TOXICOLOGICAL INVESTIGATION OF FINE PARTICLE EMISSIONS FROM OIL-FIRED POWER PLANTS*

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ABSTRACT

Detailed studies have been conducted of the stack-collected fine particles collected at a representative oil-burning power plant in Southern California. Samples were collected over a three-week period with a two-stage particle sampler designed to aerodynamically classify the particles and collect them into coarse and fine fractions, respectively. This separation was achieved with a cyclone separator with cut size of about 5 μm aerodynamic diameter. Thus, the principal respirable particles are in the fine fraction. Detailed studies were conducted of the physical and chemical characteristics of the collected particles showing the coarse particles to consist of some pitted cinder-like particles, some iron rust-like particles, and metallic sulfates. The fine particles consisted primarily of metallic sulfates and were 85% soluble in water (suggesting considerable solubility in body fluids), with an important fraction being associated with the biologically active nickel and vanadium sulfates. Biological studies of the mutagenesis of these oil fly ash particles showed that there was some slight mutagenic activity indicated (as assessed in Ames bacterial mutagenesis assays), and also there was considerable cellular toxicity demonstrated by in vitro exposure of rabbit pulmonary alveolar macrophages to these fly ash particles at different concentrations. The cytotoxicity results are consistent with the known cellular toxicity associated with soluble forms of vanadium as present in oil ash. The mutagenesis results indicate further studies are needed for more complete evaluation.

ACKNOWLEDGMENTS

The fly ash sampler was designed, constructed, and tested by Dr. Andrew McFarland and Dwight Russell at Texas A&M University, College Station, Texas. Stephen Teague and Kenneth "Danny" McFarland of the Laboratory for Energy-Related Health Research at the University of California, Davis, were principally responsible for the field equipment, operation of the sampler, the collection of ancillary samples, sample storage, and associated field study logistics. Additional technical support was given by Kenneth McFarland (aerosol analyses), Celeste Downey (macrophages), Heather McArthur (mutagenesis), Deborah Okamoto (organic chemistry), Patricia Wong (organic chemistry), Amiram Rasolt (aerosol physics), Margaret Brummer (scanning electron microscopy), Lynn Morrin (transmission electron microscopy), Ken Shiomoto (illustrations), Shirley Coffelt (photographic services), and Charles Baty (manuscript preparation). Dr. Gerald Fisher (now at Battelle Institute, Columbus, Ohio) lead the original planning of this project. The Laboratory for Energy-related Health Research is directed by Dr. Marvin Goldman.

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EXECUTIVE SUMMARY

Laboratory and field studies have been performed of the physical, chemical, and selected biological properties of smoke-stack oil fly ash collected aerodynamically from a commercial 485 MWe oil-burning steam-cycle utility electrical power plant located in Southern California. Samples were collected with a specially designed sampler system over a 21 day period of continuous operation at about 80% power level (400 MWe) to provide representative samples. The special fly ash sampler was designed to aerodynamically separate the fly ash particles into coarse (non-respirable) and fine (respirable) fractions for these studies. This separation was achieved with a cyclone separator with cut size of about 5 μm aerodynamic diameter. The fly ash collection was conducted at 140°C, the stack gas temperature, to avoid condensed sulfuric acid. Low ash, low sulfur Indonesian crude oil, typical of the type used by power companies in California, was burned throughout the sampling period.

There were several reasons for interest in the physical, chemical, and biological properties of oil fly ash released from power plants. Oil-burning power plants are used to produce the majority of electricity generated in California and they usually release to the atmosphere most of the respirable fly ash produced during the oil combustion. The quantities of fly ash released are significant. Hence, these power plants represent an important source of fly ash particles as well as pollutant gases. The potential health impact for people breathing the resultant aerosol in California requires evaluation of the biological properties associated with the physico-chemical characteristics of the respirable oil fly ash particles.

This project, therefore, involved determination of the physical properties of the fly ash particles, measurement of the chemical constituents, and studies of the effects of these particles on mammalian cells, namely pulmonary alveolar macrophages, the frontier cells of the lung that respond to inhaled particles. In addition, the potential carcinogenicity of these particles was considered by subjecting suitable extracts of chemical components to a bacterial test (Ames analysis) of cellular mutagenesis, an indicator of potential carcinogenicity.

The oil burned at the chosen power plant had a fuel ash concentration of 0.005%. For operation at 400 MWe, involving the combustion of 217,000 lbs of oil per hour, the effluent stream of about one million cubic feet of gas per minute had a fly ash concentration of 2.5 mg/m^3. This figure does not include condensable sulfuric acid that also forms part of the released aerosol upon mixing with ambient air at the exit of the smoke stack. Of this fly ash, 64% was in the fine (respirable) fraction (less than 10 μm in aerodynamic diameter).

The coarse particles consisted of some pitted cinder-like particles, some iron rust-like particles and metallic sulfates, and the fine particles consisted primarily of relatively soluble metallic sulfates and appeared to have structures typical of mixed salt crystals. About half the mass of the particles was identified as associated with the sulfate anion. The principal metals present in the fine ash were nickel (10.3%), sodium (3.7%), iron (3.8%), vanadium (2.7%) and calcium (1.2%). Also present were important quantities of manganese, zinc, cobalt, magnesium, aluminum, and potassium. About 85% of the fine ash was water soluble and about 65% of the coarse ash was water soluble, indicating high potential biological availability of the trace metals present upon inhalation deposition.

The organic analyses showed organic compounds to be only a minor component of these fly ash particles. Elaborate procedures were used to avoid organic contamination in handling or collection and to insure that the observed organic, chemical properties were representative of the combustion process. Gas chromatographic traces were made of the organic species, but background organic constituents on the Teflon filters used for collection obscured the organic analysis and limited its usefulness, because of the inherently low concentration of organic constituents in the ash.

Biological studies of the mutagenesis of these oil fly ash particles showed that there was some slight mutagenic activity in the ash (as assessed in the Ames bacterial mutagenesis assays) and considerable toxicity to the bacterial cells. These studies were performed using five tester strains, TA 100, TA 98, TA 1535, TA 1538, and TA 1537, with and without microsome activation. Extracts were prepared with an azeotropic benzene/methanol mixture and with horse serum. Except in the case of two tests with benzene/methanol extracts of 4.83 mg and 0.483 mg of fine oil ash per colony culture plate with TA 100, none of the samples showed statistically significant levels of mutagenic activity ($P < 0.1$) although some slight dose-response trends were

suggested by the data. However, the cellular toxicity demonstrated by the oil fly ash may have obscured some of the inherent mutagenic activity that may have been present.

Three sets of cellular toxicity studies with New Zealand white rabbit pulmonary alveolar macrphage were conducted that demonstrated a clear toxicity dose response relationship with increasing fly ash exposure for the fine particles. Both fine fly ash particles and the dissolvable soluble components were separately observed to be highly toxic to the cells with the equivalent of 156.7 μg of ash per ml causing cell lysis and reduced phagocytic function as observed in test sphere phagocytosis challenge tests. Exposure to 512.7 μg/ml concentration of fine ash caused cell death. Comparison with similar effects caused by vanadium implicated the vanadium portion of the fly ash to the observed cellular toxicity.

These studies show that oil fly ash is highly biologically active, is relatively toxic to cells, and may contain some mutagenic constituents. Most of the ash released from the power plant was in the respirable fine fraction. Since the fine (respirable) ash particles consisted primarily of metallic sulfates, including the biologically important trace metals vanadium, nickel, manganese, cobalt, and magnesium, and were 85% soluble in water, relatively high solubility in lung fluids upon inhalation deposition is to be expected. Oil fly ash thus provides a major contrast to coal fly ash which primarily consists of relatively inert insoluble fused aluminosilicate spheres with extremely low concentrations of trace metals. Future work should consider the magnitude of oil fly ash releases and estimates of health impact.

I. INTRODUCTION

About 70% of the utility electrical generation capability in California involves combustion of oil, and most utilities use conventional boiler steam generation systems. Some small units are diesel powered. Although convertible to natural gas, the oil burning utility boilers will probably not be operated with natural gas because of the priority for natural gas in other uses. The remainder of California's electricity generation capability is hydroelectric (about 23%), nuclear (about 4%), and geothermal (about 2%). Hence, the principal airborne emissions in generation of electricity in California are those associated with effluents from residual oil combustion (1).

Although some studies have been reported of the physical and chemical properties of oil fly ash from some sources, information is needed concerning the physical and chemical characteristics of the oil fly ash being released by power plants in California. Further, little information has been available on the biological activity, biomedical properties, or potential health impact of oil fly ash inhaled by people after it becomes part of the ambience. For these reasons this project was undertaken by the Laboratory for Energy-Related Health Research (LEHR) of the University of California, Davis (UCD).

In this study a special fly ash sampler was designed and constructed to collect fly ash samples from the breeching of a power plant smoke stack and to separate particles into respirable (fine) and nonrespirable (coarse) fractions. Samples were collected over a three-week period at a representative 485 megawatts of electricity (MWe) oil-fired unit in Southern California in June 1980. Careful studies were made of morphological, physical, and chemical properties of the collected ash, including particle size distribution of the collected particles, light and electron microscopic characteristics, elemental composition by atomic absorption spectroscopy (AAS) and instrumental neutron activation analysis (INAA). Anions were evaluated with ion chromatography (IC). Organic components were studied by benzene and benzene/methanol extraction followed by gas and liquid chromatographic analyses. Ancillary samples were collected with a Sierra cascade impactor under diluted near-ambient

conditions to provide a comparison mass size distribution of the stack-released aerosol. Small samples of condensate were collected at about 0°C to provide samples containing the condensable gaseous materials not collected with the particle sampler. Fuel samples were also collected for study. Most of the particle mass was found to be inorganic sulfate compounds of nickel, vanadium, and iron. Eighty-five percent of the respirable fraction was water soluble.

The biological activity of the collected respirable particles was studied using two short-term assay systems, measurements of bacterial mutagenesis (Ames test) and toxicity to mammalian cells using pulmonary alveolar macrophages harvested from rabbit lungs and cultured after adherence to small flat plastic slides. The mutagenesis tests were designed to measure the presence of either inorganic or organic mutagens using several extraction and testing approaches. The mutagenicity of the condensate was also evaluated. The cytotoxicity test with macrophages was designed to observe both gross toxicity and subtle alterations in cellular function with assays of macrophage viability, adherence capabilities, and phagocytic capabilities, both before and after exposure to the respirable fly ash particles. Although the results suggested some mutagenic activity in the fine particle fractions, the most important biologic reactivity of the particles was found to be high in cytotoxicity. Also, the soluble fraction (85% of the mass of the respirable particles) was found to be cytotoxic to macrophages. The level of cytotoxicity was consistent with the presence of biologically active vanadium and nickel in these samples.

II. FIELD STUDIES

Description of Power Plant

The oil burning power plant chosen for this study was a 485 MWe steam cycle electrical generation utility unit burning 260,000 lbs (34,000 gallons) of petroleum oil per hour. During the 21 days of sampling, nominal power level was maintained near 400 MWe to provide a realistic level of performance.

Intake air for the combustion chamber is drawn by giant electric motor-driven fans through a mechanical heat exchange unit that serves to preheat the air by transfer of heat energy from the hot exhaust gas stream under counter flow conditions. After combustion the resulting exhaust gases exceed one million cubic ft/min at the 140°C stack temperature and consist principally of about 74% N_2, 12% CO_2, 11% H_2O, and 3% O_2 in addition to 10 ppm CO, minor traces of hydrocarbons or other inorganic and organic vapors, 150 ppm NO_x, 150 ppm SO_2, 4 ppm SO_3 (which condenses to H_2SO_4 upon mixing with the ambient air) and solid particles of fly ash. The SO_3 dew point for these exhaust gases was about 115°C so that sampling temperatures had to exceed this temperature at all times to prevent condensation of H_2SO_4 and associated water.

The stack released gases are cooled by the ambient environment resulting in the condensation of SO_3 to H_2SO_4 which produces a fine aerosol of sulfuric acid and coats the fly ash particles. Also, certain condensable inorganic vapors such as of arsenic or selenium and organic vapors such as of polycyclic organic matter may condense to form an additional ultrafine aerosol and coat the other airborne particles in the stack plume.

The fuel burned by this power plant during the sampling period was a residual oil blend consisting primarily of oil derived from Indonesian petroleum, a particularly low sulfur and low ash source of crude oil. The results of chemical analysis of the major constituents of a sample of oil collected during the fly ash sampling period is given in Table 1. An important feature of this oil is the abnormally low ash content, found to be less than 0.01% compared to the 0.1 to 0.2% ash content of most residual oil used in oil burning power plants.

Table 1. Results of Fuel Oil Analysis for Three Samples Collected on 10 June 1980 at Power Plant

(Analysis performed by The Air and Industrial Hygiene Laboratory, California State Department of Health, Berkeley, California, for the California Air Resources Board)

Sample No.	% C	% H	% N	% S	% H_2O	% Ash	Density (g/cm^3)	BTU/lb
1	86.97	12.89	0.22	0.20	0.75	<0.01	0.905	19272
2	86.30	13.44	0.22	0.14	0.88	<0.01	0.906	19273
3	86.70	12.99	0.28	0.14	0.73	<0.01	0.903	19370
Mean	86.66	13.11	0.24	0.16	0.79	<0.01	0.905	19305
SE	0.19	0.17	0.02	0.02	0.05	---	0.001	33

Sampler Design and Testing

The fly ash sampler used in this project was designed and built under subcontract by Dr. Andrew McFarland of the Department of Environmental Engineering of Texas A&M University, College Station, Texas. A separate report containing design criteria and operating characteristics has been submitted by Dr. McFarland.

The sampler consisted basically of a cyclone separator designed to collect the coarse nonrespirable particles followed by a Teflon fabric filter designed to collect most of the fine respirable particles (Figure 1). The filter unit was mounted in a single "bag house" unit designed to be intermittently pulsed with high-pressure air to dislodge collected ash into a collection hopper below the unit. The system was equipped with a flow meter, jet ejector pump, pressure gauges, and a one-inch diameter sampler inlet probe which was inserted into the stack breeching at the power plant. The principal components of the sampler and flow system were enclosed in an insulated metal box

equipped with electrically operated thermostatically controlled heaters to maintain the sampler flow gas stream above the dew point of sulfuric acid and water.

The design criteria for the sampling system were:

(a) Iso-velocity sampling - Sampler flow rate was planned to be about 8 ft^3/min at the 140°C stack gas temperature through the one-inch diameter inlet probe that was to be inserted into the smoke stack breeching. This would provide a sampling linear velocity of about 1,500 ft/min. To provide a representative sample, this sampling velocity should be nearly equal to the average velocity of the effluent stream being sampled. The dimensions of the breeching where the sampler was installed were 35 feet wide by 13 feet high so that at one million ft^3/min the average velocity of the effluent stream was about 2,000 ft/min. As a result, the design sampling rate was slightly below the iso-velocity flow rate which may have caused the collection of somewhat more than the representative proportion of coarse ash but would not have affected the representativeness of the fine fly ash particles. At 8 ft^3/min it was anticipated that about 8 g/day of fly ash would be collected based on available ash concentration data.

(b) Fabric filter cleaning - The fine ash which was accumulated on the Teflon fabric filter was to be removed through the use of pulsed jets of clean filtered air. The air used for this purpose was zero-air, chemically clean and dry, supplied in compressed air tanks to avoid contamination of the samples. The cleaning jets of air were activated by a set of electrically operated solenoid valves which, in turn, were energized by a timer driven relay. The time interval between pulses was approximately two minutes with about 100 ms pulse length and 100 psig air pressure.

(c) Flow rate measurement - An orifice flow meter was included as part of the sampling system after the fabric filter. This allowed measurement of the sample gas volumetric flow rate during sampling by sensing the pressure drop across a small orifice. Readout was made using a calibrated pressure indicator.

Fig. 1. Sampler used in this study to collect size-classified respirable and nonrespirable oil fly ash.

