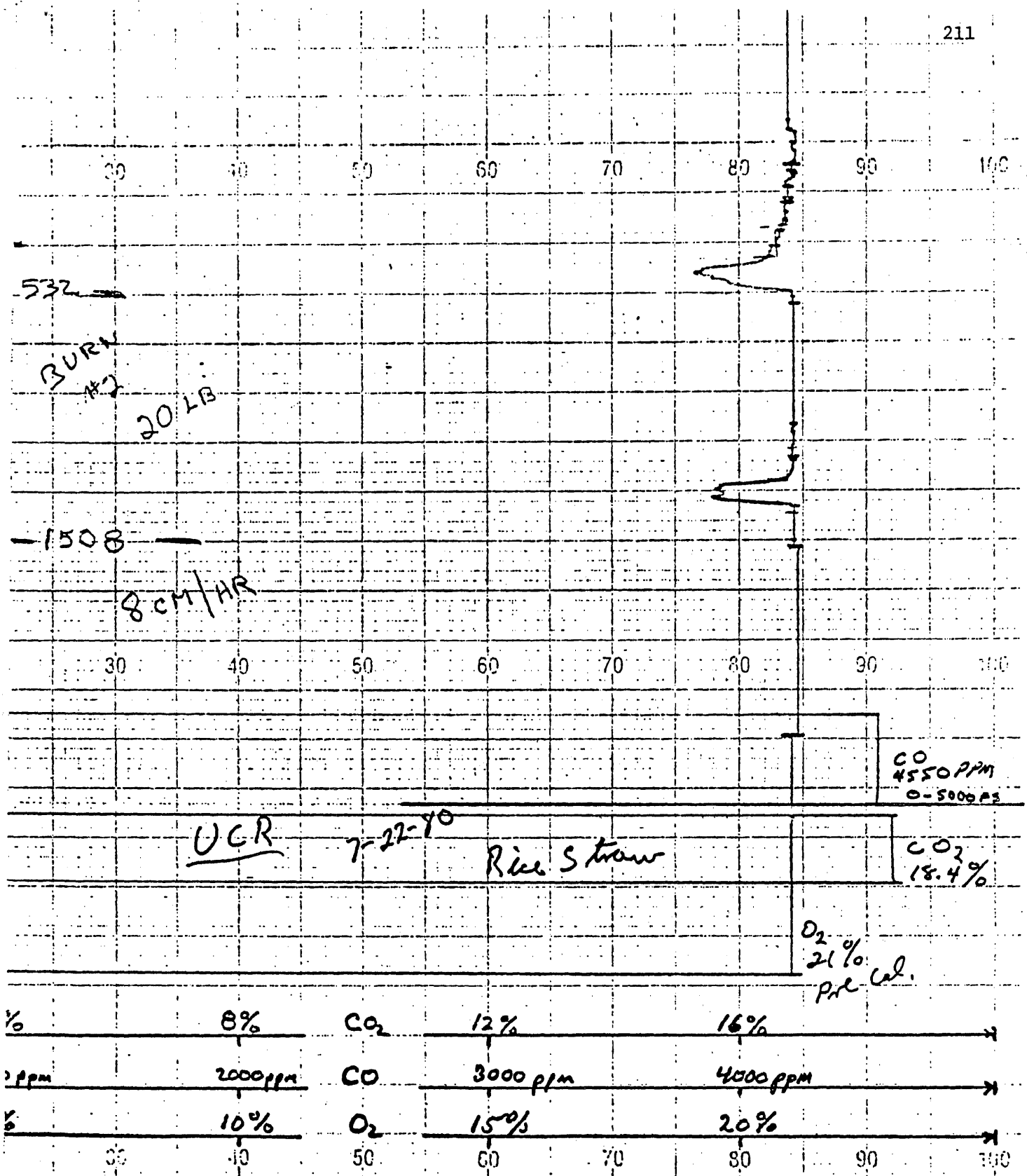
Qs

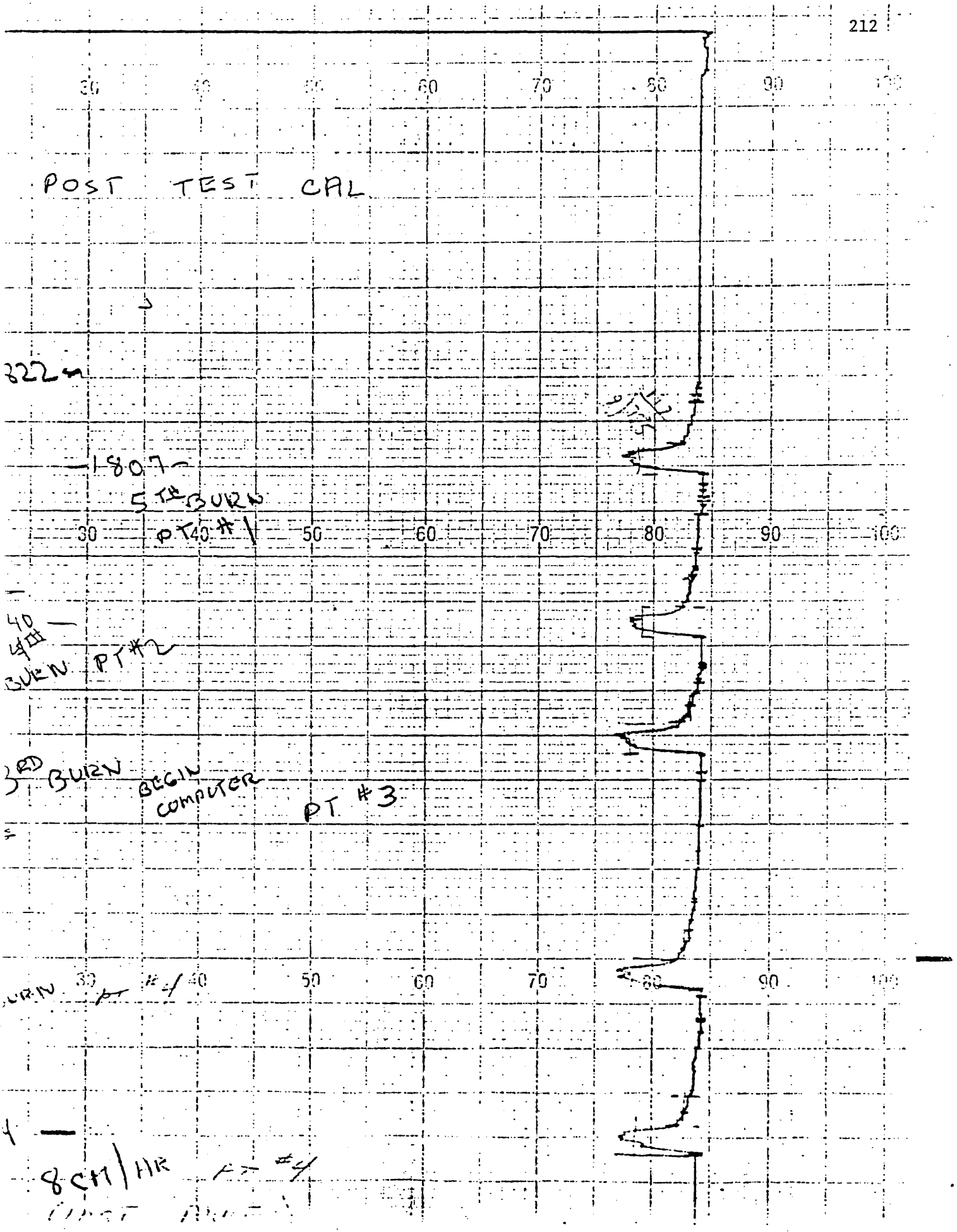
5.0

210

Q = .577

190 29 5.1


30
40
50
60
70
80
90
100
532
BURN
#2
20 LB
1508
8 CM/HR
CO
4550 PPM
0-5000 PPM
UCR
7-22-80
Rice Straw
CO2
18.4%
O2
21%
pre cal.
8%
CO2
12%
16%
2000ppm
CO
3000 ppm
4000 ppm
10%
O2
15%
20%