FLY ASH SAMPLER

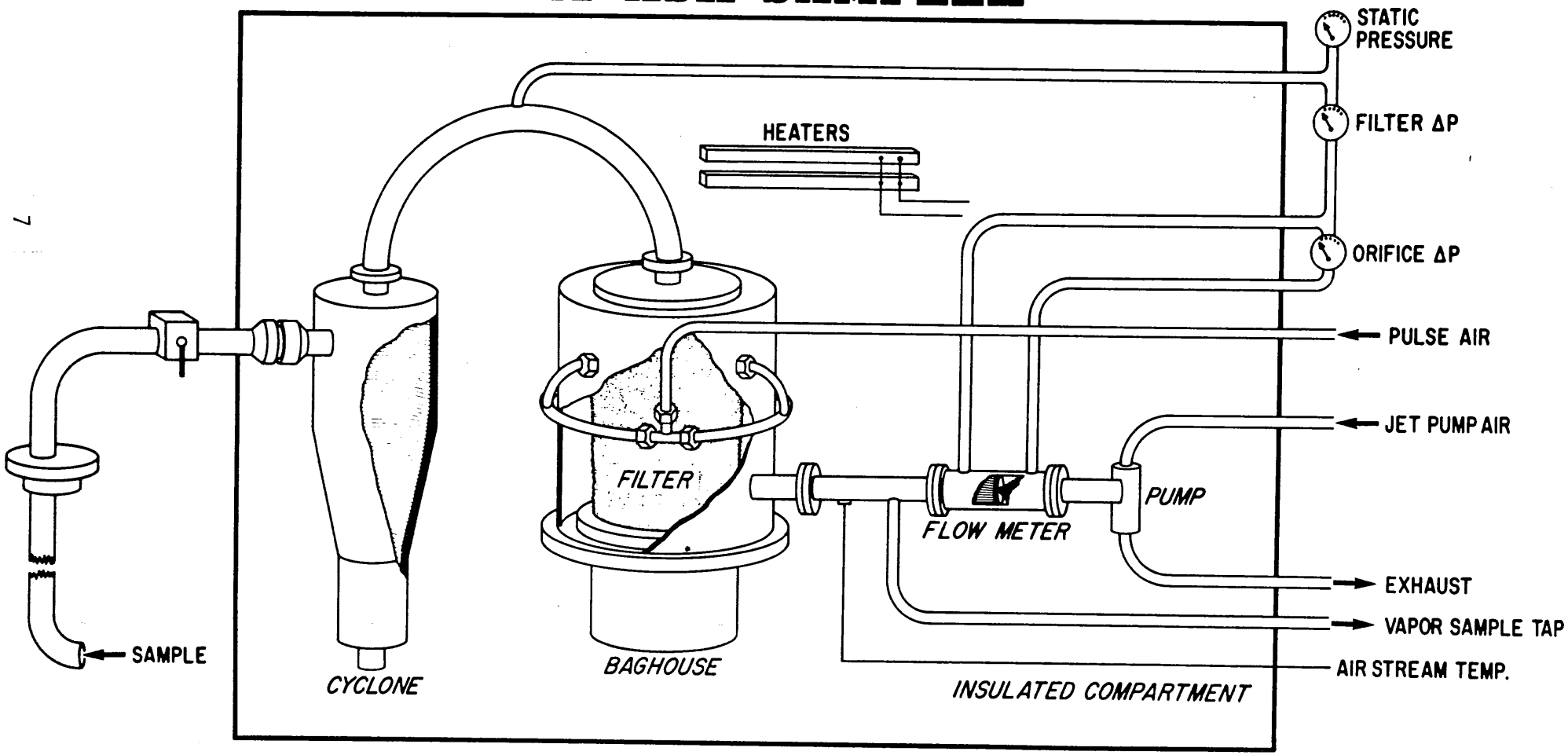


Fig. 1

(d) Flow rate control - An air ejector pump operated with power plant supplied compressed air at 125 psig was used to draw the samples of effluent gas into the sampling system and through the cyclone separator, the fabric filter, and the orifice flow meter.

(e) Temperature control - Electrical heating elements were mounted within the insulated enclosure of the sampler system to maintain the internal temperature at a level equal to that in the stack. These heaters were set up with thermostatic controls and digital readout of the temperature of the gas stream and of the enclosure during sampling.

(f) Condensate sampling - A separate vapor sample trap was installed after the fabric filter to provide a source of particle-free hot exhaust gases for collection of condensate at reduced temperature.

(g) Control console - A portable control console was designed to provide separate readouts of sampler flow rate (orifice ΔP), filter load pressure drop (filter ΔP), system static pressure (static ΔP), enclosure temperature and sample gas stream temperature, and to provide controls for the thermostatic heater system, bag-house pulse solenoid and timer. The jet pump supply air regulator and the filter baghouse pulse air pressure control system were also included.

Sampler Operation and Collection of Oil Fly Ash and Ancillary Samples

It was hoped that about 75 g of oil fly ash could be collected in about 10 days of sampling. Sample containers of pure Teflon and glass were cleaned at UC Davis to insure freedom from both inorganic and organic contamination and all necessary tools, containers, reagents, cables, and other needed sampling supplies were purchased and assembled at UC Davis. The sampler and its associated filters were the responsibility of Dr. McFarland at Texas A&M. The arrangements were completed and necessary approval obtained from the power company in May 1980.

The sampling operation was rescheduled to begin on or about May 30. The staff at UCD LEHR (Dr. Otto Raabe, Mr. Steve Teague and Mr. Danny McFarland) loaded the UC Davis sampling van at LEHR the week of May 26 and departed for the plant site on May 30. Simultaneously, Dr. Andrew McFarland and his assistant, Mr. Dwight Russell, shipped the completed sampling unit from Texas A&M in College Station, Texas, and traveled by air to California. Andrew McFarland, Otto Raabe, Steve Teague, Dwight Russell, Danny McFarland, and Mr.

Dane Westerdahl of the California Air Resources Board completely set up the sampling system and began the operation of the unit on May 31, 1980.

The sampler probe was inserted vertically downward into a horizontal run of the stack breeching duct through a 3-inch diameter port. The sampler was placed on top of the breech duct. The first sample was taken with the sampler heated to 95°C. However, filter loading (as indicated by the pressure drop across the sampling filter bag) was not normal, and it became apparent on the second day that this first sample run needed to be aborted. Upon removal of the filter bag from the baghouse unit, it was found that the filter was wet, presumably with condensed sulfuric acid and water. The 95°C chosen for sampling was clearly below the dew point for sulfuric acid and water in this case. The sampler was reloaded and operated at a temperature close to that observed for the exhaust duct, approximately 140°C, to avoid condensation problems. However, it was found that there were still some condensation problems associated with the cooling occurring in the sampling probe. Equipment was sent by air from UC Davis to set up a temperature controller on heating tape surrounding the sampling probe in order to maintain the probe at a temperature above the dew point to avoid this condensation.

During the first two days of operation it was noted that a few hundred milligrams of fly ash was collected in the cyclone hopper. This was considerably below the anticipated 5 g per day that was expected to be collected by the cyclone based on data supplied to us, calculations, and the literature asssociated with combustion of oil. It then became apparent that the oil being used in this unit at the time of sampling was of exceedingly low ash content, far lower than the fuel oil we believe to be commonly used elsewhere for electrical power generation.

Several problems occurred with the Teflon fabric filters originally planned for use in the sampler. The filters were of special Teflon-coated Dacron composition which was found to decompose at the required collection temperature of 140°C. On June 4, we began using pure Teflon felt filters in place of the Dacron-supported filters. The pure Teflon filters were used for the remainder of the sampling period.

Because of the low mass loading, all fine particles collected on filters were in fact adherent to those filters, and there was no ash collected in the

pulse filter unit. This difficulty during the collection phase limited some of the studies performed with these samples, for although ash was collected by the cyclone separator for coarse particles, the fine particles collected by the baghouse filter remained adherent to the filter and had to be removed for all studies performed. Also on the filters were small marks of marking pencil wax used by the manufacturer as guide marks which interfered with the organic analysis of the particles trapped in the filter. In addition, the adherence of particles to the filter was not planned and not expected in the original design of the sampling unit, so that the exact weights of particles collected on these filters was not always measurable. In one case, filters collected over a period of six days were weighed to estimate the small particle loading.

Cascade impactor samples were taken directly from the effluent stream by Andrew McFarland with the assistance of Danny McFarland, using a Hill impactor inserted directly into the effluent stream. Additional ancillary cascade impactor samples were taken by Otto Raabe with the assistance of Danny McFarland using effluent stream samples directly from the main sampler probe unit. Condensate samples were collected at near zero °C (using an ice water bath) via the vapor sample tap on the main fly ash sampler (Fig. 1).

A team of investigators from the Air Resources Board arrived at the plant site on June 3, 1980 and set up a system using EPA method 5 to sample from the effluent stream. Those from the Air Resources Board involved in this study were Ken Jones, Peter Ouchida, Jack LaBrue, Bud Thoma, Dwight Warner, Don Fitzell, Pat Sullivan, and Don Bratton. The ARB took samples using the Hill impactor directly from the effluent stream. A few days after the beginning of the sampling, Andrew McFarland and his assistant, Dwight Russell, returned to Texas A&M, and the sampling was continued by the UCD team. Danny McFarland of UCD was in charge of sample operation and was present at the power plant every day of the collection period. Periodically during the course of the sampling, Drs. Brenda Kimble and Otto Raabe visited the sampling site and reviewed the data collection and sample collection procedures in use.

On June 22, 1980, Mr. Ami Rasolt traveled from Davis to the power plant and helped Danny McFarland to disassemble the sampling unit and to load it into the van for return to Davis. All parts of the system were loaded for return to Davis and the area was cleaned prior to departure from the power plant site.

The collected samples are summarized in Table 2. The quantities of fly ash collected when determined are given in Table 2, and the resultant apparent collection rates and exhaust gas particle concentrations are given in Table 3. The total concentration of ash was found to be about 2.5 mg/m^3. For operation at 400 MWe involving combustion of 217,000 lbs of oil per hour and with an effluent stream of 1.2×10^6 ft^3/min, this concentration of fly ash indicates a fuel ash concentration of 0.005%. This is in agreement with the fuel sample analysis (Table 1).

Table 2. Samples Collected at Power Plant

	Sample/LEHR Code	Lab Code	Time From	Time To	Quantity
	FUEL OIL				
1.	Oil 2 x 25 ml, #10 tank LB/OIL/80155/FUEL	LB/FO/1	June 2, 1980		2 x 25 ml
2.	Oil 2 x 25 ml, Burner #6 LB/OIL/80158/FUEL	LB/FO/2	June 5 :0920		2 x 25 ml
3.	Oil 4 x 25 ml, #9 tank Burner #6 LB/OIL/80172/FUEL	LB/FO/3	June 20		4 x 25 ml
4.	Oil 3 x 25 ml #9 tank, Burner #6 LB/OIL/80173/FUEL	LB/FO/4	June 21		3 x 25 ml
5.	Oil 3 x 25 ml #9 tank, Burner #6 LB/OIL/80174/FUEL	LB/FO/5	June 22 :1240		3 x 25 ml
6.	1 Vial-Fuel oil control	LB/FO/6			
	COARSE ASH				
1.	LB/OIL/80153/SFA-SS1	LB/SCA/1	May 31 :1635	June 1 :0940	40 mg
2.	LB/OIL/80154/SFA-SS1	LB/SCA/2	June 1 :1330	June 2 :0830	60 mg
3.	LB/OIL/80155/SFA-SS1	LB/SCA/3	June 2 :1048	June 3 :0900	140 mg
4.	LB/OIL/80156/SFA-SS1	LB/SCA/4	June 3 :1250	June 4 :0800	360 mg
			June 4 :1133	June 5 :0841	

Table 2 Continued

Sample/LEHR Code	Lab Code	Time From	Time To	Quantity
5. LB/OIL/80158/SFA-SS1	LB/SCA/5	(1) June 5 :1008	June 6 :0833	
		(2) June 6 :0943	June 7 :1713	
		(3) June 7 :1832	June 9 :0949	710 mg
		(4) June 9 :1007	June 10 :0910	
6. LB/OIL/80162/SFA-SS1	LB/SCA/6	(1) June 10 :1806	June 11 :1224	
		(2) June 11 :1250	June 12 :1148	
		(3) June 12 :1231	June 13 :1314	1.380 g
		(4) June 13 :1335	June 16 :1026	
7. LB/OIL/80168/SFA-SS1	LB/SCA/7	(1) June 16 :2022	June 17 :0808	
		(2) June 17 :0833	June 18 :1258	
		(3) June 18 :1327	June 19 :0926	2.04 g + about 0.14 g
		(4) June 19 before 1349	June 20 :0955	
		(5) June 20 :1014	June 21 :1221	
		(6) June 21 :1237	June 22 :1248	
TOTAL COARSE ASH		480.7 hrs		4.87 g

Table 2 Continued

Sample/LEHR Code*	Lab Code	Time From	Time To	Quantity
FINE ASH				
1. New Teflon-coated Dacron filter LB/OIL/80153/SFA-SS2 (a) Aqueous extract (b) Filter + residue (lost or reused)	LB/FA/1	May 31 :1635	June 1 :0940	(a) 3/4 gallon
2. New Teflon-coated Dacron filter LB/OIL/80154/SFA-SS2 (a) Aqueous extract (b) Filter + residue (lost or reused)	LB/FA/2	June 1 :1330	June 2 :0830	(a) 1/2 gallon
3. New Teflon-coated Dacron filter LB/OIL/80155/SFA-SS2	LB/FA/3	June 2 :1048	June 3 :0900	Not weighed
4. Used Teflon-coated Dacron filter (either 1 or 2 reused for this filter LB/OIL/80156/SFA-SS2	LB/FA/4	June 3 :1250	June 4 :0800	Not weighed
5. Pure Teflon filter LB/OIL/80157/SFA-SS2 (a) Catch cup sample (b) Filter w/o side seam (c) Side seam	 LB/FA/5(a) /5(b) /5(c)	June 4 :1133	June 10 :0910	Not weighed
6. Pure Teflon filter LB/OIL/80162/SFA-SS2 (a) Large piece of filter w/o bottom piece (b) Bottom piece of filter	 LB/FA/6(a) /6(b)	June 10 :1806	June 16 :1026	2.42 g
7. Pure Teflon filter stitched--no silicon LB/OIL/80168/SFA-SS2	LB/FA/7	June 16 :2022	June 22 :1248	Not weighed
8. Control--Pure Teflon filter Silicon-sealed seam	LB/FA/8			--
9. Control--Pure Teflon filter Silicon-sealed seam	LB/FA/9			--
10. Control--Pure Teflon filter Triple stitched-no silicon	LB/FA/10			--
TOTAL FINE ASH	--	480.7 hrs		About 8.6 g

*Samples 1 through 5 may contain O-ring material

Table 2 Continued

Sample/LEHR Code	Lab Code	Time From	Time To	Quantity
CONDENSATES				
1st Collection				
Condenser #1 LB/OIL/80159/CON 1	LB/C1/1	June 6 :1100	June 6 :1300	About 73 ml
Condenser #2 LB/OIL/80159/CON 2	LB/C2/1	June 6 :1100	June 6 :1300	About 25 ml
2nd Collection				
Condenser #1 LB/OIL/80168/CON	LB/C/2(a-f) (a thru f)	June 16 :1904 collected about 5 hrs ea day for 6 days	June 22 ?	About 3000 ml
Condenser #2 LB/OIL/80172/CON	LB/C2/2	June 20	June 22	About 460 ml
PROBE TIP DEPOSIT	LB/PTC/1 LB/PTC/2			

MISCELLANEOUS SAMPLES

1. ARB joint grease Halocarbon 25-59
2. Dow Corning High vacuum grease Batch #B10954-4
3. Silicon O-ring
4. Glass powder funnel used to transfer cyclone ash to containers

Table 3. Fly Ash Collection Data

Period	Time (hrs)	Rate (mg/hr) Coarse	Rate (mg/hr) Fine[a]	Concentration (mg/m3)[b] Coarse	Concentration (mg/m3)[b] Fine
1	17.1	2.3	--	0.21	--
2	19.1	3.1	--	0.28	--
3	22.2	6.3	--	0.57	--
4	40.3	8.9	--	0.81	--
5	116.3	7.7	--	0.70	--
6	134.9	10.2	17.9	0.92	1.62
7	130.8	16.6	--	1.50	--
TOTAL	480.7	10.1	--	0.92	1.62

[a]Fine ash trapped on filter, only one reliable gravimetric determination made for period 6.

[b]Concentration at 20°C.

III. PHYSICAL CHARACTERIZATION OF OIL FLY ASH

Microscopic Characterization

All physical characterization was performed on fine ash sample #6 and coarse ash sample #6 (Table 2). Because of the low concentration of respirable ash in the power plant effluent stream, the ash collected on the baghouse fabric filter could not be removed by pulsing with air as planned. Samples of the respirable ash were therefore trapped in the filter and had to be harvested after returning to the laboratory. To avoid dissolution of the inorganic constituents, harvesting was accomplished by ultrasonic agitation in benzene (Nanograde, Mallinckrodt), a highly nonpolar liquid. After repeated agitation over a few days, the recovered ash was gently dried to remove the benzene and weighed.

Although considerable clumping occurred because of the extraction process, the fine ash could be seen by light microscopy on glass slides to have a uniform granular appearance. At a magnification of 125X, no coloration was apparent and the ash grains were translucent and very refractile with various interference patterns. Their sizes were estimated at 2 to 5 μm.

At 500X magnification, small black inclusions were seen in all fine particles; a small number of very small, red inclusions was also observed. The particles appeared somewhat crystalline. Overall, the uniformity of shape, texture, size, and color was the prominent morphological feature of the fine oil ash.

The coarse ash removed from the cyclone hopper contained a variety of morphological types, including small, red-brown granules identifiable as rust particles. The size distribution ranged from very small particles under 5 μm to huge particles up to 265 μm in diameter, but the agglomeration of the particles made sizing difficult. The colors of these particles included white, black, ruby red, golden, and greenish yellow. Although agglomeration obscured some of the particle distinctions, a morphological classification was made. Of a sample of 110 particles were found one large, nearly square-looking 265 μm particle, one irregular 185 μm by 115 μm particle, 11 white (sugar-like) particles between 55 and 185 μm having an oblong appearance and

rounded corners, 15 irregular, black aggregate-like particles between 40 and 115 μm, 24 black, spherical, carbon-like particles of about 25 μm geometric diameter, two red (rust-like) particles of about 50 μm, and 56 black particles smaller than 15 μm.

When the coarse ash was illuminated under dark field conditions in the light microscope, surface colors could be examined. The majority of the particles were black, of various sizes, and of roughly spherical and angular shape. The second most common color was white. Some of the red particles had a gem-like appearance; others were of a golden cast. Some of the large black particles appeared to have red surfaces.

Electron micrographs were prepared of both the fine and coarse ashes using an ETEC Autoscan VI scanning electron microscope (ETEC Corp., Hayward, California). Samples were mounted on carbon stubs and coated with either Au or C in a vacuum evaporator. Low power micrographs of each are contrasted in Figure 2. Both ashes show agglomeration, but the fine ash was much more difficult to deagglomerate and remained primarily as clumps of fine crystalline particles. The presence of some fibers was noted. These were identifiable as originating with the fabric filter of the sampler system and were not part of the fly ash aerosol. The coarse ash showed a greater variety of particle types including roughly spherical, pitted, cinder-like particles, crystalline selenite-like aggregates as are characteristic of sulfates, and sharp edged fluted crystalline particles (Fig. 3). Both the fine ash and coarse particles were studied under higher magnifications as shown in Figures 4-6.

Particle Size Analysis and Size Distribution Studies

The physical projected size distribution of collected oil fly ash was studied with samples coarse #6 and fine #6 (Table 2) using both light and electron microscopy. However, because the fine ash was imbedded in the Teflon felt filter, an extraction procedure was required to remove the particles. This procedure involved ultrasonic agitation for several hours submerged in benzene, a highly nonpolar liquid. Following filtration the particles were removed from the filters and dried. Unfortunately, this caused the ash particles to agglomerate into large clusters (Fig. 2).

Ash bulk density was determined by a gravimetric method using ash buoyancy in cyclohexane. A small amount of ash of each type was weighed and placed in a small weighed beaker. Cyclohexane was added to the beaker and the ash was degassed under vacuum conditions. The beaker and contents were then weighed while completely submerged in cyclohexane. The empty beaker was also weighed while submerged. From these data the resultant density of the ash was found to be 2.69 for the coarse ash and 2.52 for the fine ash. A sample of coal fly ash previously found to have a density of 2.36 using an alcohol buoyancy measurement was found to have a density of 2.48 by the cyclohexane method, so that the possible experimental error is estimated to be less than 5% for these determinations. This is reasonable considering the small quantity of ash available in these studies.

Ash particle size distributions were measured by the Coulter method using a Particle Data Elzone™ System (Particle Data, Inc., Elmhurst, Illinois). This involves measurement of the particle volume by electrical conductivity analysis as the particles suspended in an electrically conductive liquid pass through a small orifice in a glass barrier. Data were collected for both the count distribution and the volume distribution for both the coarse ash #6 and the fine ash #6. Since some dissolution was possible in the conductive liquid, the data were analyzed as collected and as well by assuming a 42% dissolution of the fine ash and a 32% dissolution of the coarse ash, which values are approximately one-half of the observed respective water solubilities of these ash samples. The data were fit graphically to log-normal distribution to obtain median diameters and geometric standard deviation. Aerodynamic diameters (4) were calculated from the data. The results are given in Table 4.

Size data were also collected from both photo and electron micrographs. These data were collected with a Zeiss particle size analyzer (Model TGZ3, Carl Zeiss Co., New York, NY) and log-normal functions were fit to the size distribution data by use of the grouped data of projected-area diameters and the maximum likelihood methods described by Raabe (5,6). These results are also found in Table 4. Unfortunately, the microscopic data suffered from considerable particle agglomeration so that the scanning electron micrographs of the fine ash were unusable for sizing (Fig. 2) and the light microscopic results were clearly over-estimates of the actual original size of the fine ash and coarse ash aerosols as collected by the sampler.

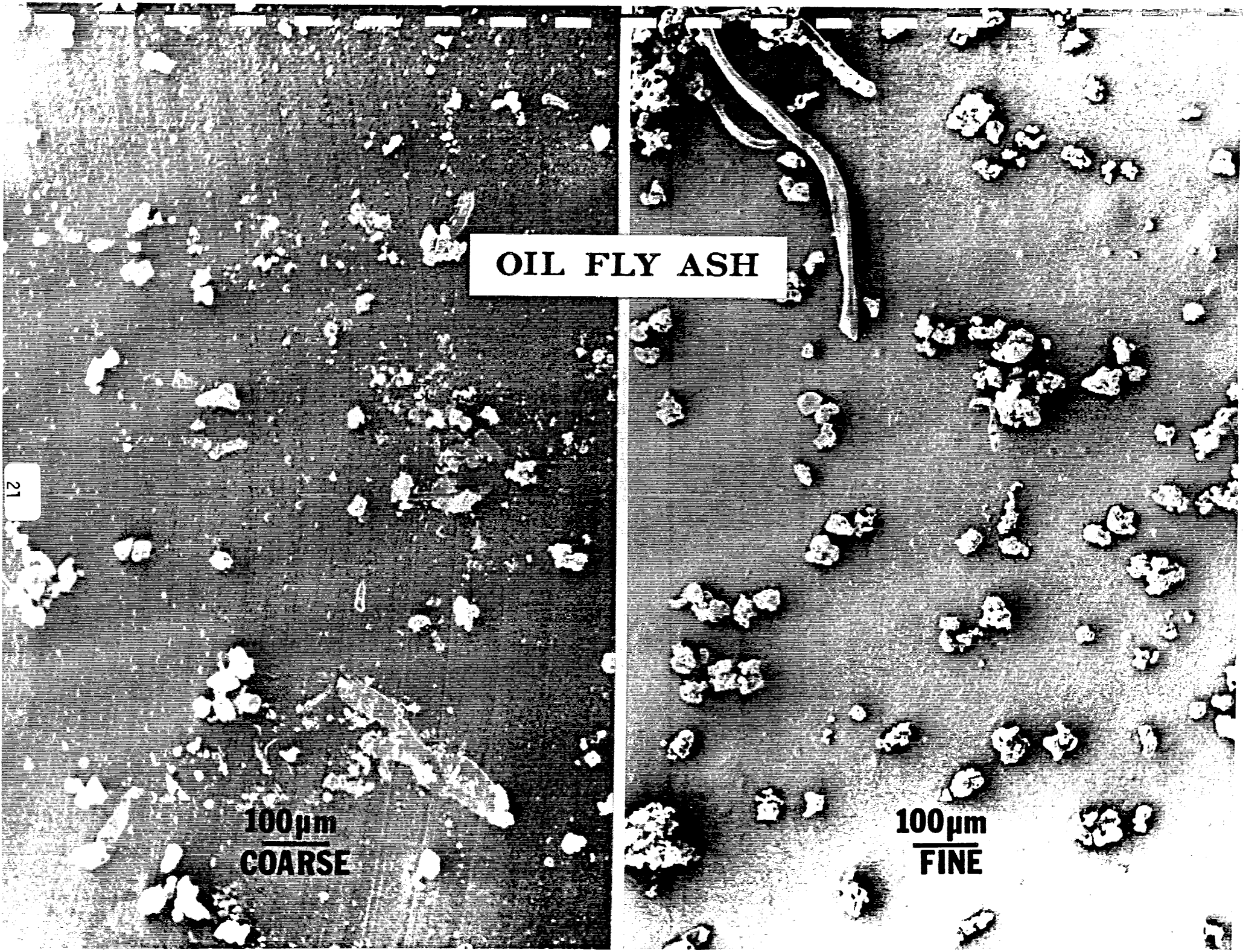
Fig. 2. Scanning electron micrographs at low power of coarse and fine oil fly ash collected in this study showing the individual particles to be agglomerated into large clusters after collection so that the apparent size distributions are not representative of the particles as sampled.

Fig. 3. Scanning electron micrograph of cyclone collected coarse oil fly ash showing different particle types.

Fig. 4. Scanning electron micrographs of both coarse and fine oil fly ash collected in this study.

Fig. 5. Scanning electron micrographs of both coarse and fine oil fly ash collected in this study.

Fig. 6. Scanning electron micrographs of both coarse and fine oil fly ash collected in this study.


OIL FLY ASH
100µm
COARSE
100µm
FINE

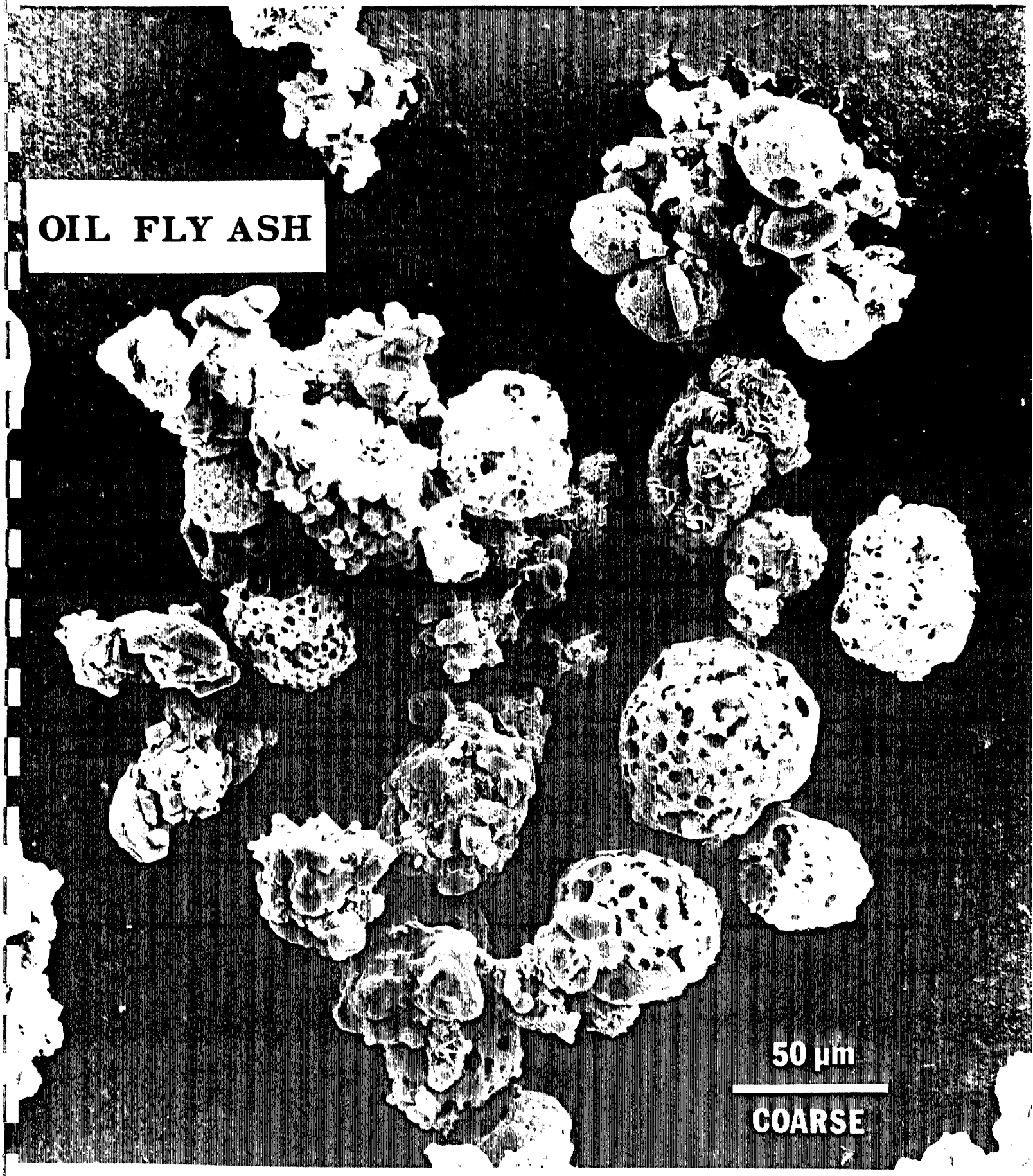


Fig. 3

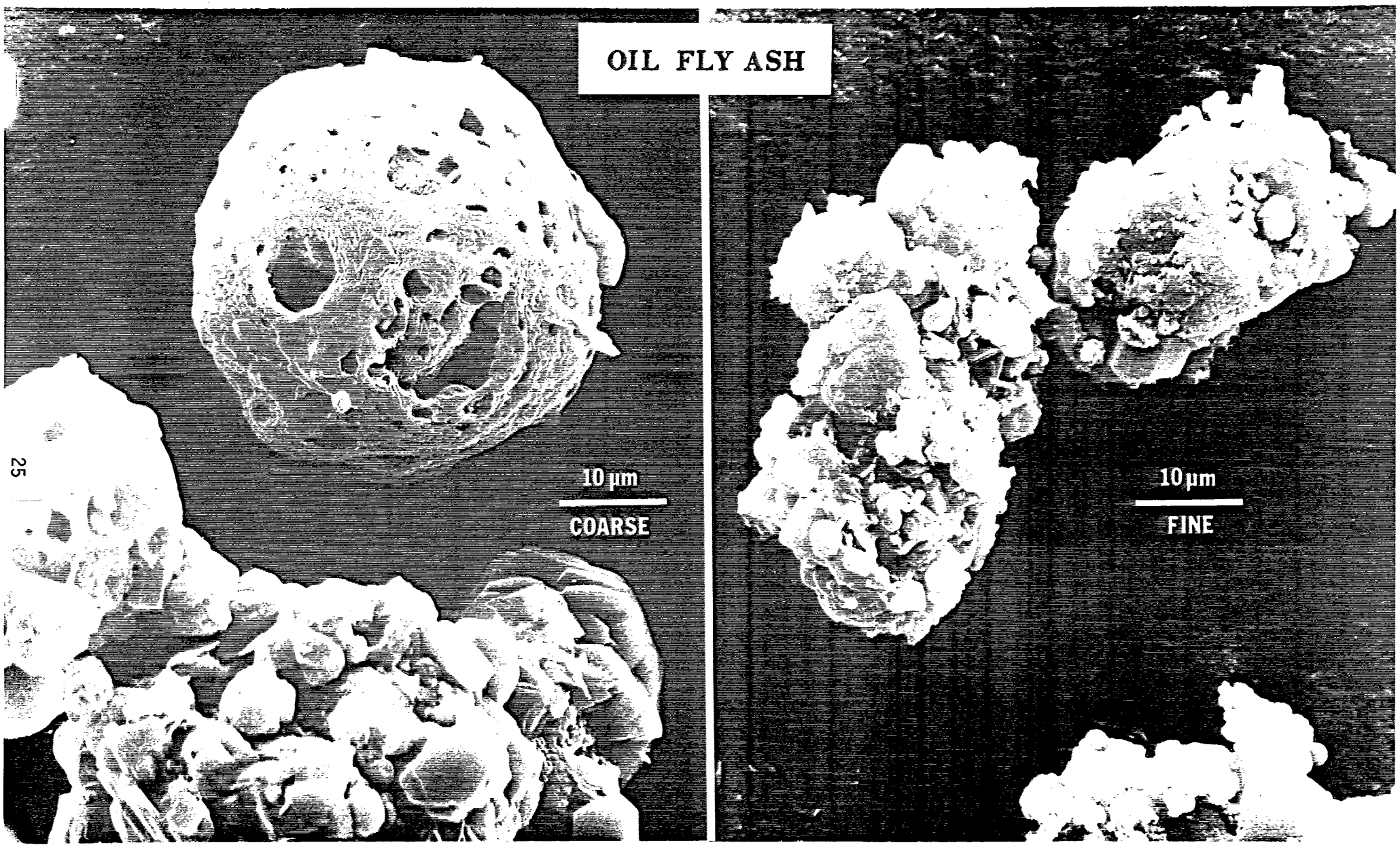
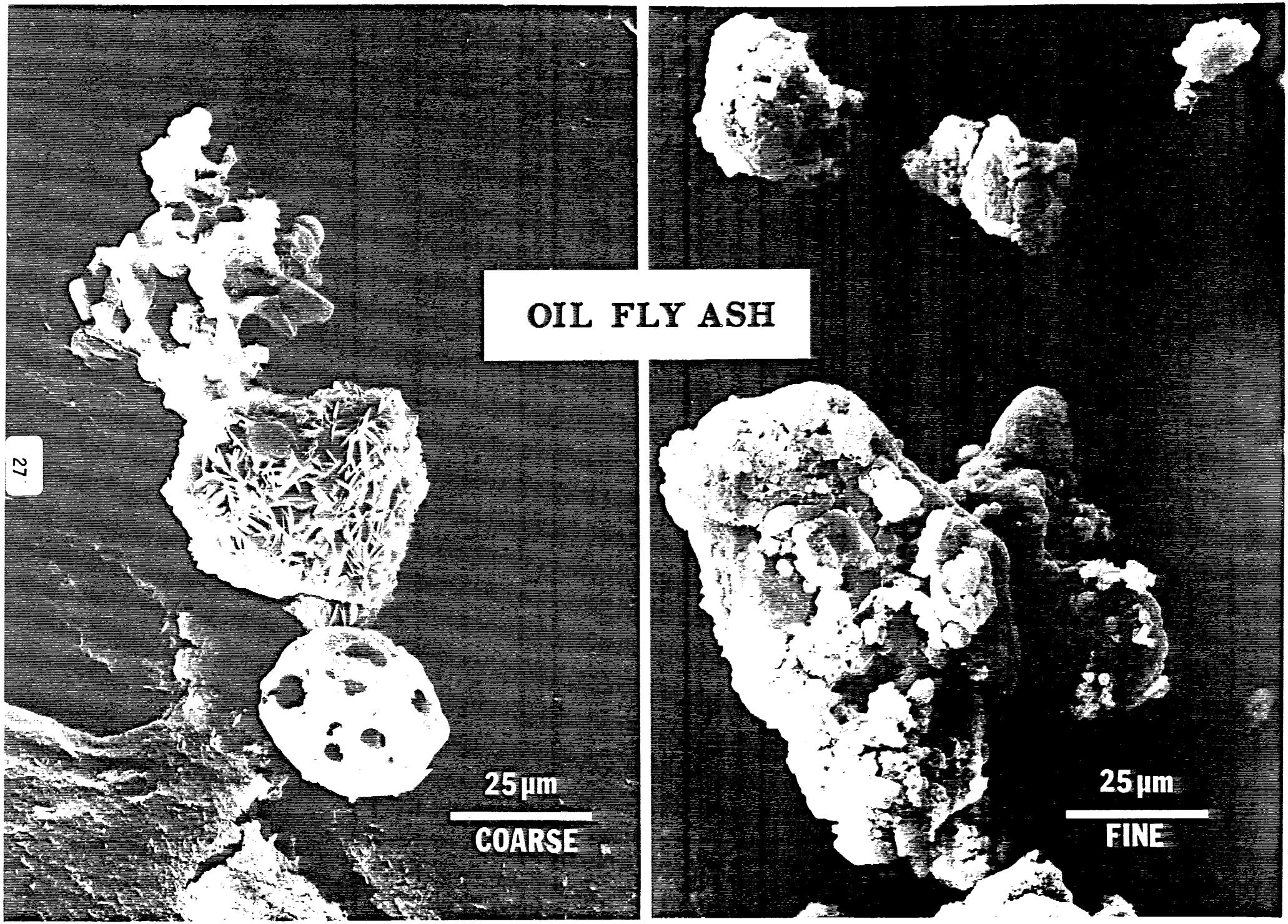