POST TEST CAL
822 m
1807
5TH BURN
PT #1
30
40
50
60
70
80
90
100
4TH BURN PT #2
3RD BURN
BEGIN COMPUTER
PT #3
8 CM/HR

4TH BURN PT #2

SO_2 SWITCHED TO SAMPLE

3RD BURN BEGIN COMPUTER PT #3

SO_2 SET TO ZERO BY MISTAKE

30 40 50 60 70 80 90 100

WT BURN PT #4

1614

PT #4

START 8CM/HR (1ST POWER)

30 40 50 60 70 80 90 100

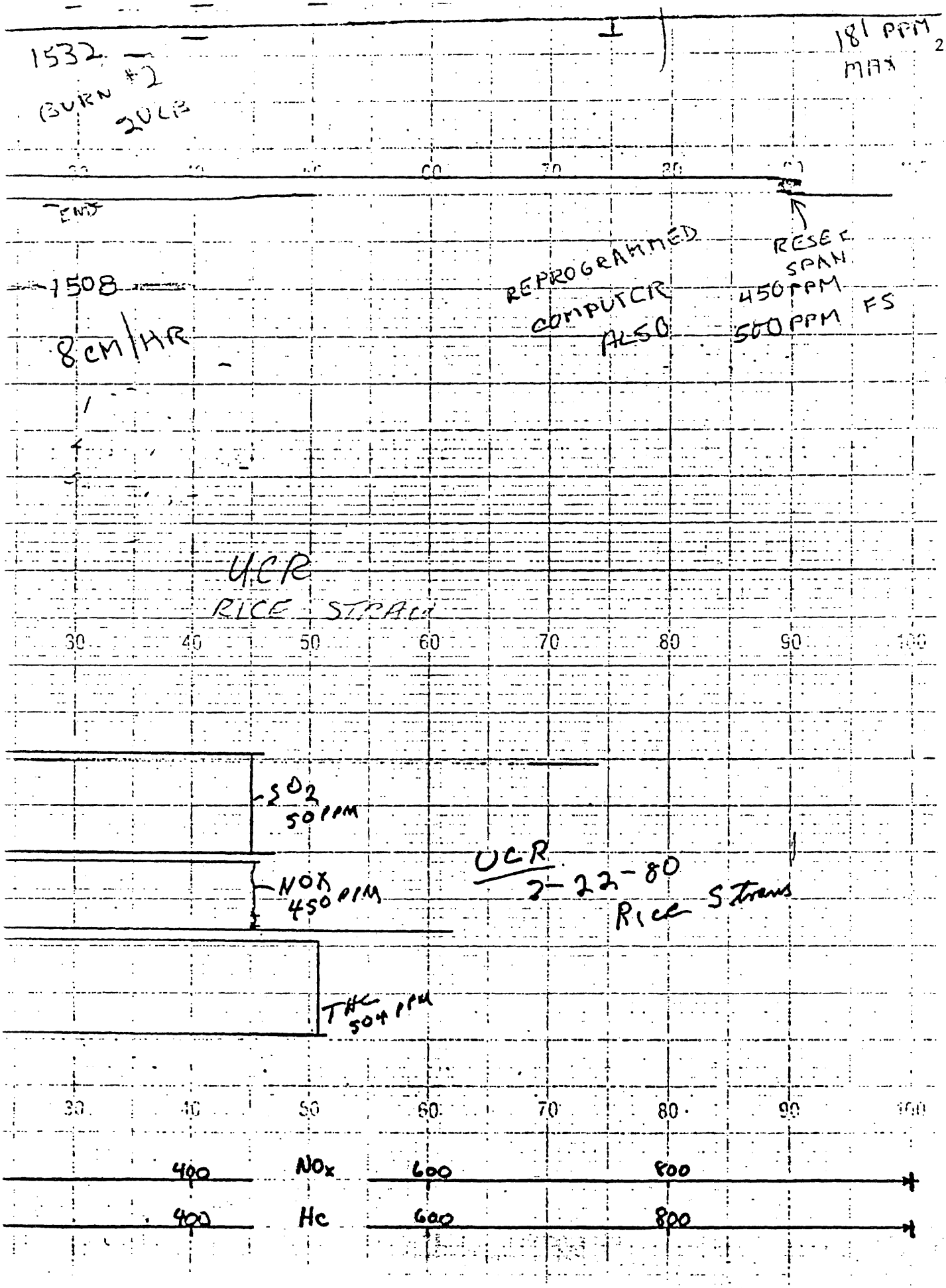
RESET SO_2 SPAN

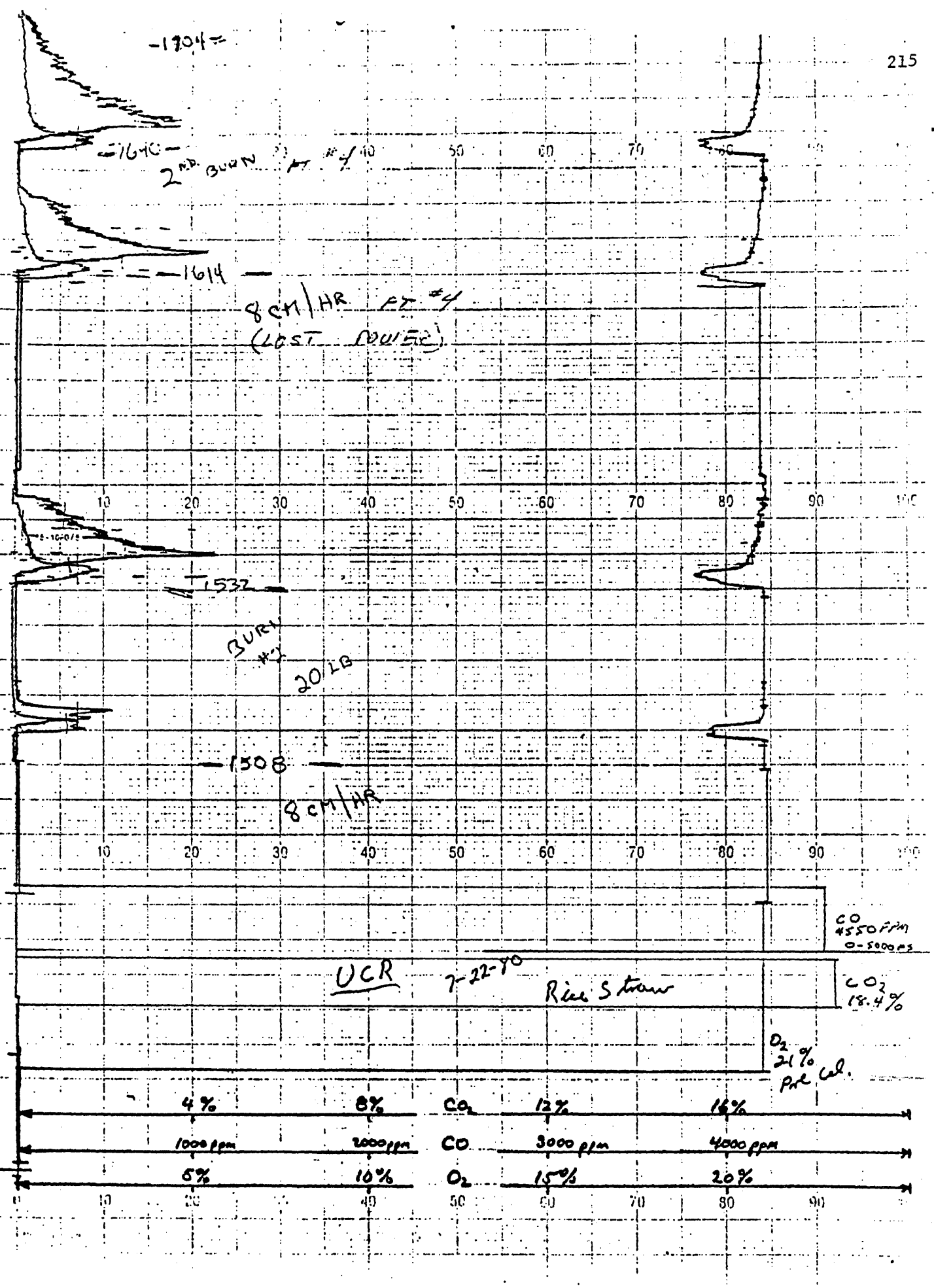
80 SO_2 120 160

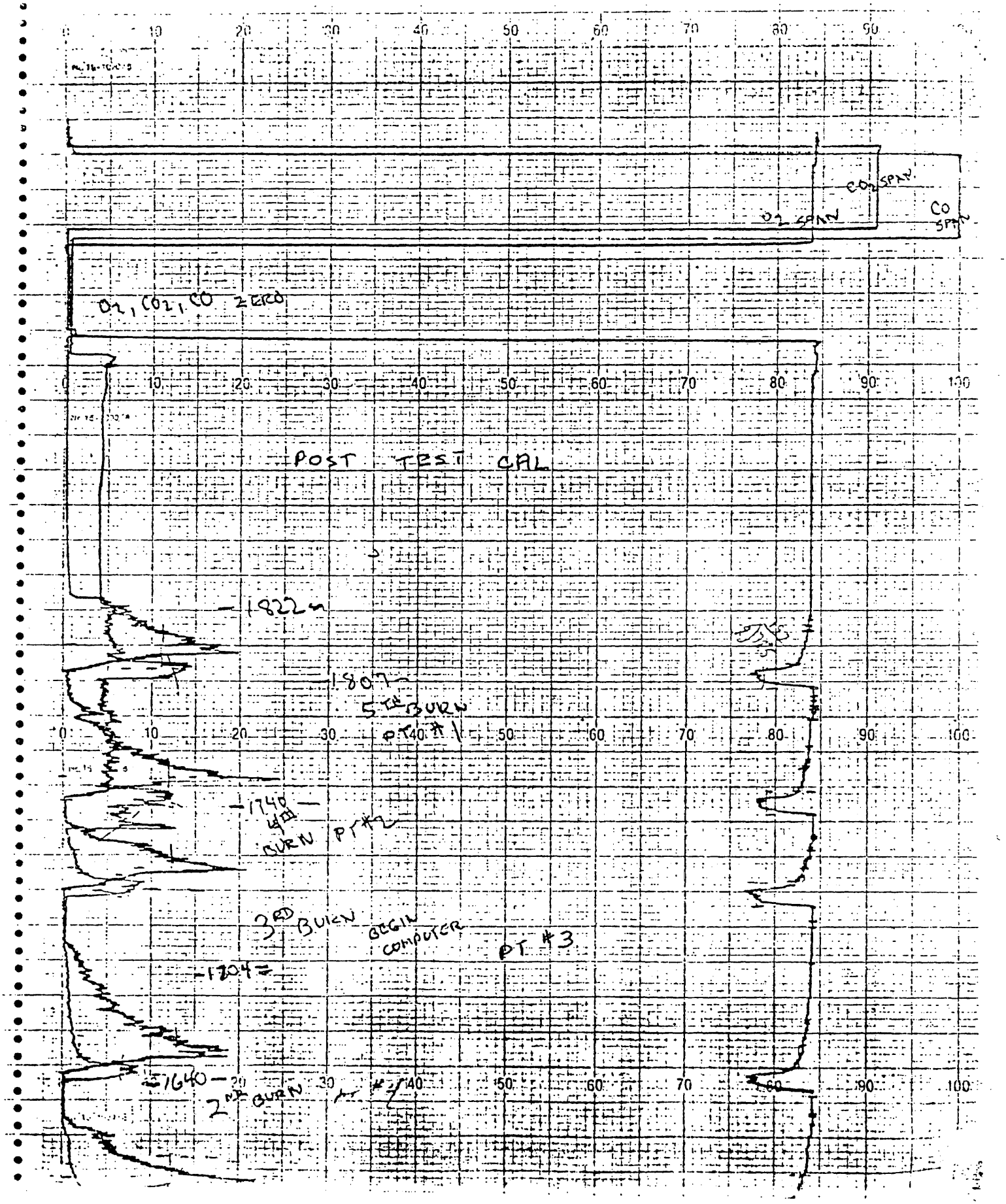
1532

BURN #2 PULP

181 PPM MAX


1532
BURN #2
20LB
181 PPM MAX
END
1508
8 CM/HR
REPROGRAMMED COMPUTER AL50
RESET SPAN 450 PPM 500 PPM FS
U.C.R.
RICE STRAW
30 40 50 60 70 80 90 100
SO2 50 PPM
NOX 450 PPM
THC 50 PPM
U.C.R.
2-22-80
Rice Straw
30 40 50 60 70 80 90 100
400 NOx 600 800
400 Hc 600 800


-1704-
-1640-
2ND BURN AT #4
-1614-
8 CM/HR #4
(LOST POWER)
10 20 30 40 50 60 70 80 90
-1532-
BURN #3
20 LB
-1508-
8 CM/HR
10 20 30 40 50 60 70 80 90
CO
4550 PPM
0-5000 PPM
UCR 7-22-70
Rice Straw
CO2
18.4%
O2
21%
Pre Cal.
4% 8% CO2 12% 16%
1000 ppm 2000 ppm CO 3000 ppm 4000 ppm
5% 10% O2 15% 20%
0 10 20 30 40 50 60 70 80 90


CO2 SPAN
O2 SPAN
CO SPAN
O2, CO2, CO ZERO
POST TEST CAL
1822
1807
5TH BURN
PT #1
1740
4TH BURN PT #2
3RD BURN
BEGIN COMPUTER
PT #3
1804
1640
2ND BURN

AFP/SSCB - DATA ACQUISITION SYSTEM PROJECT
CONTINUOUS EMISSIONS MONITORING, PART. TEST #2
DATA:

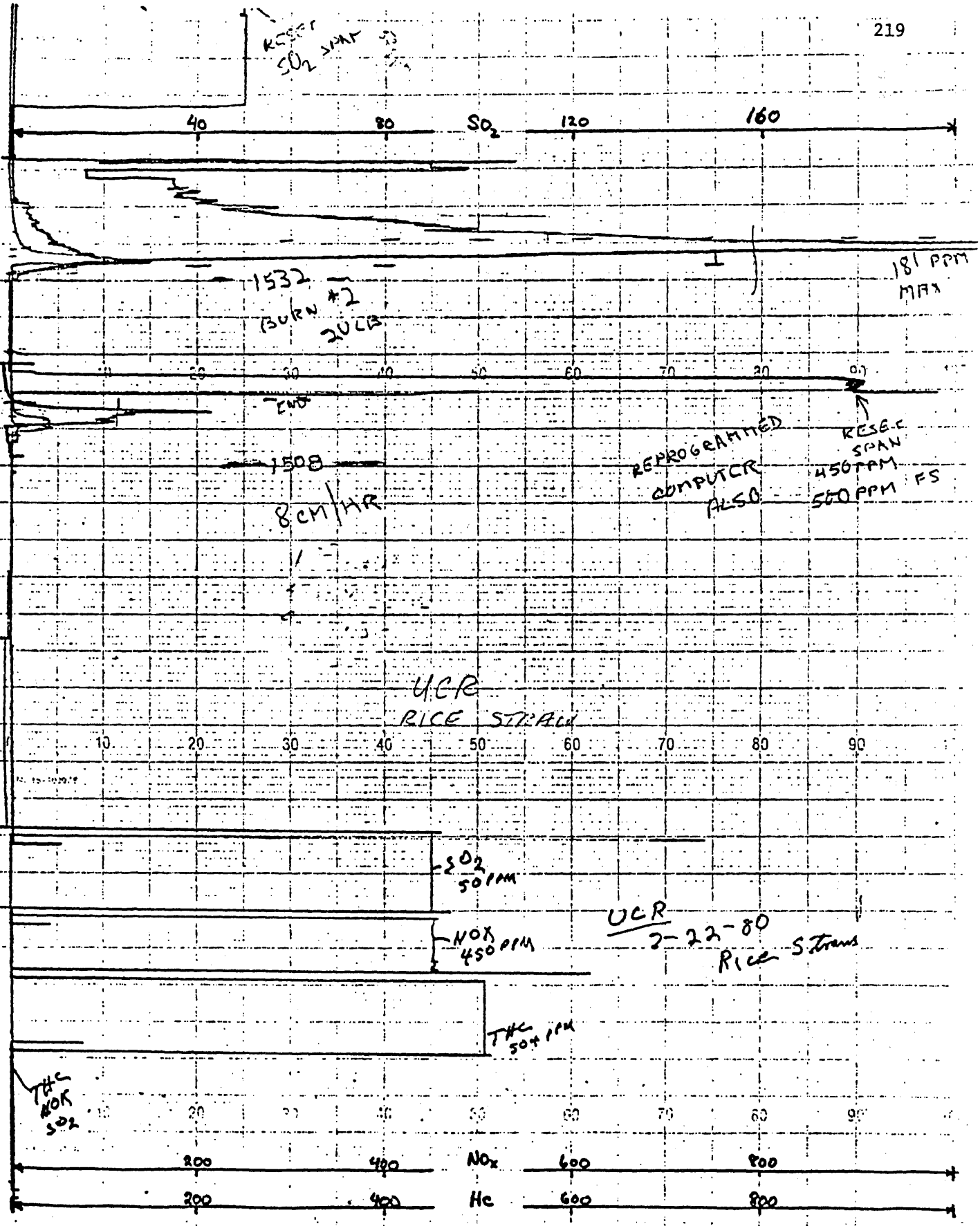
(hydrocarbons expressed as : propane)

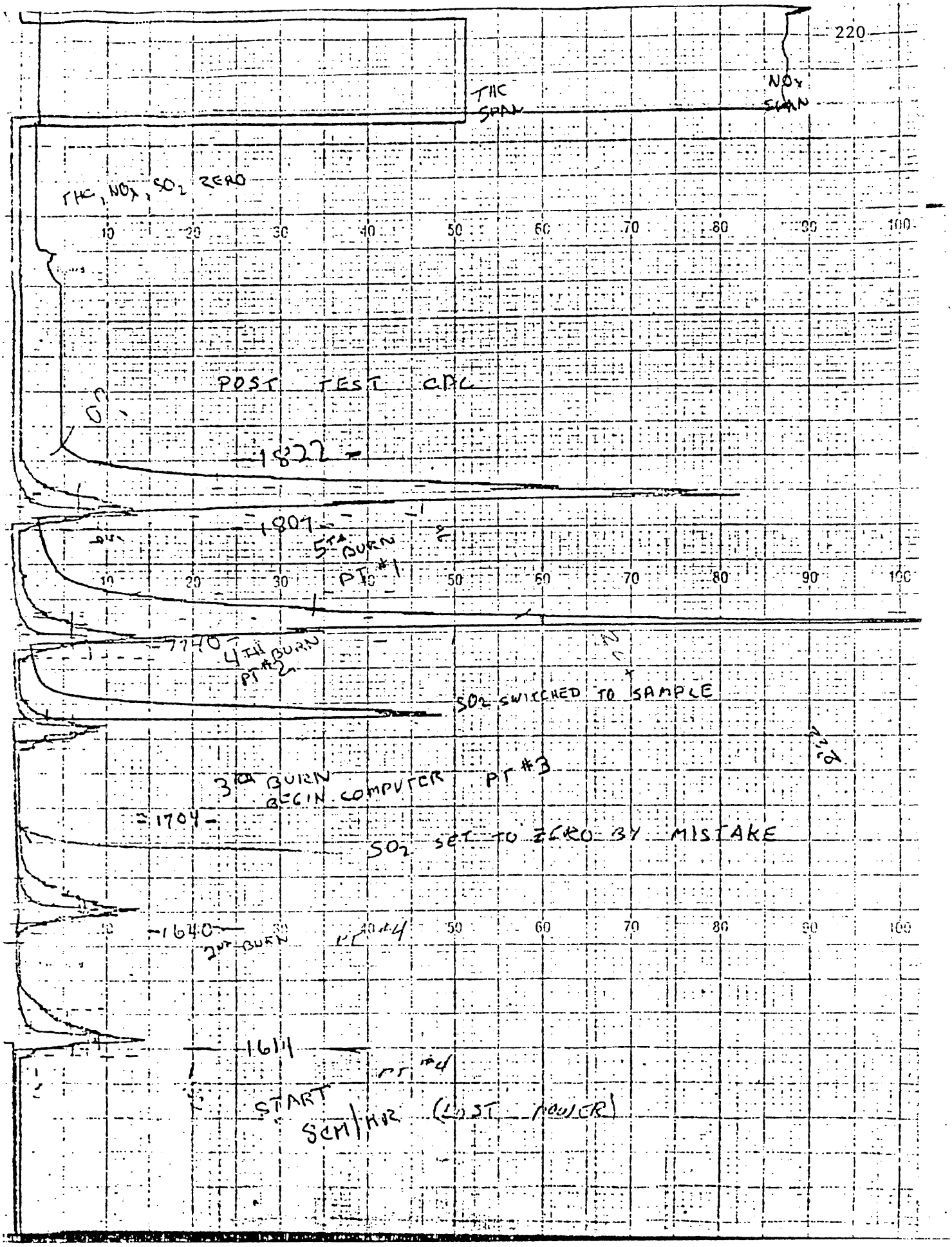
Oxygen	Carbon Dioxide	Carbon Monoxide	Hydro-carbons	Nitrogen Oxides	Sulfur Dioxide	Temperature
percent	percent	ppmv	ppmv	ppmv	ppmv	Deg. C
from 07/23@1322:57hrs to 07/23@1322:57hrs :						
20.9	0.135	-9.49	-0.585	4.84	-1.07	0.00
AVERAGE VALUES DURING TEST:						
20.3	0.596	504.85	54.944	15.84	66.29	0.00
percent	percent	ppmv	ppmv	ppmv	ppmv	Deg. C