Fig. 4


OIL FLY ASH
25 µm
COARSE
25 µm
FINE

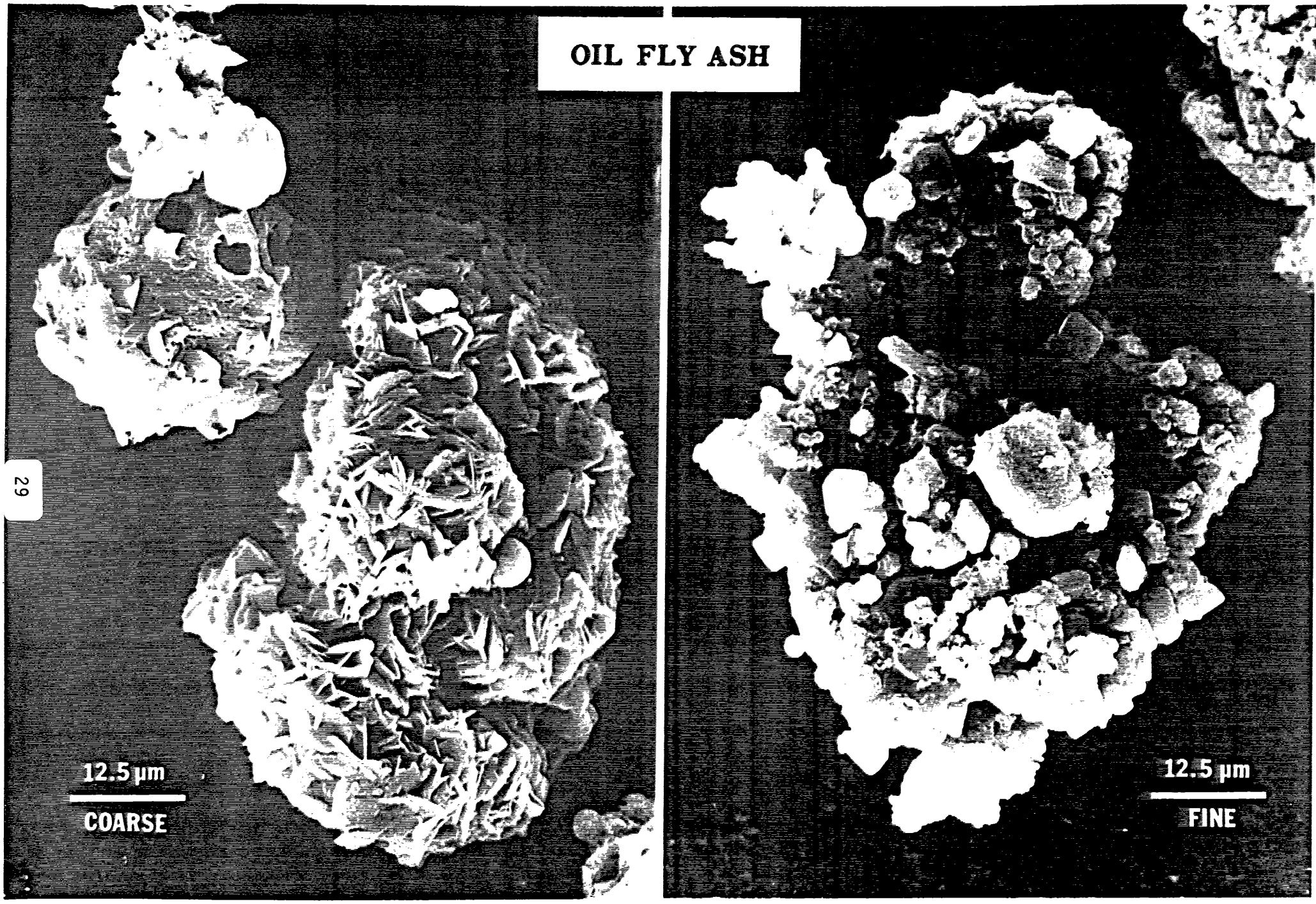


Fig. 6

Table 4. Size Distributions of Collected Oil Fly Ash[a]

	Fine Fraction			Coarse Fraction		
	Median (μm)		σ_g	Median (μm)		σ_g
	Raw Data	+42%		Raw Data	+32%	
Coulter Analysis						
CMD	1.2	1.4	1.6	2.3	2.6	1.8
VMD	3.1	3.7	NLN	21.6	24.6	2.1
VMAD	5.0	6.0	NLN	35.6	40.4	2.0
Smallest D	0.58	0.70	---	1.38	1.57	---
Smallest AD	(1.04)	(1.23)	---	(2.39)	(2.705)	---
Scanning electron micrographic Zeiss analysis (5)						
CMD	Too aggregated for resolution				2.3	2.2
VMD					(43.9)	NLN
VMAD					(72.1)	NLN
Photomicrographic Zeiss Analysis (5)[b]						
CMD		2.58	1.8		15.1	2.1
VMD		(6.87)	(1.8)		(79.5)	(2.1)
Density (Buoyancy Method)	2.52 (± 0.01) g/cm^3			2.69 (± 0.01) g/cm^3		

[a]CMD = count median diameter, VMD = volume median diameter, VMAD = volume median aerodynamic diameter, D = physical diameter, AD = aerodynamic diameter, NLN = not log-normal. Values in parentheses are calculated values.

[b]Samples suffer from aggregation of particles yielding larger sizes than when resolved into separate sizes by other methods (5).

Since the smallest aerodynamic diameter of particles collected by the sampler cyclone (coarse ash, Table 4) was between 2.39 and 2.71 μm, the effective cut-size of the sampler cyclone was seen to be between 4.78 and 5.42 μm AD rather than the smaller planned cut size. This is consistent with the flow rate measurements, viscosity, and density of the exhaust gases at the 140°C of the sampler system. Since 63% by mass of the oil fly ash in sample 6 was fine ash (Table 1), the mass median aerodynamic diameter is indicated to be 3.9 μm if the cyclone cut-size is assumed to be 5 μm and the ash geometric standard deviation is assumed to be 2.0. The size distribution of stack released fly ash is thus seen to be primarily in the respirable size range.

Ancillary samples were collected with a Sierra cascade impactor of the effluent stack aerosol by drawing a sampler from the main sampler probe through a ^{85}Kr discharger and mixing it with clean, dry air (zero gas from a compressed air tank) at a ratio of three parts clean air to one part exhaust gases. In this process of dilution, the sample stream was reduced to 22°C and about 80% relative humidity, a near ambient temperature and humidity, so that the mixing of stack released gases and particles with the atmosphere was simulated. The impactor data were fit to a log-normal distribution graphically, and the aerosol was found to have a mass median aerodynamic diameter of only 0.17 ± 0.06 μm, with geometric standard deviation of 18 ± 4. These meaningless results show that the aerosol collected by the impactor was not log-normal. Study of the data suggested that the actual aerosol consisted primarily of ultrafine particles probably formed by condensation of sulfuric acid and absorbed water. It is known that sulfuric acid is self-nucleating and will therefore form a condensation aerosol without requiring other condensation nuclei upon temperature reduction of the effluent stream below the dew point of the sulfuric acid upon emission from the smoke stack. This result is important in understanding the actual properties of the stack released aerosols: a bimodal distribution with solid particles (coated with some sulfuric acid) larger than 0.6 μm, similar to those collected with the heated fly ash sampler used in this study; and a mist of ultrafine droplets (smaller than 0.5 μm) consisting principally of sulfuric acid, formed upon mixing with the ambient air at the outlet of the smoke stack.

IV. INORGANIC CHEMICAL CHARACTERIZATION STUDIES

Elemental Analysis of Oil Fly Ash

The principal elements present in oil fly ash were determined by analysis of 47 elements using several instrumental and analytical techniques, including instrumental neutron activation analysis (Washington State University), atomic absorption spectrometry (AAS), and ion chromatography (IC). Coarse ash from sample LB/SCA/6 was studied directly, but the fine ash had to be extracted from the Teflon felt filter media with a nonpolar solvent, benzene (Nanograde, Mallinckrodt), prior to analysis. About 200 mg was used for preparative chemistry for AAS.

The fine ash was removed from a section of filter sample LB/FA/6(a). It was treated by ultrasonic agitation periodically over several days (about 15 hrs agitation) in benzene (Nanograde) in a Teflon container. The masses of coarse ash and fine ash used for preparative wet chemistry for AAS analysis studies were 204 mg and 220 mg, respectively.

Each ash was weighed and dissolved in 100 ml double distilled water shaken for 24 hours. A portion of the filtrate was analyzed for anions by IC and the rest analyzed for metals by AAS. The insoluble fraction was filtered with a polycarbonate membrane filter (0.1 μm, Nuclepore Corp., Pleasanton, CA). After weighing, these particles were dissolved in 100 ml 0.01 N HNO_3 (redistilled grade, G. Frederick Smith Co., Columbus, OH), shaken for 24 hours and filtered with a 0.1 μm Nuclepore filter and analyzed by AAS. After weighing, the water and nitric acid insoluble particles were dissolved with HF and boric acid and analyzed by AAS using the procedure by Silberman and Fisher (7). The insoluble residue, carbon, was filtered and weighed. A flow chart of the analysis preparative procedures for AAS is given below.

The results of the AAS and IC studies are summarized in Table 5. Most of the inorganic material present is clearly in the form of sulfates with 42.6% and 50.4% of the mass of the ash being accounted for by the SO_4^{2-} anion alone for coarse and fine ash, respectively. The presence of considerably more iron in the coarse ash could be explained by the obvious presence of rust particles in the coarse ash and the magnetic properties of about 10% of the particles when near a magnet. These rust particles probably originated with

Inorganic Analysis Procedures for Oil Fly Ash and Atomic Absorption Spectroscopy (AAS)

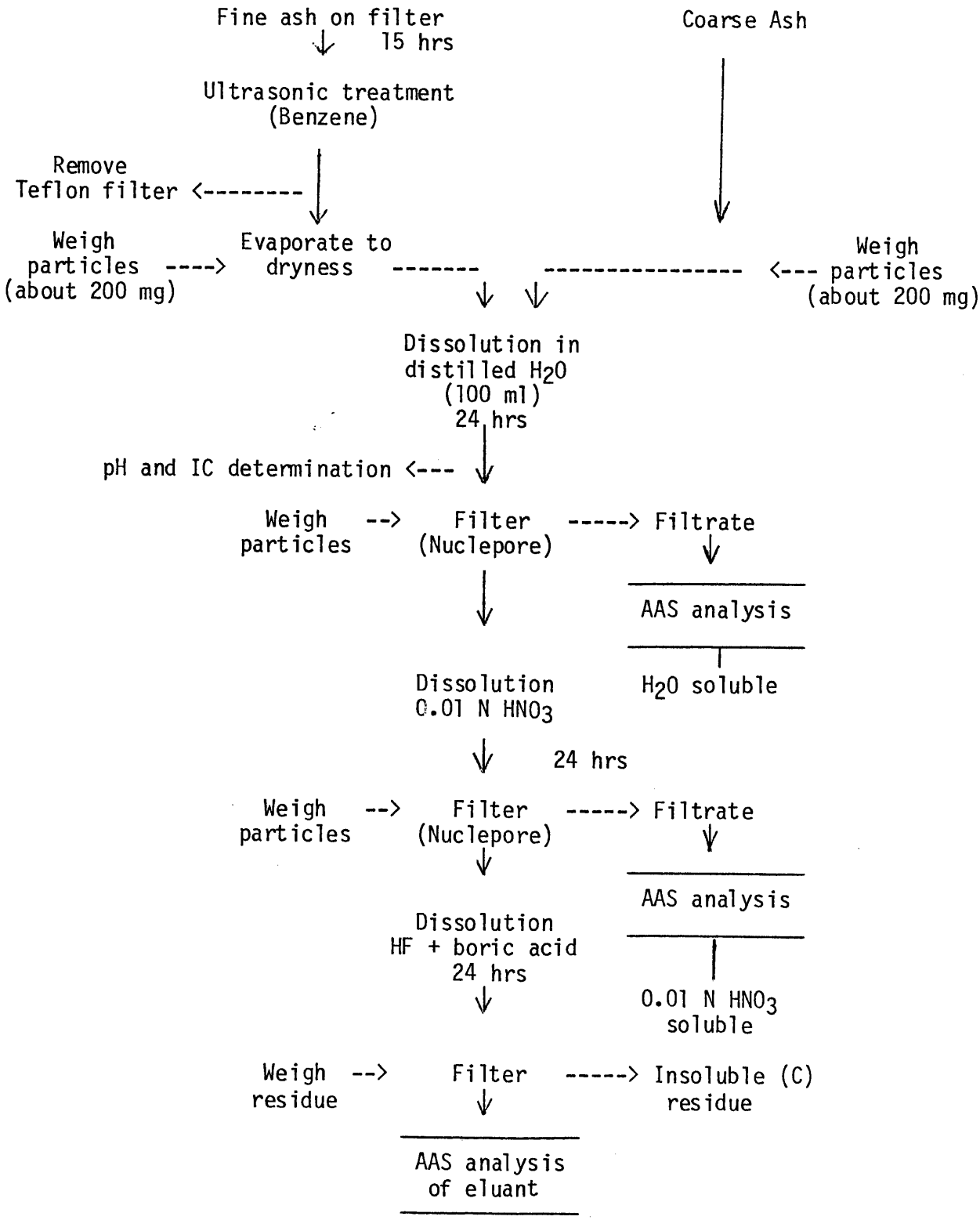


Table 5. Results of Atomic Absorption Spectrometry (AAS) for Elements and for Ion Chromatography (IC) for Anions for Oil Fly Ash Components

Component	Water Soluble (μg/g)		0.01 N HNO_3 Soluble (μg/g)		Bulk Total (μg/g)	
	Coarse	Fine	Coarse	Fine	Coarse	Fine
SO_4^{2-}	426 000	504 000	--	--	426 000	504 000
Fe	25 000	26 800	5 440	7 700	112 000	38 300
Ni	30 400	98 400	40	0	68 200	103 000
Na	27 500	36 600	27	33	28 100	36 800
Al	24 300	3 690	36	12	30 300	4 460
V	7 250	20 300	3 590	6 110	20 500	27 300
Ca	6 270	11 500	65	18	7 320	11 700
Si	125	4	4	4	11 500	8 880
Mg	2 950	5 920	9	2	4 900	6 650
Zn	2 190	2 920	4	3	3 360	3 040
Co	1 150	3 070	0	0	2 240	3 160
K	1 360	2 180	9	4	1 620	2 210
Cr	241	233	13	0	947	271
Mn	309	348	4	0	622	357
Pb	110	64	372	328	572	426
Cu	466	383	8	2	833	389
P	99	36	159	203	636	555
Sr	126	240	0	45	137	290
Ti	0	0	0	149	201	221
Mo	13	55	5	61	48	150
Li	13	22	0	0	15	22
Be	5	6	0	0	6	7
Rb	0	1	0	0	0	1
C	(Insoluble residue)				71 500	60 500

the power plant ducts or heat exchanger rather than from the fuel. Rust-like particles were less apparent in the fine ash, supporting their suggested mechanical origin.