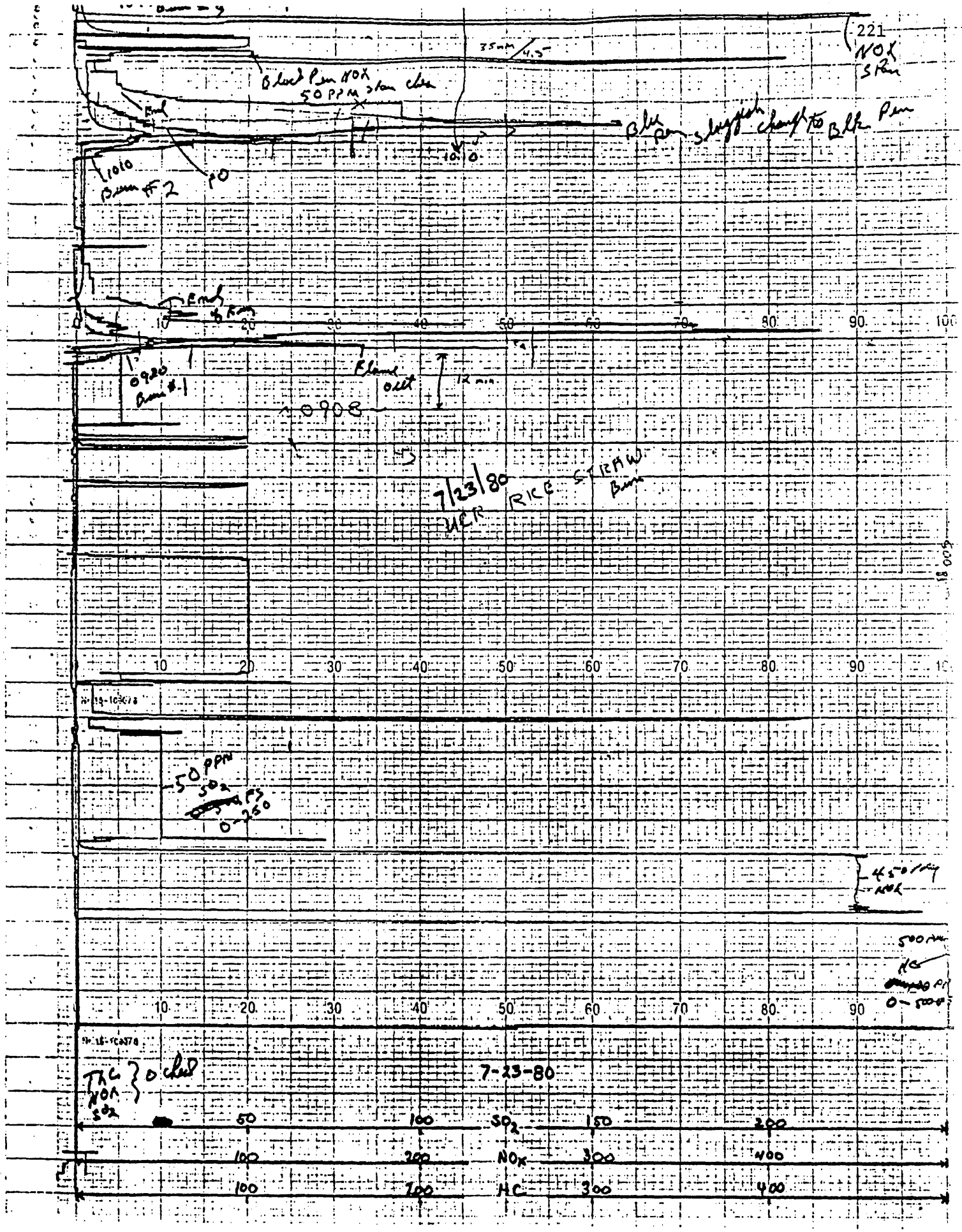
EPA/ORD - DATA ACQUISITION SYSTEM PRINTOUT
OCI Rice Straw Burner
DATE:
(Hydrocarbons expressed as : propane)

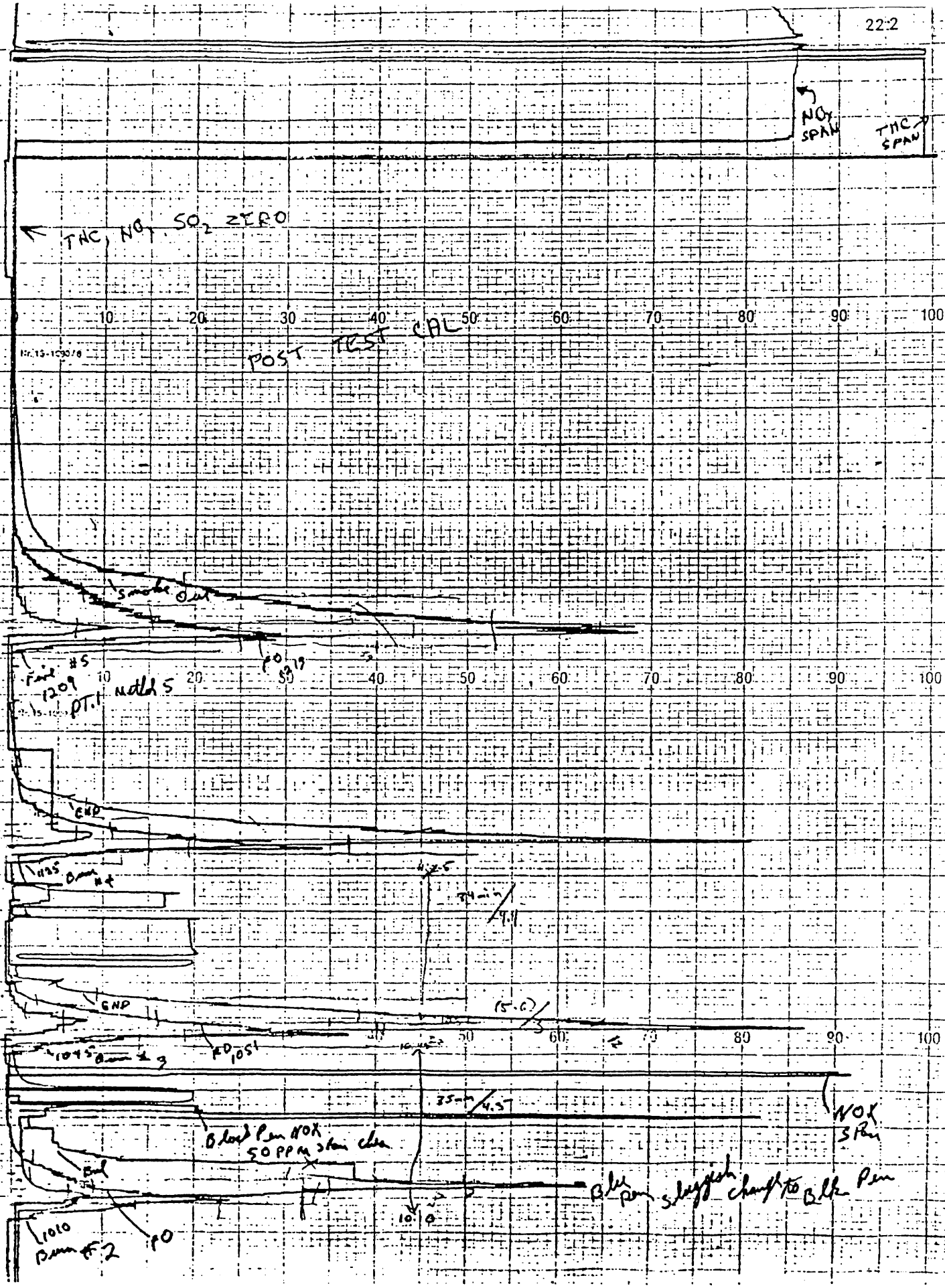
Oxygen	Carbon Dioxide	Carbon Monoxide	Hydro-carbons	Nitrogen Oxides	Sulfur Dioxide	Temperature
Percent	Percent	PPMV	PPMV	PPMV	PPMV	Deg. C
from 07/23@1444:14hrs to 07/23@1449:11hrs :						
19.8	0.958	575	78.7	36.9	107	0
from 07/23@1449:11hrs to 07/23@1454:11hrs :						
19.8	0.237	399	54.7	6.42	66.2	0.0
from 07/23@1454:11hrs to 07/23@1456:15hrs :						
19.9	0.207	256	22.8	3.87	27.2	0.0
(pause to span instruments: 07/23@1456:15hrs to 07/23@1521:41hrs)						
from 07/23@1521:41hrs to 07/23@1526:38hrs :						
19.0	1.14	594	97.5	33.6	132	0
from 07/23@1526:38hrs to 07/23@1531:38hrs :						
19.8	0.384	539	105	10.9	140	0
from 07/23@1531:38hrs to 07/23@1534:05hrs :						
20.0	0.275	312	38.1	4.84	48.9	0.0
(pause to span instruments: 07/23@1534:05hrs to 07/23@1605:31hrs)						
from 07/23@1605:31hrs to 07/23@1610:28hrs :						
19.0	1.26	601	100	36.1	138	0
from 07/23@1610:28hrs to 07/23@1615:28hrs :						
19.9	0.495	434	70.8	7.98	93.8	0.0
from 07/23@1615:28hrs to 07/23@1618:19hrs :						
20.0	0.444	282	26.0	4.86	30.6	0.0
(pause to span instruments: 07/23@1618:19hrs to 07/23@1650:46hrs)						
from 07/23@1650:46hrs to 07/23@1701:43hrs :						
19.1	1.37	612	86.2	28.3	111	0
from 07/23@1701:43hrs to 07/23@1706:43hrs :						
19.9	0.547	561	72.8	6.83	91.2	0.0
from 07/23@1706:58hrs to 07/23@1708:10hrs :						
20.0	0.456	333	24.5	3.79	33.3	0.0
(pause to span instruments: 07/23@1708:10hrs to 07/23@1708:33hrs)						
Percent	Percent	PPMV	PPMV	PPMV	PPMV	Deg. C