Untreated ash (except for the extraction from the filter of fine ash) was sent to the Washington State University reactor facility for INAA. The results are shown in Table 6 along with the estimated standard deviations based upon statistical factors. For those elements measured by both INAA and AAS, there was reasonable agreement (mostly within 15%) between the two analyses, with the largest discrepancy being 167% and 45% differences between the fine ash values for copper and chromium, respectively. The reasons for this discrepancy is not apparent. For those elements listed in Tables 5 and 6, the AAS values are believed to be most accurate, however.

Most of the ash consists of soluble sulfate salts of several important metals, with about half of the mass of the ash samples being associated with the sulfate anion. The results of the solubility studies are shown in Table 7. Presuming the sulfate form for the elements found to dissolve in water (Tables 5 and 7), the resultant mass fraction of the ash associated with each form were computed. The results, using chemically plausible guesses as to principal chemical speciation, are given in Table 8. The presumed forms are in good agreement with the observed dissolution of ash and measured total sulfate when water of hydration is considered. In actuality, other soluble forms such as $NaVO_3$ may to some extent be represented in the ash, however. For the respirable fine ash 26% is seen to be identified with soluble nickel sulfate, 7 to 10% is associated with soluble iron sulfate, and 6.5% is associated with vanadyl sulfate (the presumed form of vanadium). High biological activities and potential for toxicity are known to be associated with certain nickel and vanadium compounds. These chemical analyses suggest these two elemental components as the principal potentially toxic species in the respirable oil ash for which additional studies are needed.

Table 6. Results of Instrumental Neutron Activation Analysis (INAA) of Untreated Oil Fly Ash Samples with Estimated Statistical Confidence Limits Given as the Standard Deviations (±SD)

Element	Coarse (μg/g)	Fine (μg/g)	Element	Coarse (μg/g)	Fine (μg/g)
Fe	148,000±1000	40,500± 500	Sb	8.8±0.8	8.6±0.9
Ni	54,500± 600	91,300±1100	Sm	3.7±0.1	8.8±0.1
Na	25,800± 139	39,800± 217	La	50 ± 1	80 ± 2
Al	35,200±5000	<5,000	W	<7	<11
V	22,300±1000	28,700±1300	Ga	105 ± 7	253 ± 13
Ca	<20,000	<11,000	Se	19 ± 2	5 ± 1
Zn	2,520± 173	2,550± 176	Cs	1.1±0.3	3.4±0.5
Co	1,730± 10	2,720± 14	Sc	1.2±0.0	2.3±0.0
K	<1,700	2,050± 600	Tb	1.0±0.2	1.6±0.2
Cr	980± 3	149± 2	Eu	0.9±0.1	2.1±0.1
Mn	543± 21	352± 19	Ce	41 ± 0.5	78 ± 1
Cu	988± 31	1,040± 40	Th	20 ± 0.2	51 ± 0.3
Ba	1,200± 107	1,160± 112	U	16 ± 1	15 ± 2
Sr	<500	<900	Hf	<0.7	<1.2
Ti	<8,700	<6,400	Ta	<0.9	<1.6
Mo	67± 16	112± 25	Zr	<100	<100
Cl	865± 294	<580	Ag	5 ± 1	<6.5
Dy	6± 0.7	14± 1.4	Hg (ppb)	242 ± 36	<200
As	251± 4	238± 4	Au (ppb)	<9	<16

Table 7. Solubility of Inorganic Components of Oil Fly Ash in Sequential 100 ml Volumes of Distilled Water, 0.01 N HNO_3, and $HF-H_3BO_3$ for 204 mg Coarse Ash and 220 mg Fine Ash, Respectively

Component	Water Soluble (%)		0.01 N HNO_3 Soluble (%)		$HF-H_3BO_3$ Soluble (%)	
	Coarse	Fine	Coarse	Fine	Coarse	Fine
SO_4^{2-}	100.0*	100.0*	--	--	--	--
Fe	22.3	70.1	4.9	20.1	72.8	9.8
Ni	44.6	95.6	0.1	0.0	55.4	4.4
Na	98.0	99.6	0.1	0.1	1.9	0.3
Al	80.1	82.7	0.1	0.3	19.8	17.0
V	35.4	74.2	17.6	22.3	47.0	3.5
Ca	85.6	98.4	0.9	0.2	13.5	1.5
Si	1.1	0.0	0.0	0.0	98.9	100.0
Mg	60.2	89.1	0.2	0.0	39.8	10.9
Zn	65.2	96.1	0.1	0.1	34.6	3.8
Co	51.1	97.3	0.0	0.0	48.9	2.7
K	84.0	98.8	0.6	0.2	15.5	1.1
Cr	25.4	86.0	1.4	0.0	73.3	14.0
Mn	49.7	97.5	0.6	0.0	49.5	2.5
Pb	19.2	15.0	65.0	77.0	15.9	8.2
Cu	55.9	98.5	1.0	0.5	43.1	1.0
P	15.6	6.5	25.0	36.6	59.4	56.8
Sr	92.0	82.8	0.0	15.5	8.0	1.7
Ti	0.0	0.0	0.0	67.4	100.0	33.0
Mo	27.1	36.7	10.4	40.7	62.5	22.7
Li	86.7	95.5	0.0	0.0	13.3	4.5
Be	83.3	85.7	0.0	0.0	16.7	14.3
Rb	0.0	100.0	0.0	0.0	0.0	0.0

*Presumed to be consistent with overall results, but some small quantity of insoluble sulfates, such as of Ba, may be present.

Table 8. Suggested Sulfate Form of Major Inorganic Water Soluble (Tables 5 and 7) Components of Oil Fly Ash Compared to Observed Total Soluble Fractions Using Chemically Plausible Species of the Measured Cationic Elements

Presumed Form Water Soluble	Mass Fraction in Presumed Form (%)		SO_4^{2-} Mass (%)	
	Coarse	Fine	Coarse	Fine
$FeSO_4$	6.80	7.30	4.30	4.61
$Fe_2(SO_4)_3$*	(8.95)	(9.60)	(6.45)	(6.92)
$NiSO_4$	8.02	25.95	4.98	16.11
Na_2SO_4	8.51	11.32	5.75	7.65
$Al_2(SO_4)_3$	15.38	2.34	12.95	1.97
$VOSO_4$	2.32	6.49	1.59	4.46
$CaSO_4$	2.13	3.90	1.50	2.75
$MgSO_4$	1.46	2.93	1.17	2.34
$ZnSO_4$	0.54	0.72	0.32	0.43
$CoSO_4$	0.30	0.79	0.19	0.49
K_2SO_4	0.30	0.48	0.16	0.26
$MnSO_4$	0.08	0.10	0.05	0.06
$CuSO_4$	0.12	0.10	0.07	0.06
pH		--	(2.73)	(2.63)
H_2SO_4			4.38	5.08
Total Dissolution	45.96	62.41	37.41	46.3
(Total Dissolution)*	(48.11)	(64.72)	(39.56)	(48.6)
Observed Dissolution$^{\Delta}$	64.66	85.14	42.55	50.44

*Alternate form of iron

$^{\Delta}$Differences between observed dissolution and total calculated dissolution are assumed to some extent to be associated with the presence of water of hydration with some of the sulfate species listed, such as $NiSO_4 \cdot 6H_2O$ or $H_2SO_4 \cdot 8H_2O$

X-ray diffraction (XRD), which is a non-destructive technique, was used to try to identify the major crystalline components of the coarse and fine oil ash. Due to the limited amounts of ash available the results of this work are qualitative. Nearly all the x-ray peaks found in the coarse ash are also found in the fine ash but the fine ash has several unmatched peaks. It appears that there are about 2-4 times more crystalline components in the fine ash based on increased peak heights of similar patterns found in both fractions. This is in general agreement with the inorganic chemical data.

XRD patterns clearly indicated the presence of $NiSO_4 \cdot 6\ H_2O$ in both fractions. $NaVO_3$ is also suggested to be present in both fractions but with a lower degree of certainty. Suspected compounds in the fine ash include $VOSO_4 \cdot 5\ H_2O$, $Al_2(SO_4)_3 \cdot 12\ H_2O$, some highly hydrated form of $Fe_2(SO_4)_3 \cdot X\ H_2O$ and traces of $NiSO_4 \cdot 7\ H_2O$. Quartz could not be verified due to peak overlaps of $VOSO_4 \cdot 5\ H_2O$ and $NiSO_4 \cdot 6\ H_2O$. If more samples had been available, XRD on the water insoluble fraction would have been done since the soluble vanadyl and nickel sulfates would not be present to interfere.

V. MICROBIAL MUTAGENESIS STUDIES OF OIL FLY ASH AND CONDENSATE

Mutagenesis Studies

The Ames microbial mutagenesis assay was done with all five standard histidine-requiring tester strains of Salmonella typhimurium at various concentrations of ash extract or collected condensate extract to provide dose-response data and an indication of potential toxicity of the extract to the bacterial cells. For these samples three replicates were evaluated for each concentration on three different days to reduce the inherent plate replicate variation and variation between days. Also, standard positive mutagens including mutagenic samples of coal fly ash were run at each time to test the normal level of response of the bacteria to known mutagens and experimental and laboratory controls were studied to evaluate the normal background level of revertants. The basis of the test is the evaluation of the production of colonies of revertants of Salmonella typhimurium not requiring histidine supplementations after the treatment of histidine requiring strains with potential mutagens. The results are indicative of a possible link to human cancer since mutagenic agents identified in this test are usually also carcinogenic to mammalian species.

Two extraction systems were used for the sample: (a) extractions with benzene/methanol (as separately discussed in the organic chemistry analysis section of this report), followed by evaporation and addition of dimethylsulfoxide (DMSO), and (b) extractions in horse serum that more directly simulate biological mobilization of potential mutagens.

Horse Serum Extract Preparations

The filter ash was removed from 20.15 g of sample LB/FA/6(a). It was removed by ultrasonicating for 2 min part of the filter in 300 ml dichloromethane (Mallinckrodt, Nanograde, St. Louis, MO) in Teflon bottles. The ash suspension was then filtered through solvent-washed Millipore Teflon filter (pore size 0.2 μm, type G, lot no. COD 291A9A, Bedford, MA). The filtered solvent was reused to remove more ash particles from the filter. This removal process was continuously repeated until as much ash was removed as possible. A total of 639 mg fine filter ash was harvested by using this procedure. Both the unused filter and separate solvent control samples were

prepared following the same procedures to check background level of revertant formation.

After the volume was greatly reduced by evaporation under N_2, the CH_2Cl_2 solvent was transferred to a serum bottle and then evaporated to dryness. The filter ash (639 mg) was then transferred to this bottle and ready for extraction with horse serum.

To prevent serum protein coagulation due to the acidity of the fly ash, the samples were neutralized first with 1.56 M NaOH solution before autoclaving; 1.2 ml and 0.95 ml of NaOH solutions were added to 634 mg cyclone ash and 639 mg filter ash, respectively. After extraction at 37°C for 7 days at a concentration of 30 mg ash per ml of horse serum (Microbiological Associates, lot no. 92743), the samples were centrifuged at 35,000 x g for 30 min. The supernatants were filtered through a 0.45 μm membrane filter to remove particulate matter. The filter control, the solvent control, and horse serum control samples were prepared following the same procedures.

Results of Mutagenesis Tests

The results of these mutagenesis studies are summarized in Tables 9, 10, and 11. Coarse oil fly ash extracts and effluent condensate samples did not demonstrate statistically significant levels of mutagenic activity at the 10% significance level (one-tailed) in any of the test systems. However, in two cases of a benzene/methanol extract from fine oil ash, one at the highest concentration with TA 100 with S-9 and one at the intermediate concentration with TA 1539 without S-9, statistically significant levels of mutagenic activity were demonstrated. However, the oil fly ash was highly toxic to the bacteria in these tests so that it is possible that the inherent mutagenic potential of the ashes was obscured by cellular toxicity in the majority of the tests and there may be in fact more mutagenic potential than is clear from these results. The highly toxic character of the fine ash samples, especially at a concentration of 4.83 mg/plate to the *Salmonella* bacterium, is suggestive of the potential toxicity of these materials to mammalian cells as well.

Preparation of condensate samples for the Ames mutagen test

The condensate samples were studied by nine different preparative methods as outlined below:

A. Sample no. 1

15 ml condensate, pH 2.3

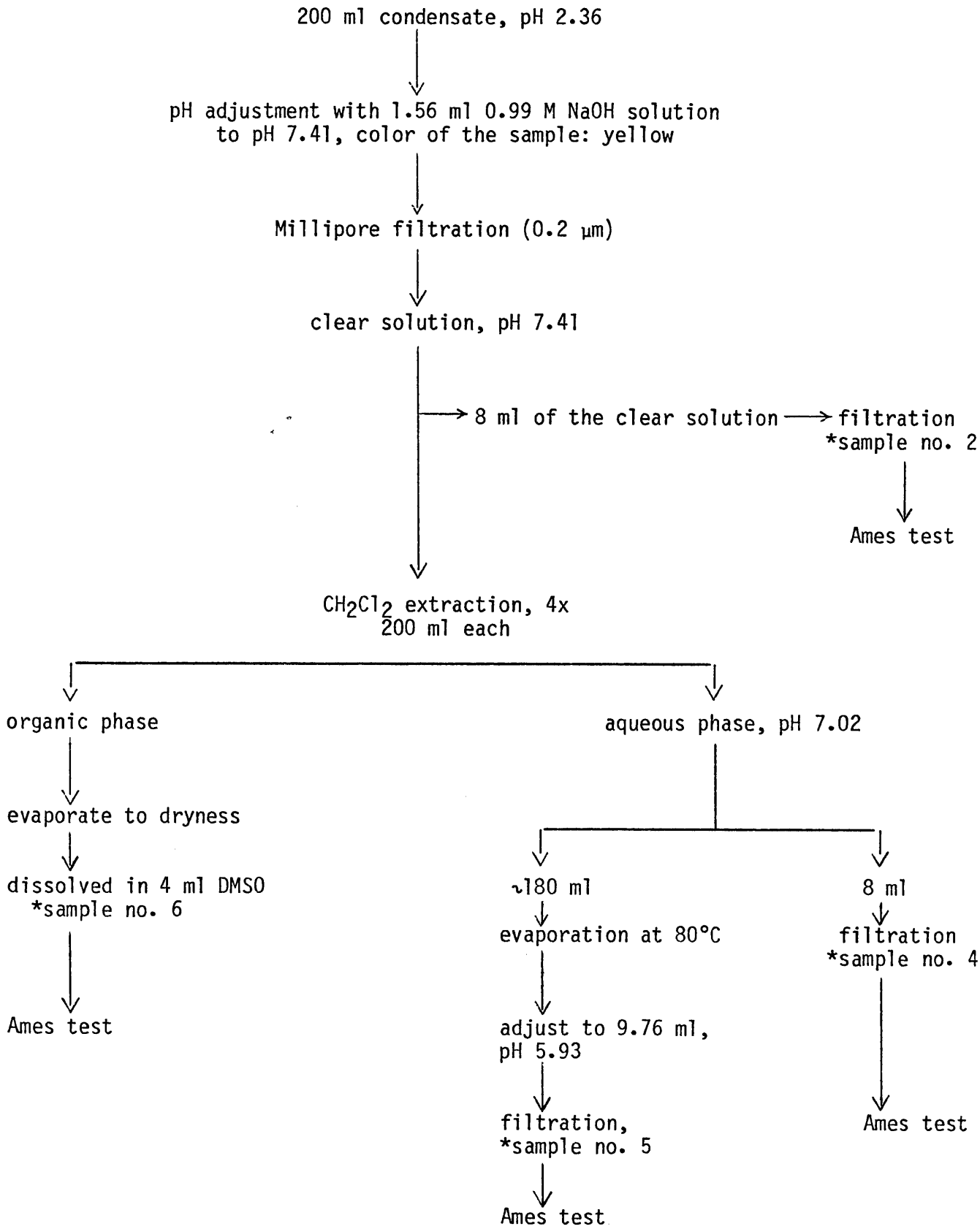
↓

filtration, *sample no. 1

↓

Ames test

B. Samples No. 2, 4, 5, and 6



C. Sample no. 3

308 ml condensate, pH 2.30, colorless

↓

pH adjustment with 0.99 M NaOH solution to 7.4
(2.5 ml NaOH solution was added)
color of solution: yellow

↓

evaporation at 80°C

↓

sample has orange-reddish-brown precipitate

↓

volume adjusted to 16.7 ml, pH 7.65

↓

centrifugation, 2,000 rpm, 5 min

↓

Millipore filtration (0.2 μm)
*Sample no. 3

↓

Ames test

D. Samples No. 7, 8, and 9

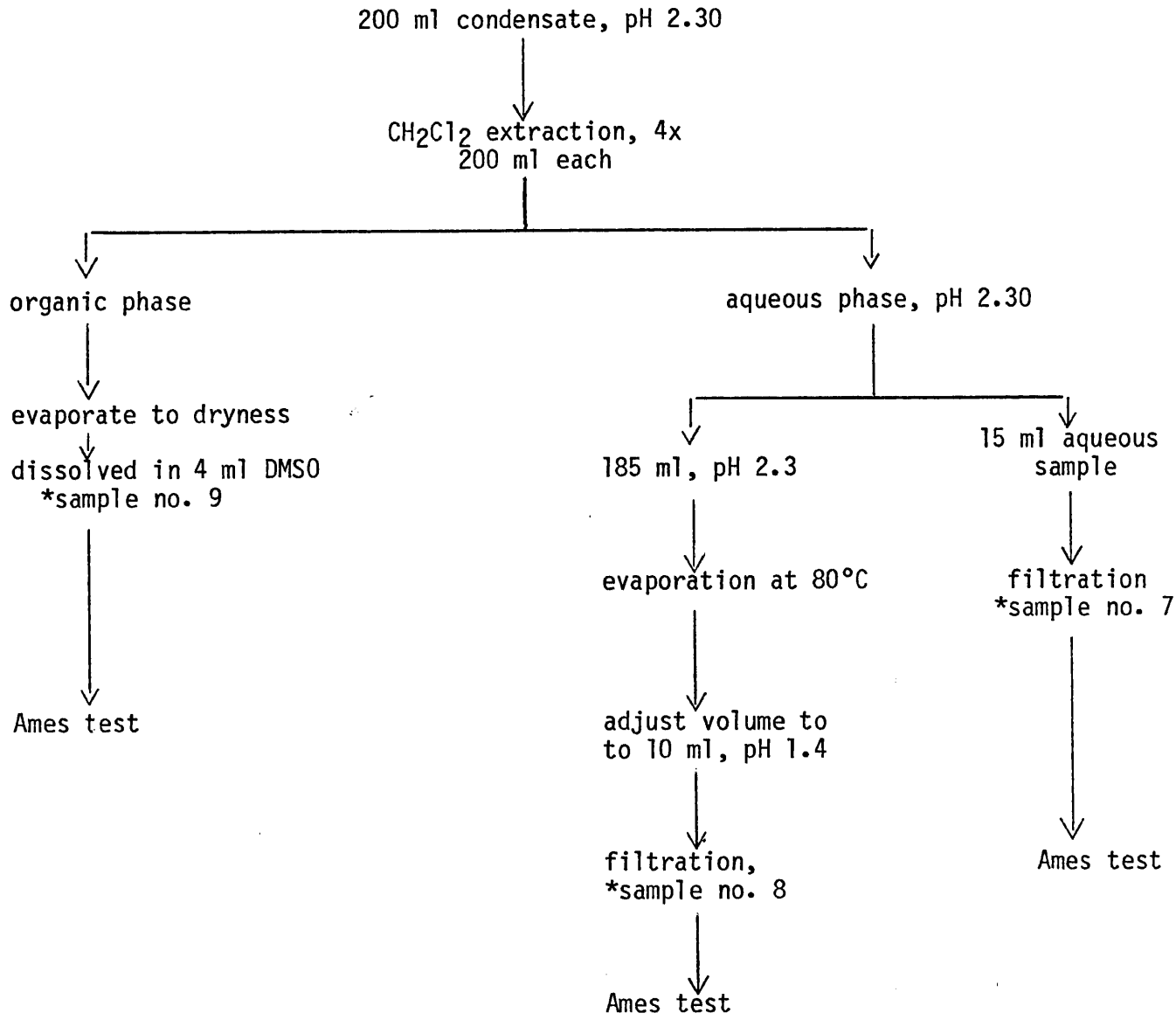


Table 9. Mutagenicity of Benzene/Methanol Extracts of Oil Fly Ash

Sample[b]	Number of Revertants (With/Without Metabolic Activation)[a]				
	TA 100	TA 1535	TA 98	TA 1538	TA 1537
Coarse Oil Ash					
5.1 mg	85 ± 11/ 63 ± 2	10 ± 4/11 ± 1	33 ± 3/ 30 ± 3	16 ± 3/16 ± 4	5 ± 1/ 8 ± 4
0.51 mg	76 ± 17/ 69 ± 11	7 ± 4/10 ± 1	21 ± 5/ 21 ± 3	16 ± 1/ 9 ± 3	8 ± 3/ 8 ± 3
0.051 mg	60 ± 10/ 65 ± 6	9 ± 3/11 ± 4	28 ± 3/ 24 ± 4	10 ± 1/11 ± 3	4 ± 2/ 6 ± 2
Fine Oil Ash					
4.83 mg	150* ± 33/Toxic	Toxic/Toxic	45 ± 26/Toxic	26 ± 16/Toxic	Toxic/Toxic
0.483 mg	81 ± 15/ 63 ± 8	5 ± 1/ 6 ± 4	29 ± 5/ 28 ± 11	14 ± 3/17* ± 3	8 ± 2/ 7 ± 3
0.0483 mg	71 ± 10/ 65 ± 5	9 ± 4/12 ± 4	18 ± 4/ 18 ± 5	13 ± 6/11 ± 7	6 ± 2/ 8 ± 3
Filter Control					
0.1 ml	68 ± 4/ 67 ± 13	8 ± 3/ 7 ± 2	22 ± 2/ 26 ± 12	11 ± 4/ 5 ± 2	----
Coal Ash (Cut 2)[c]					
1.8 mg	188 ± 18/126 ± 23	15 ± 6/ 8 ± 6	103 ± 27/127 ± 4	65 ± 13/53 ± 15	12 ± 4/17 ± 2
DMSO only					
0.1 ml	73 ± 8/ 73 ± 4	10 ± 2/ 9 ± 2	23 ± 3/ 14 ± 3	5 ± 3/ 6 ± 2	7 ± 1/ 8 ± 4

[a]Mean ± standard deviation from triplicate plates in Ames Salmonella mutagenicity assay.

[b]Dose per plate.

[c]Known mutagenic coal ash used as positive control.

*Statistically significant $p < 0.1$ with the one-tailed Mann-Whitney non-parametric test.

Table 10. Mutagenicity of Horse Serum Filtrates of Fly Ash

Sample[b]	Number of Revertants (with/without S-9)[a]				
	TA 100	TA 1535	TA 98	TA 1538	TA 1537
Spontaneous reversion	112±19/107±12	12±3/11±3	29±5/29±9	14±9/9±2	4±0/10±2
Horse Serum Control					
0.1 ml	130±18/116±12	12±4/15±2	30±2/26±4	21±6/16±4	9±4/ 5±3
Solvent Control					
0.1 ml	114± 5/113± 9	9±2/15±1	33±2/28±5	24±4/24±5	5±1/ 9±6
Filter Control					
0.1 ml	145± 6/100±22	9±4/13±5	31±4/28±5	26±6/27±4	4±0/10±1
Coarse Oil Ash					
3 mg	152± 8/142± 3	11±2/ 8±1	22±2/16±3	16±2/17±2	6±1/11±3
2 mg	117±38/119± 4	13±1/ 7±1	22±2/13±3	16±4/15±2	3±1/ 6±1
1 mg	114± 6/123± 9	6±2/ 7±2	30±4/20±7	17±2/11±2	9±3/11±2
0.5 mg	122± 7/109± 8	7±1/ 8±3	27±4/22±1	14±3/16±2	8±3/16±3
0.1 mg	123±18/102±14	12±4/10±3	24±9/21±2	14±4/13±4	11±2/10±2
Fine Oil Ash					
3 mg	102±24/ 71± 9	toxic/toxic	14±2/16±2	31±4/13±1	5±3/ 3±2
2 mg	91± 5/ 92±11	toxic/toxic	17±4/17±2	13±1/23±2	3±3/ 4±4
1 mg	100±18/111±28	5±3/ 6±3	18±3/28±4	13±3/15±2	5±3/ 8±2
0.5 mg	131±11/105±39	8±2/ 6±3	20±3/24±3	23±3/27±1	6±2/12±3
0.1 mg	108±17/123± 9	10±2/11±1	32±4/22±3	27±5/27±1	2±2/12±2
Coal Ash, cut 3[c]					
4 mg	261±41/214±16	9±3/12±4	148±4/154±2	204±19/276±25	17±7/23±4

[a]Mean ± standard deviation from triplicate plates in Ames *Salmonella* mutagenicity assay.
[b]Dose per plate.
[c]Known mutagenic coal ash used as positive control.

Table 11. Mutagenicity of Various Condensate Samples in 5 *Salmonella typhimurium* Tester Strains

Sample[b]	Number of Revertants (with/without S-9)[a]				
	TA 100	TA 1535	TA 98	TA 1538	TA 1537
Spontaneous reversion	112±19/107±12	12±3/11±3	29± 5/29±9	14±9/9±2	4±0/10±2
Phosphate Buffered Saline	111±11/113±17	8±3/16±2	30± 2/27±7	22±4/22±4	5±1/14±2
DMSO Control	115±15/100±11	11±3/16±2	30± 8/28±3	27±2/12±4	7±3/ 4±2
Condensate					
1	103±14/ 96±27	10±2/11±1	31± 2/30±3	17±1/15±1	6±1/13±6
2	101±12/ 84±19	9±0/11±3	34± 7/24±2	18±1/16±1	5±4/15±4
3	112±10/120±10	11±2/15±1	35± 6/21±3	16±4/16±1	8±3/10±1
4	111±15/111± 5	13±2/10±3	33±10/31±4	22±6/17±4	6±2/10±2
5	114± 1/116± 7	16±2/16±2	25± 5/25±2	17±3/17±4	6±2/14±2
6	110±17/ 90±31	12±2/12±1	29± 7/14±1	12±2/13±2	5±1/13±3
7	117± 6/103±12	11±1/12±7	34± 7/23±4	16±6/18±3	7±4/14±4
8	81±20/toxic	toxic/toxic	21± 3/16±1	toxic/toxic	4±2/toxic
9	91±10/ 97±12	12±3/18±2	25± 3/21±2	16±2/16±1	8±2/10±2

[a]Mean ± standard deviation from triplicate plates.

[b]Dose per plate.

VI. MAMMALIAN CELL TESTING - PULMONARY ALVEOLAR MACROPHAGES

In Vitro Studies of Pulmonary Alveolar Macrophages

The first line of defense of the lung to inhaled particles is the action of the pulmonary alveolar macrophage scavenger cells to collect the particles by engulfment and prevent their direct contact with other cells. In these studies pulmonary alveolar macrophages were harvested from the lungs of rabbits and cultured with and without various concentrations of respirable fine oil fly ash and challenged with carbon coated plastic macrospheres to measure viability changes, cellular attachment capabilities, phagocytosis, and the possible cytotoxicity of the fly ash particles.

Fine respirable ash was harvested as described in the inorganic chemistry section of this report and suspended in maintenance media (having 20% rabbit serum). Six different concentrations (a) 1.71 μg/ml, (b) 5.7 μg/ml, (c) 17.1 μg/ml, (d) 51.3 μg/ml, (e) 56.7 μg/ml, and (f) 512.7 μg/ml were studied for dose-response determinations. Also studied were the samples of the filtered eluent and resuspended filtered particles to evaluate the effect of the soluble ash compounds independently of the less soluble particulate fraction.

Controls included plain media and media containing inert particles of fused montmorillonite clay at a concentration of 7.72 mg/ml. All samples were run in triplicate or quadruplicate.

Materials and Methods for Macrophage Studies

New Zealand white rabbits weighing 5-6 pounds (2.3-2.7 kg) were used to obtain living macrophages. These respiratory-disease free animals were obtained from H.A.R.E. Rabbits for Research (Hewlitt, NJ). The animals were placed in a metal restrainer and anesthetized by intramuscular injection of Rompum (Xylazine, Haver-Lockhart, 20 mg/ml) at 5 mg/kg. The second injection given was Ketamine (Parke-Davis, 100 mg/ml) at 50 mg/kg (Current Vet. Therapy VII Sm. Animal Pract. 1980). The animals were then sacrificed by an air embolism via marginal ear vein (Mryvik et al.).

The abdominal and thoracic area were shaved and disinfected with 70% alcohol. The thoracic area was then exposed and the lung excised. The excised lungs were placed in a sterile petri dish. The trachea was cannulated with a 16 ga blunt tip needle held in place by suture. A ringstand and clamp were used to position the lung (with hemostat) in order to facilitate the lavaging of the lungs. To harvest and maintain the alveolar macrophages, the lung was lavaged six times with 30 ml volumes of prewarmed 37°C Hanks solution (Balanced Salt Solution) using a 60 ml syringe. Lavage fluid was collected in 50 ml conical centrifuge tubes (on ice) and centrifuged at 1400 rpm (Sorvall RC-58) for 15 min at 5°C. The supernatant was decanted, cells were pooled for those obtained from a single rabbit and resuspended in cold Attachment medium (Begman et al.). The cells were counted using a hematocytometer and viability was checked using the trypan blue exclusion test. Costar plastic Leighton tubes containing 9 x 55 mm cover slips were seeded with 0.1 ml of 1.5×10^7 macrophages/ml in 1 ml of Attachment media (199-GIBCO) with 25 mM Hepes, Hanks Salts, L-glutamine; penicillin 10,000 units, streptomycin 10,000 mcg/ml and fungizone 25 mcg/ml. The Leighton tubes were incubated for 2 hours at 37°C in an atmosphere of 4% CO_2 enriched air. Upon completion of 2 hour incubation time, the Leighton tubes were washed 2 times with Hanks solution (pre-warmed 37°C) and 1 ml Maintenance media was added containing different concentrations of fine oil ash particles. Prior to adding ash to the Maintenance media (media plus 20% rabbit serum), the pH was adjusted to the physiological range of about 7.3 to 7.4 by addition of sodium hydroxide.

Macrophage cultures containing oil ash particles at the chosen concentrations were incubated for 20 hr. After completion of the 20 hr incubation period, the Leighton tubes were washed with prewarmed Hanks and viabilities were measured using the trypan blue exclusion viability test. Fresh prewarmed (37°C) Maintenance media was added to the remaining cells with microspheres ("carbonized plastic" - 3M Company) of 3.6 μm ± 1 μm diameter at a ratio of 20:1 (20 microspheres per 1 macrophage). The Leighton tubes were returned to 37°C 4% CO_2 atmosphere for 1 hr. The coverslips were removed from Leighton tubes, washed with nonsterile phosphate buffered saline, and forced hot air dried. Coverslips were then stained and mounted for viewing to measure phagocytosis.

Results of Macrophage Studies

The results of three studies of the effects of fine oil fly ash are summarized in Table 12. In reviewing these results it is important to note that they represent three separate studies with different control baselines which must be separately evaluated. Both the fly ash particles and the dissolved soluble components of the fly ash particles were found to be highly toxic to cells with the equivalent of 156.7 μg of ash per ml causing cell lysis and reduced phagocytic function as observed in the plastic sphere phagocytosis challenge test and 512.7 μg/ml causes cell death. These results are consistent with those previously reported for vanadium salts (Fisher et al., 1978). They found reduced viability and reduced phagocytosis in mouse macrophages at 7 μg vanadium per ml and cell death at 9 μg/ml. 156.7 μg of fine oil ash contains about 4.3 μg of vanadium and 512.7 contains about 14 μg of vanadium, so that the observed response of macrophages was in the range of total vanadium concentration of 4.3 μg/ml to 14 μg/ml. Since 74.2% of the vanadium dissolved, the residual particles suspension should contain 3.2 to 10.4 μg vanadium per ml. The other biologically active trace metals, particularly nickel, may also have a role in the observed cellular toxicity of respirable oil fly ash and dissolvable components. However, our ancillary studies show that a clear correlation between the presence of vanadium and observed cellular toxicity.

Table 12. Results of Three Separate Studies of Fraction of Cells Alive (Viability), Nonadherence to Cover Slips and Associated Surfaces in the Test System, and Phagocytosis Measurements for the In Vitro Pulmonary Alveolar Macrophage Studies of Oil Fly Ash With Rabbit Macrophages

	Test No.[a]	Viability (20 hr, %)	Nonadherence (20 hr, %)	Phagocytosis (20 hr, %)		
		Mean	Mean	Mean	SE	
Controls	1	97	0.2	63	2.0	3
	2	100	0.2	65	6.0	3
	3	92	2.3	27	9.0	4
Oil ash 1.71 μg/ml	1	95	0.2	52	6.0	3
	2	99[c]	0.1	66	5.0	3
	3A	96	2.2	25	5.5	4
	3B	95	2.2	43	5.0	4
Oil ash 5.7 μg/ml	1	93	0.2	56	6.3	3
	2	99[c]	0.0	54	4.0	3
	3A	94	2.2	22	6.0	4
	3B	93	2.3	48	2.0	4
Oil ash 17.1 μg/ml	1	89	0.2	34*	3.8	3
	2	79*[c]	0.1	53	1.0	3
	3A	100	2.1	38	7.5	3
	3B	98	1.7	47	5.5	4
Oil ash 51.3 μg/ml	1	99	0.3	12*[b]	4.2	3
	2	78*[c]	0.2	31*[b]	6.2	3
	3A	93	2.1	29	4.5	4
	3B	90	2.5	34[b]	6.4	3
Oil ash 156.7 μg/ml	1	85*	0.2	16*[b]	5.3	3
	2	74*[c]	0.1	22*[b]	2.4	2
	3A	--	--	--	--	-
	3B	--	--	--	--	-
Oil ash 512.7 μg/ml	1	--	--	--	--	-
	2	--	--	--	--	-
	3A	100	2.2	27[b]	5.0	4
	3B	91	2.0	4*[b]	2.0	4

[a]Test 1 - Ash particles + leachate, Test 2 - Ash particles + leachate, Test 3A - Ash particles without leachate, Test 3B - Leachate alone.