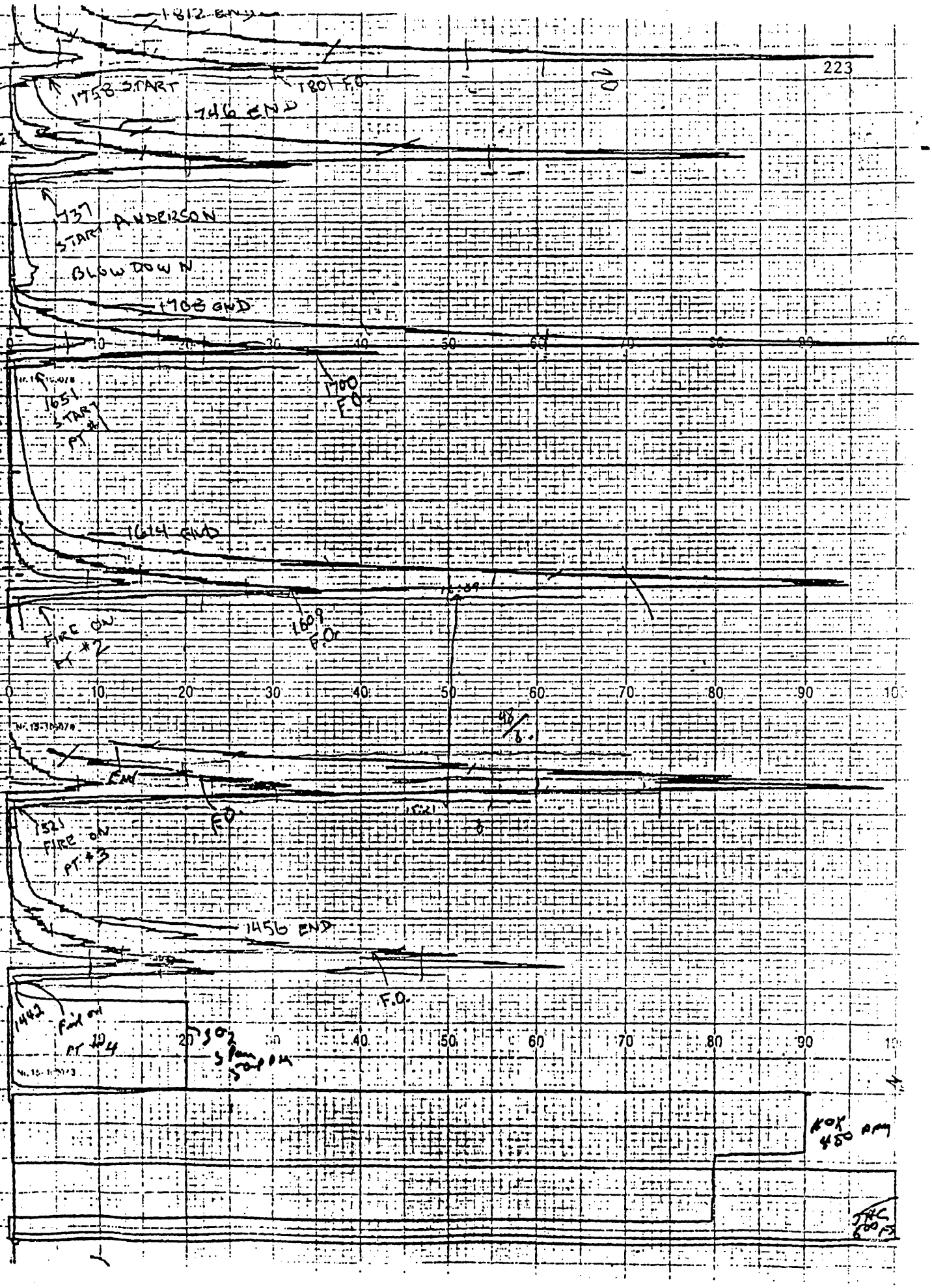
(continued next page)


RESET
SO2 SPAN
40
80
SO2
120
160
1532
BURN #2
2UCR
181 PPM
MAX
10
20
30
40
50
60
70
80
90
END
RESET
SPAN
450PPM
500 PPM FS
REPROGRAMMED
COMPUTER
ALSO
1508
8CM/HR
UCR
RICE STRAW
10
20
30
40
50
60
70
80
90
SO2
50 PPM
NOX
450 PPM
UCR
2-22-80
Rice Straw
THC
50+ PPM
THC
NOX
SO2
200
400
NOx
600
800
200
400
HC
600
800


220
THC SPAN
NOx SPAN
THC, NOx, SO2 ZERO
10
20
30
40
50
60
70
80
90
100
POST TEST CAL
1822
1807
5TH BURN
PT #1
1740
4TH BURN
PT #2
SO2 SWITCHED TO SAMPLE
3RD BURN
BEGIN COMPUTER PT #3
1704
SO2 SET TO ZERO BY MISTAKE
1640
2ND BURN
PT #4
1614
START
SCM/HR (LOST POWER)


221
NOX
Blend Pen NOX
50 PPM
Blue Pen slightly change to Blue Pen
1010
Burn #2
CO
Flame out
0920
Burn #1
0908
7/23/80
HCR RICE STRAW
Burn
50 PPM
SO2
0-250
THC
NOX
SO2
7-23-80
SO2
NOX
HC


NOx SPAN
THC SPAN
THC, NO, SO2 ZERO
10
20
30
40
50
60
70
80
90
100
POST TEST CAL
Smoke Out
Ford #5
1209
PT.1
Middle 5
GMP
4.2-5
7½ min
4.1
(5.0)
GMP
FD 105
35-min
4.3
NOX SPAN
Blue Pen NOX
50 PPM span check
Blue pen sluggish
change to Blue Pen
1010
Pen #2
10.0


1812 END
1758 START
1801 F.O
1746 END
1737 START ANDERSON
BLOW DOWN
1708 END
1651 START PT #2
1700 F.O
1619 END
FIRE ON PT #2
1609 F.O
FW
F.O
1521 FIRE ON PT #3
1456 END
F.O.
1442 FIRE ON PT #4
KOK 450 psig
0
10
20
30
40
50
60
70
80
90
100