[b]Toxic to cells. Most cells were lysed; values are for surviving cells.

[c]After phagocytosis of microspheres.

*Statistically significant, $p < 0.05$.

VII. ORGANIC CHARACTERIZATION STUDIES

Since only a limited quantity of oil fly ash could be collected, some of the proposed organic analyses were precluded. For all of the experiments described in this section, an experimental control sample (blank) was prepared for each laboratory procedure. This was an essential precaution in the trace organic analytical studies due to the presence of low levels of high molecular weight contaminants in the solvents used and the relative ease with which trace samples can become contaminated in the field or laboratory with plasticizers, grease, oils, etc., unless scrupulous procedures are employed. As the analytical results described in this section demonstrate, the analytical laboratory procedures were, in fact, carried out without the introduction of any contaminants or artifacts. The results show that the Teflon fabric filter used to collect the fine oil fly ash was possibly contaminated with organic material. The filter control (blank) had been handled in the same manner as the filter used to collect the fine oil fly ash sample and was found to be seriously contaminated (probably by marking crayon used by the manufacturer). Details of all the experimental procedures for this work are presented in the Appendices to this section.

1. Total Organic Extracts for Mutagenicity Testing

To determine the possible mutagenicity of any organic components on the surface of the oil fly ash samples, extractions were carried out with an azeotropic mixture of benzene/methanol (60:40 by weight). This solvent mixture is commonly used in organic geochemical studies to provide a total solvent-soluble organic mixture of materials such as oil shales, sediments, etc. This mixture of a relatively nonpolar solvent (benzene) together with a polar solvent (methanol) will satisfactorily dissolve both nonpolar and polar organic molecules. There is some indirect evidence that this solvent mixture also dissolves some surface-coated inorganic species--presumably because of the high polarity of the methanol.

The samples prepared for mutagenicity testing are summarized in Table 13 together with other appropriate information. The samples were extracted three times with azeotropic benzene/methanol. After centrifugation, the supernatants were filtered using nitrogen gas through pure Teflon filters (0.2 μm

pore size) held in a stainless steel holder with Teflon-coated O-rings. The filtrates were combined, evaporated just to dryness, and the resulting extracts were each suspended in 6 ml of dimethylsulfoxide (DMSO) for Ames testing.

Table 13. Benzene/Methanol Extracts of Oil Fly Ash for Mutagenicity Testing (See Appendix A1 for experimental details)

Sample	Weight	Volume of Extraction Solvent
Coarse ash	306 mg	3 x 10 ml
Control	--	3 x 10 ml
Fine ash (ash + filter)	∿290 mg (9.62 g*)	a) 1 x 60 ml b) + 1 x 35 ml c) + 1 x 35 ml
Control filter	(11.32 g**)	a) 1 x 60 ml b) + 1 x 35 ml c) + 1 x 35 ml

*Initial weight of total filter	= 78.08 g
Weight of total ash + filter	= 80.50 g
Weight of total ash collected	= 2.42 g
Fraction of total ash + filter used for extraction	∿ 1/8
Weight of ash + filter used for extraction	= 9.62 g = 12% of total
Estimated weight of fine ash extracted	= 0.12 x 2.42 g = 290 mg
**Weight of total filter	= 86.56 g
Fraction of total filter used for extraction	∿ 1/8
Weight of filter used for extraction	= 11.32 g = 13% of total

Due to the limited availability of the samples for extraction, it was not possible to obtain weights of the extracted organic material. Quantitative data, therefore, are reported in terms of the original weight of the ash extracted. The results from this experiment are presented and discussed in the Microbial Mutagenesis Section.

2. Characterization of Nonpolar Components by Gas Chromatography

The relatively nonpolar components of the oil fly ash and their corresponding control samples were obtained by extraction of the samples with benzene. This solvent would be expected to dissolve compound classes such as straight-chain and branched-chain hydrocarbons, esters, ketones, and aromatic hydrocarbons, including benzo-a-pyrene and related compounds.

The samples used for extraction are summarized in Table 14, together with other appropriate information. These samples were extracted three times with benzene and, after centrifugation, the supernatants were filtered in a nitrogen atmosphere with pure Teflon filters (0.2 μm pore size) held in a stainless

Table 14. Extracts of Oil Fly Ash for Gas Chromatography and High Performance Liquid Chromatography

Sample	Weight	Volume of Extraction Solvent (Benzene and Benzene/Methanol)
Coarse ash	999 mg	3 x 10 ml
Control	--	3 x 10 ml
Fine ash (ash + filter)	∿ 770 mg (26.14 g*)	3 x 120 ml
Control filter	(26.85 g**)	3 x 120 ml

*Initial weight of total filter	= 78.08 g
Weight of total ash + filter	= 80.50 g
Weight of total ash collected	= 2.42 g
Fraction of total ash + filter used for extraction	∿ 1/3
Weight of ash + filter used for extraction	= 26.14 g = 32% of total
Estimated weight of fine ash extracted	= 0.32 x 2.42 g = 770 mg
**Weight of total filter	= 86.56 g
Fraction of total filter used for extraction	∿ 1/3
Weight of filter used for extraction	= 26.85 g = 31% of total

steel holder having Teflon-coated O-rings. The filtrates were combined, concentrated under a stream of dry nitrogen to a volume of 100 μl, and then inspected visually. The color of these solutions was found to be:

coarse oil fly ash	clear
control	clear
fine oil fly ash + filter	light yellow
control filter	yellow-brown

These colors indicate that the most organic material was contained in the control filter and the next highest amount in the extract of the fine oil fly ash + filter. It could not be determined visually whether or not the remaining two extracts contained any organic material. This simple experiment demonstrated that a significant amount of organic material was extracted from the control filter which had not been installed or used in the sampler. As the filter used for the collection of the fine oil fly ash had been handled in the same manner as the control filter, it must be concluded that the filter on which the ash was deposited was probably also contaminated with organic material, although much less total organic material appeared to be extracted from the fly ash filter. This means, therefore, that any organic material extracted from the fine oil fly ash + filter sample could come from either the contaminated filter or the fly ash collected on the filter or from both the filter and the fly ash; experience with other analytical studies of environmental samples has shown that in these problem cases, it is frequently impossible to adequately assess the relative contributions to the total extracted organic material. In view of this, any results from the extract of the fine oil fly ash + filter sample must be evaluated very cautiously. As the coarse ash sample was not collected using a Teflon fabric filter and because the laboratory control sample was determined to be free from contamination problems, it should be assumed that the results from the organic extracts of the coarse ash do not involve contamination.

The benzene extracts were analyzed by capillary gas chromatography with simultaneous detection by a flame ionization detector (FID) and by a

thermionic specific detector (TSD) operated in the nitrogen mode. The traces from the flame ionization detector represent all the organic components in the mixture, whereas the traces from the thermionic specific detector represent only the nitrogen-containing components for which this detector is selective. All chromatograms were obtained under identical conditions (see Appendix Section A3) to facilitate subsequent comparisons between the samples. The chromatographic parameters were designed so that all components that would elute between benzene (the injection solvent) and high molecular weight components such as coronene or n-$C_{40}H_{82}$ alkane could be determined.

The chromatograms from the procedural control sample are shown in Figure 7-b and demonstrate that essentially no extraneous components were introduced during the weighing, extraction, concentration and other analytical procedures. The slight rise in the baseline in the FID trace is due to minimal column bleed at high temperatures and does not reflect the material in the sample. Each of these chromatograms represents 5% of the total benzene extract obtained (see Table 15).

Table 15. Capillary Gas Chromatograms of Benzene Extracts

Sample	Total Number of Chromatograms Obtained (FID + TSD)	Representative Chromatograms Illustrated in Figure	Amount of Fly Ash Extracted for each Chromatogram*	% of Total Extract/ Detector**
Coarse oil fly ash	2	7-a	25 mg	2.50
Control	1	7-b	--	5.00
Fine oil fly ash + filter	4	7-c	∿3 mg	0.40
Control filter	3	7-d	--	0.02

*Each individual chromatogram (FID or TSD in Figure) represents the benzene extract from this weight of oil fly ash.

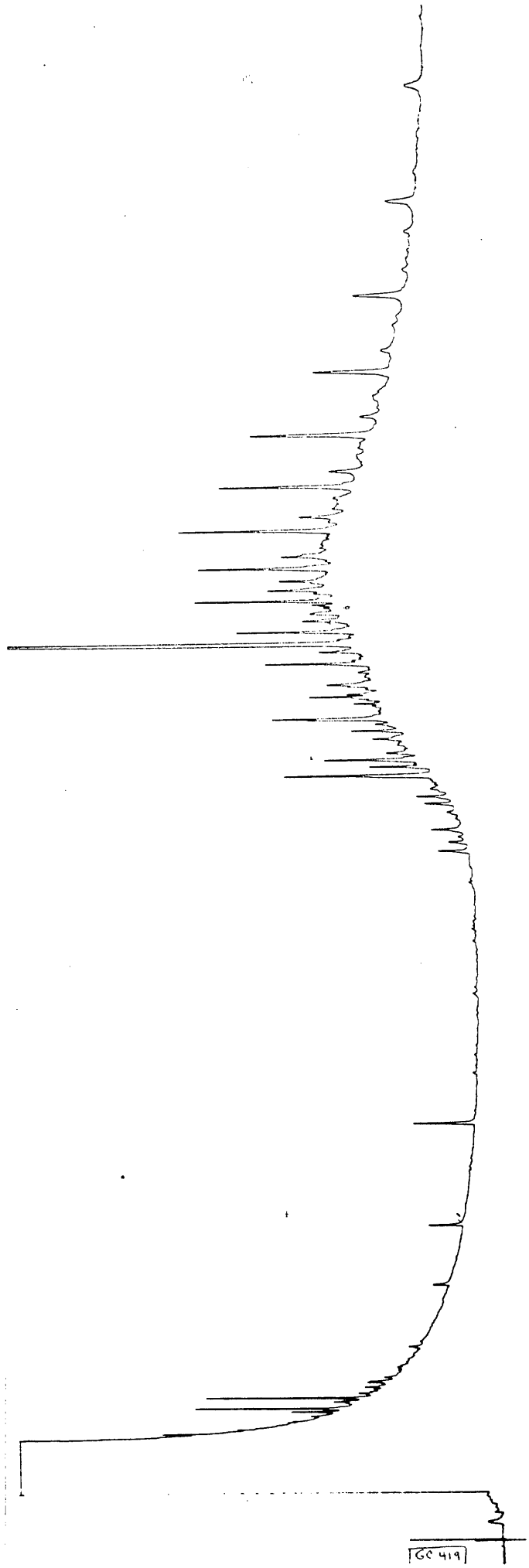
**Each individual chromatogram (FID or TSD in Figure) represents this percentage of the total benzene extract

Fig. 7-a. Capillary gas chromatograms of the benzene extract of coarse oil fly ash.

Upper trace: flame ionization detector

Lower trace: thermionic specific detector, nitrogen mode

Each chromatogram represents the benzene extract from 25 mg of coarse oil fly ash (2.5% of total benzene extract).


GC 419

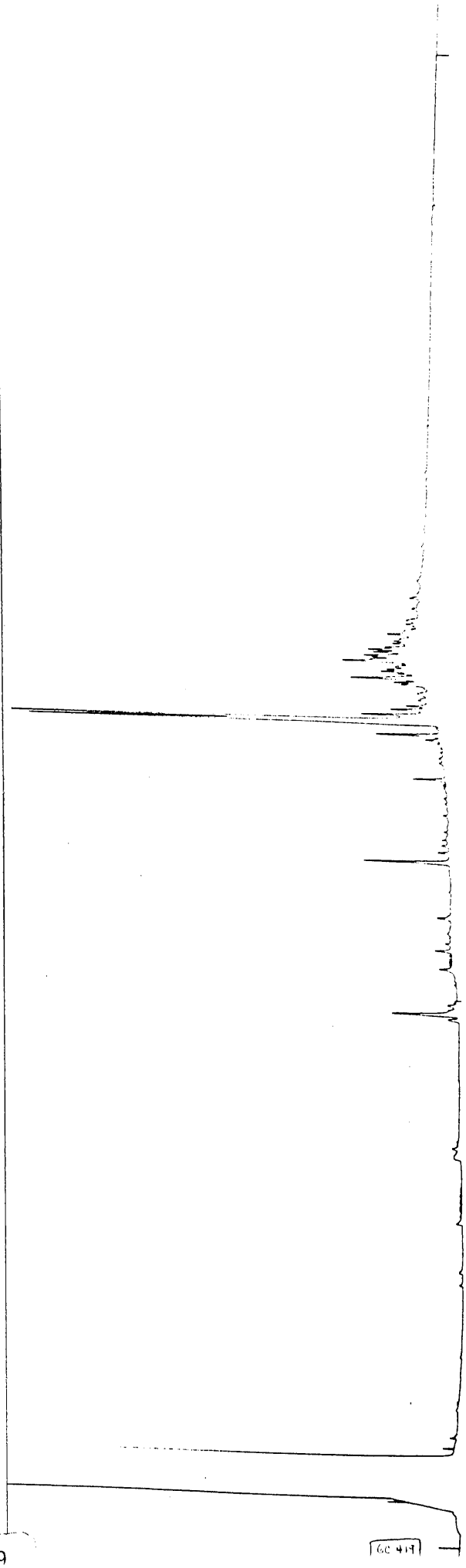
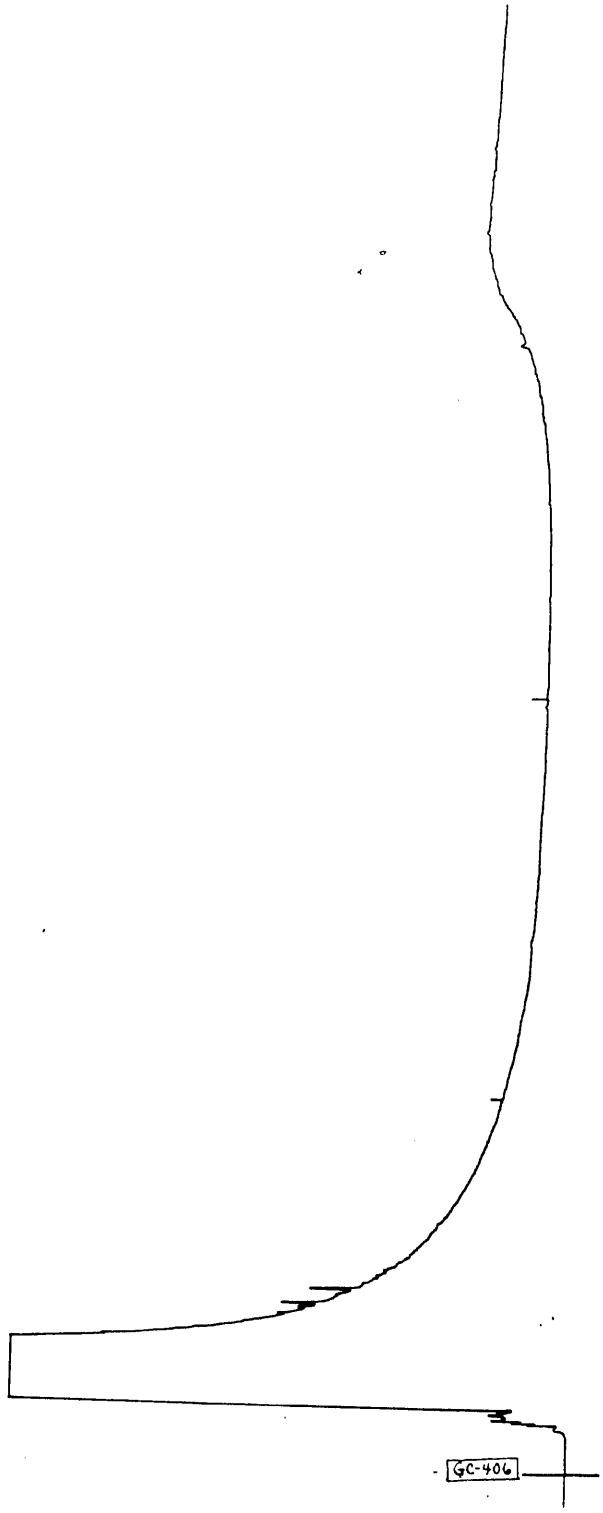

GC 419

Fig. 7-b. Capillary gas chromatogram of the benzene extract of control sample.

Upper trace: flame ionization detector

Lower trace: thermionic specific detector, nitrogen mode

Each chromatogram represents 5% of the total benzene extract.