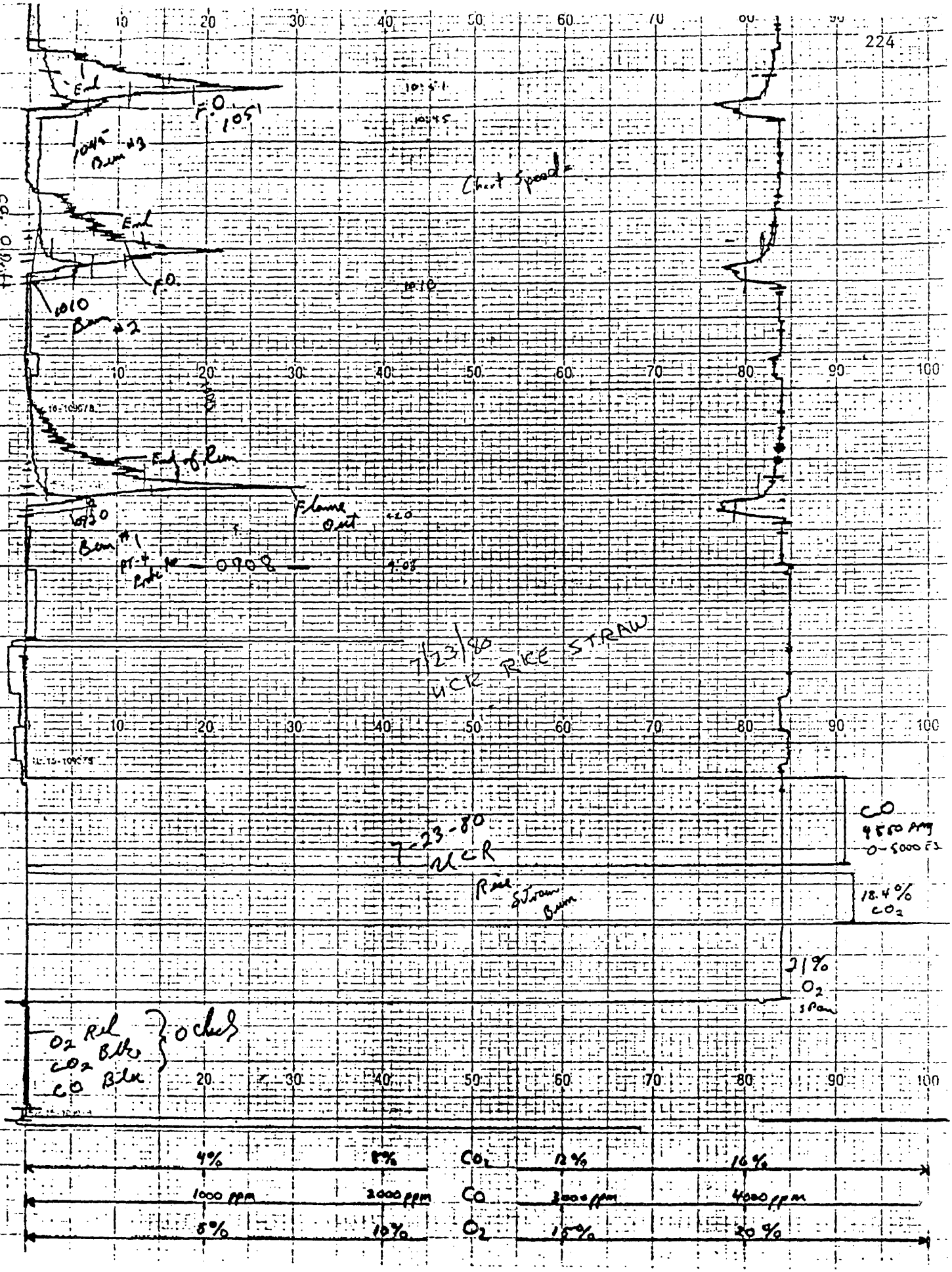
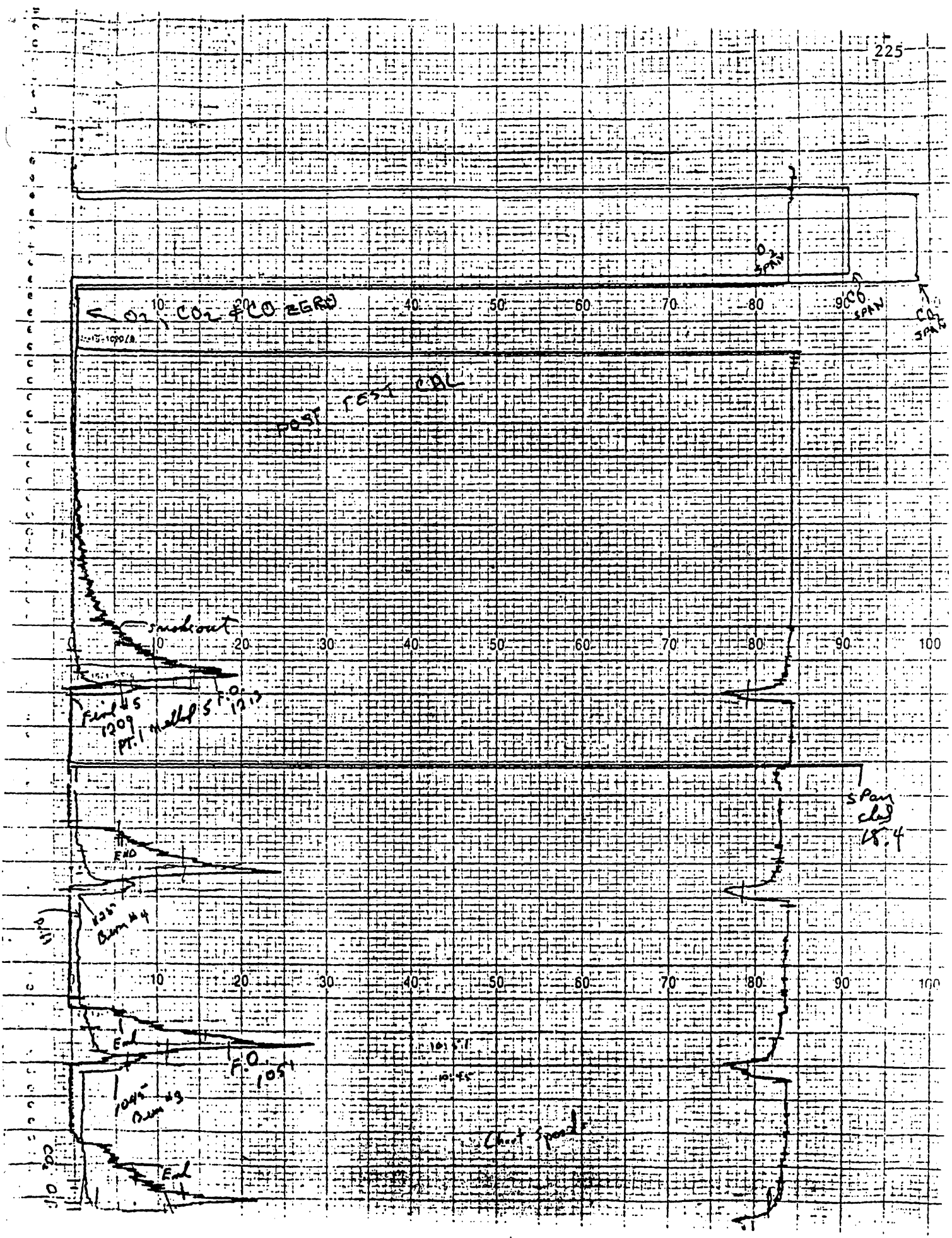
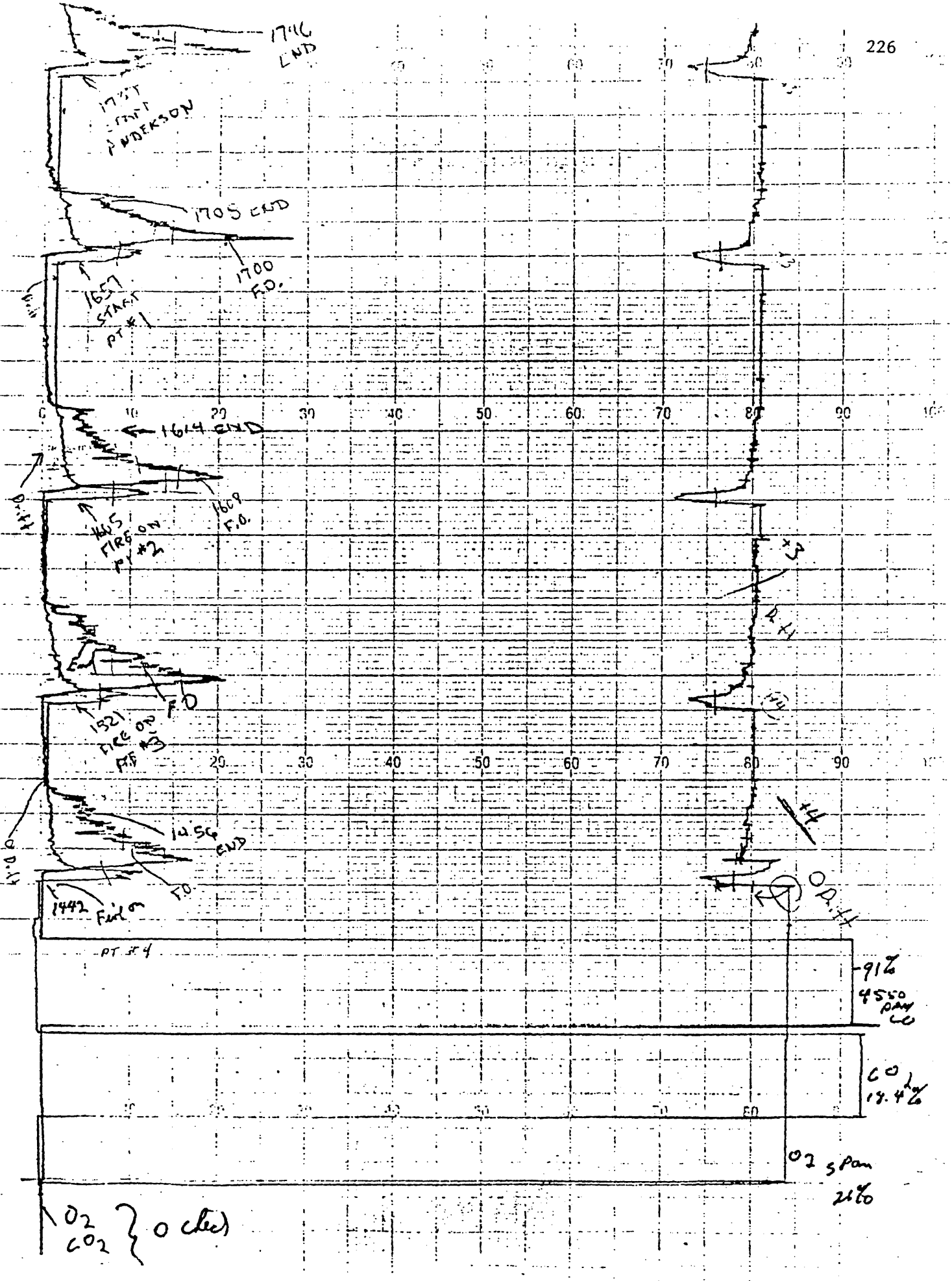
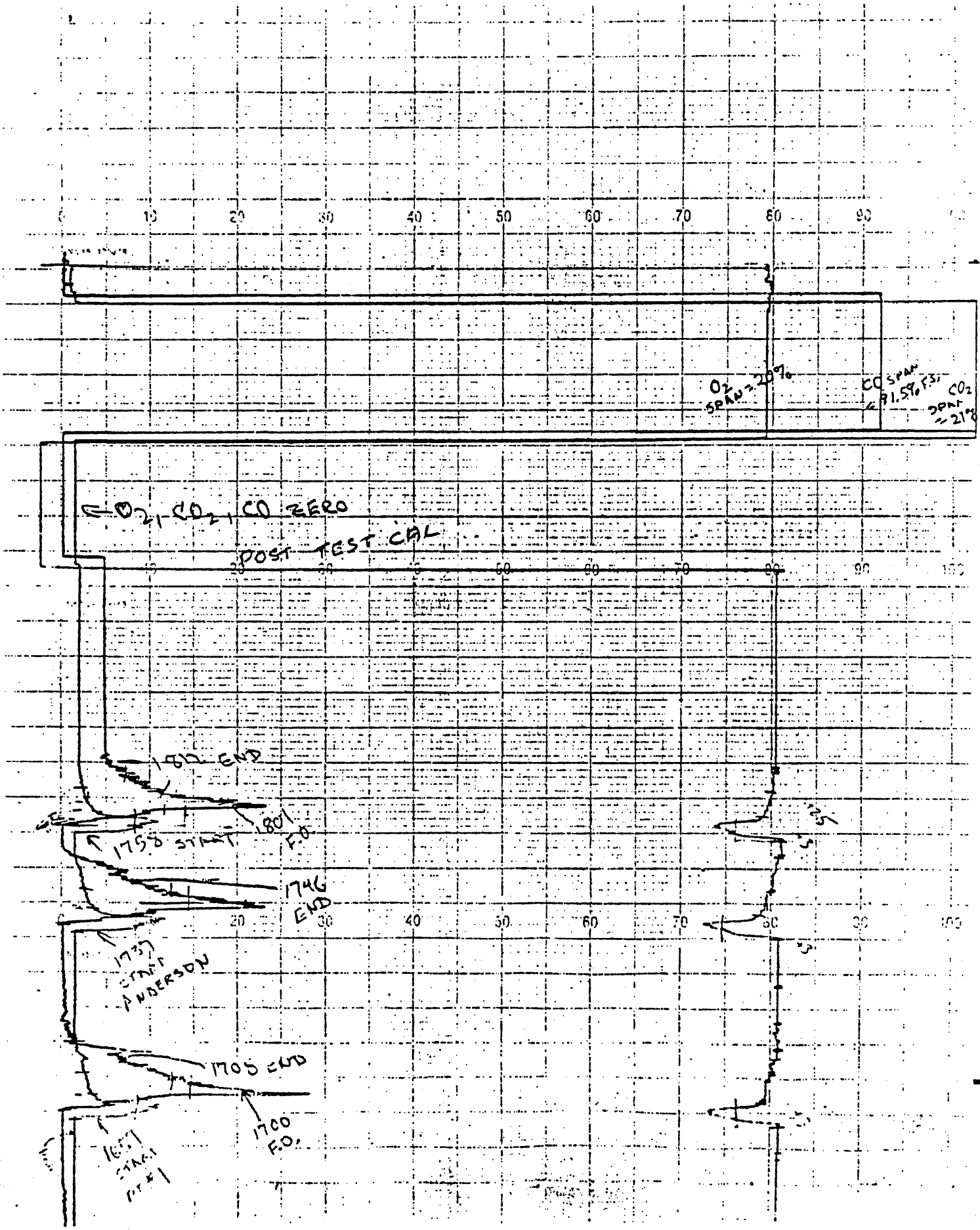


Chart Speed =
End
F.O.
1051
1044
Burn #3
End
CO
1010
Burn #2
End of Run
Flame Out
1020
Burn #1
0908
7/23/80
NCR RICE STRAW
7-23-80
NCR
Rice Straw Burn
CO
9550 Amp
0-5000 FS
18.4%
CO2
21%
O2
Span
O2 Ref
CO2 Blk
CO Blk
0 Check
4%
8%
12%
16%
CO2
1000 ppm
2000 ppm
3000 ppm
4000 ppm
CO
8%
10%
15%
20%
O2


O2 SPAN
CO SPAN
O2, CO2 & CO ZERO
post test cal
smoke out
Feed #5
1209
pt. 1 melted
F.O.
Span chk
48.4
END
Burn #4
F.O.
105°
Burn #3
Chart speed
O2
CO2
CO


1716 END
1705 END
1700 F.O.
1657 START PT #1
1614 END
1609 F.O.
1605 FIRE ON PT #2
F.O
1521 FIRE ON PT #3
1456 END
F.O
1442 FIRE ON
PT #4
-91%
4550 ppm CO
CO 18.4%
O2 span 21%
O2
CO2
0 elec


O2 SPAN = 20%
CO SPAN ~ 1.5% F.S.
CO2 SPAN ~ 21%
O2, CO2, CO ZERO
POST TEST CAL
1802 END
1755 START
1801 F.O.
1746 END
1735 START ANDERSON
1705 END
1700 F.O.
1651 START PT #1