GC-406

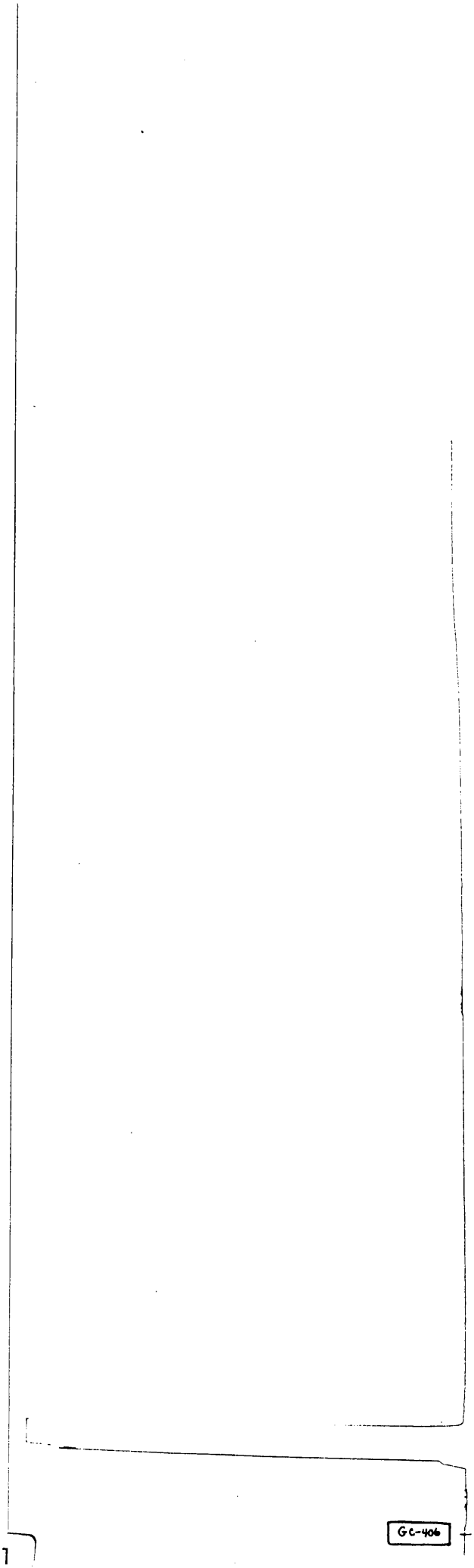
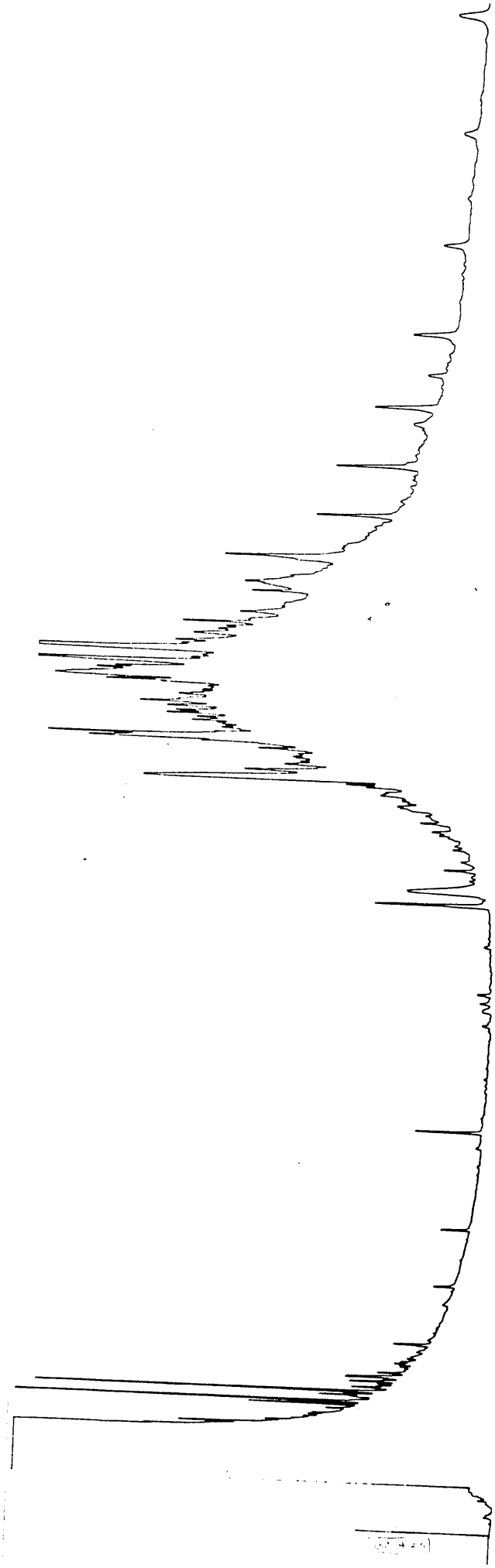

GC-406

Fig. 7-c. Capillary gas chromatogram of the benzene extract of fine oil fly ash + filter.

Upper trace: flame ionization detector

Lower trace: thermionic specific detector, nitrogen mode

Each chromatogram represents the benzene extract from ~3 mg of fine oil fly ash (estimated, see Appendix Section A2), or 0.4% of the total benzene extract.



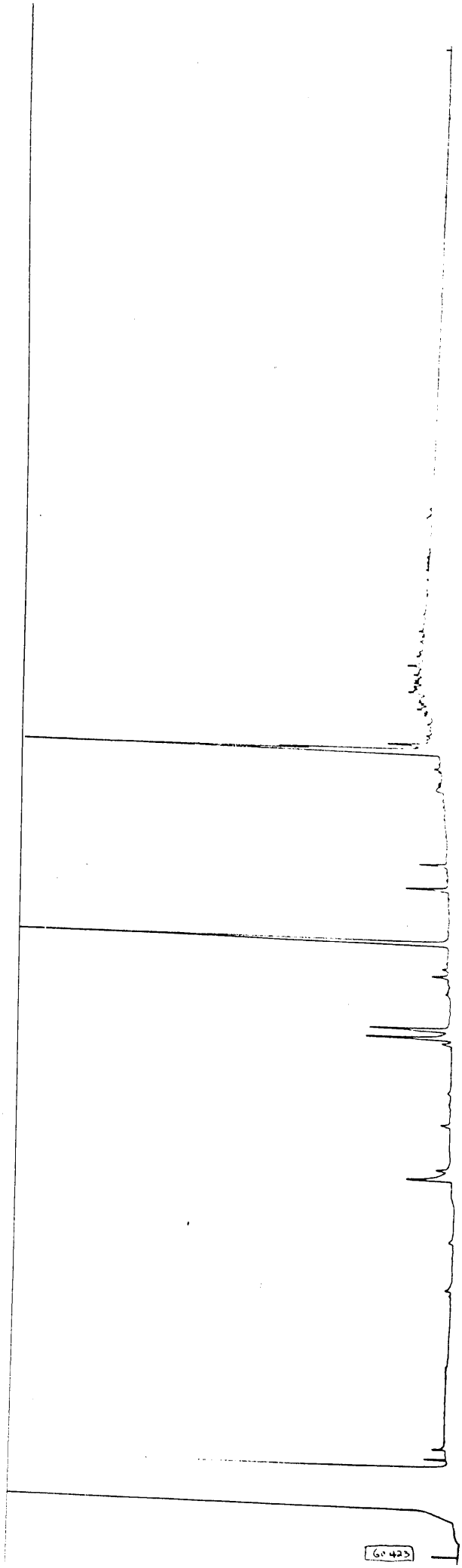
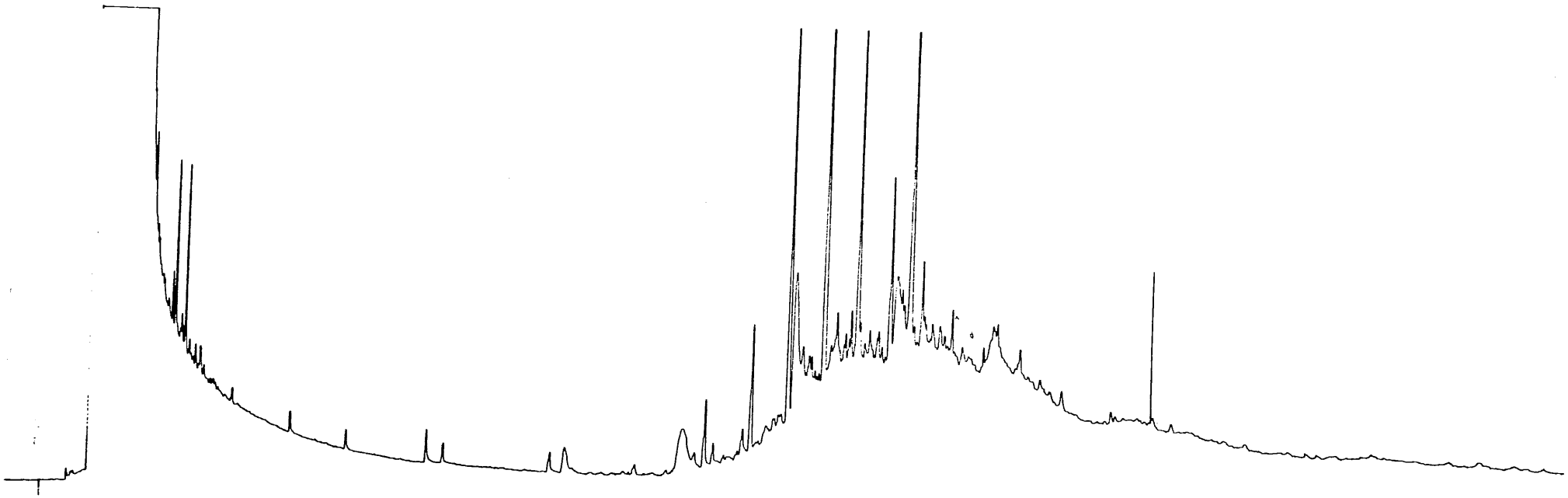

60473

Fig. 7-d. Capillary gas chromatogram of the benzene extract of control filter.

Upper trace: flame ionization detector

Lower trace: thermionic specific detector, nitrogen mode

Each chromatogram represents 0.02% of the total benzene extract.



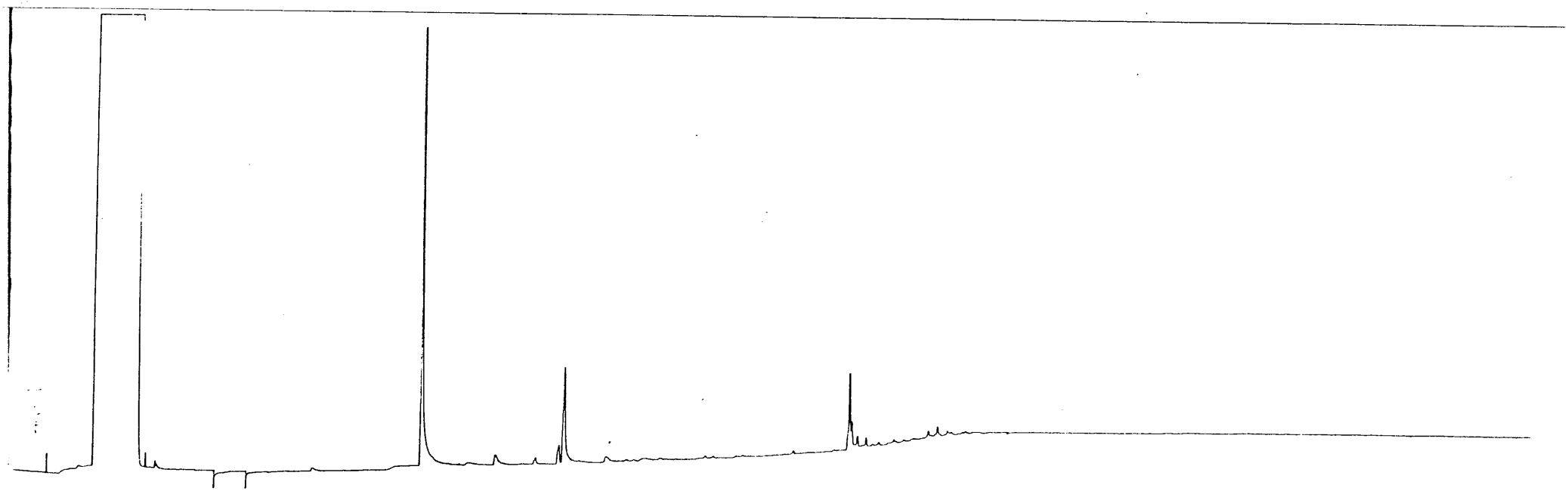


Figure 7-a shows the FID and TSD chromatograms for the benzene extract of the coarse oil fly ash. Each chromatogram represents the benzene extract from 25 mg of the ash or 2.5% of the total extract obtained. Comparison of these traces with those from the procedural control show that a significant number of components had been extracted, predominantly in the high molecular weight region in the FID trace, and over the intermediate molecular weight range for the nitrogen-containing components detected by the TSD. The FID trace shows that the extract contains a complex mixture of organic compounds since the major portion of the 'hump' results from a large number (perhaps a few thousand) of unresolved components. Above the 'hump', several features should be noted. First is the presence of a single large off-scale peak; this may represent a single major component, but analysis by combined gas chromatography/mass spectrometry (GC/MS) would be required to verify this. The structural identification of this peak would also require GC/MS analysis. The second feature of note in this chromatogram is the series of peaks extending from just before the maximum of the hump out to the end of the chromatogram, and which are derived from a homologous series of components. Again, GC/MS would be required to identify these components. There are also a reasonable number of other peaks which can be discerned above the unresolved hump which might be identified by GC/MS.

The TSD trace representing the nitrogen-containing components of the mixture also shows a fairly complex mixture for a selective detector, although it is obviously not as complex as the FID trace. The trace shows a double peak as the main feature. Although visibly this would appear to represent two partially resolved components, it is also possible that it really represents only a single very large nitrogen-containing component which is producing a distorted peak shape due to saturation effects in the detector. In addition to several discrete peaks in this trace, there is also evidence of a small hump representing an unresolved mixture of nitrogen-containing species.

Figure 7-c shows the chromatograms obtained from the benzene extract of the fine oil fly ash and its filter. Each chromatogram represents the extract from approximately 3 mg of fly ash or 0.4% of the total benzene extract. The FID chromatogram shows an extremely complex mixture of high molecular weight components together with some broad peaks representing multi-component and/or

on-scale hump and two-to-four off-scale peaks. As summarized in Table 15, however, the FID trace in Figure 7-c (fine oil fly ash + filter) is derived from 20 times more of the total extract than the FID trace in Figure 7-d. This demonstrates, therefore, that an order-of-magnitude estimate shows the benzene extract of the control filter to contain approximately 20 times more material than the benzene extract of the fine oil fly ash + filter. This means, of course, that the control filter was seriously contaminated with benzene-soluble organic compounds. Further discussion of this problem has been presented above and is discussed again below in the Summary section.

3. Characterization of Polar Components by Liquid Chromatography

The polar components of the oil fly ash and their corresponding control samples were obtained by extracting the previously-extracted samples with azeotropic benzene-methanol. The first extraction with benzene removed the relatively nonpolar components which could be analyzed directly by gas chromatography. The subsequent extraction with the polar solvent mixture then removed the remaining, polar, organic materials.

The samples remaining from the benzene extraction procedure (see Table 14) were extracted three times with azeotropic benzene/methanol and, after centrifugation, the supernatants were filtered using nitrogen through pure Teflon filters (0.2 μm pore size) held in a filter holder made of stainless steel and using Teflon-coated O-rings. The filtrates were combined and concentrated under a stream of dry nitrogen.

For the fine ash sample, when the volume of the solution was about 2 ml, the solution appeared as two distinct layers--the lower one containing crystals and colored a light greenish-brown, and the upper layer colored a darker greenish-brown. The three other samples were inspected visually at a volume of 100 μl; the color of these solutions was found to be:

coarse oil fly ash	greenish-brown
control	clear
control filter	brown

This observation indicates (as for the corresponding benzene extract) that considerable material could be extracted from the control filter, whereas the analytical control sample was clear as would be expected if no contaminants or artifacts were introduced during the analytical procedures.

The benzene/methanol extracts were blown just to dryness with N_2 and the weights of the extracted material were determined to be:

coarse oil fly ash	73.7 mg
control	0.0 mg
fine oil fly ash + filter	802.8 mg
control filter	12.2 mg (oily)

The extract from the fine oil fly ash + filter occupied a volume of about 1 ml and appeared as a lightish green solid. The extract from the control filter appeared as 20 μl of an oily-brown material, again indicating severe contamination problems as an extract from an organically-clean Teflon fabric filter would be expected to contain no extractable material. Examination of new filters showed wax crayon markings used by the manufacturer to design the stitching of the filter bags. Addition of 2 ml of acetonitrile to the control filter extract gave an oil as the lower layer and a yellow-brown upper layer. The upper layer was used for subsequent HPLC analysis. The remaining three extracts were each taken up in 100 μl of acetonitrile for HPLC analysis.

These polar extracts dissolved in acetonitrile were analyzed by high performance liquid chromatography with UV and fluorescence detection. The two detectors were connected in series (UV then fluorescence) and their responses were monitored on a dual-pen recorder. The UV detector was operated at 254 nm and thus it detects essentially all UV absorbing species except for some alkenes; alkanes would not be recorded by this detector. The fluorescence detector was operated with excitation and emission wavelengths which are optimized for benzo-a-pyrene; this detector will, therefore, respond to polycyclic aromatic hydrocarbons if present. Some of the chromatograms obtained are shown in Figure 8 and summarized with relevant information in Table 16.

The chromatograms from the procedural control sample are shown in Figure 8-b and demonstrate that no UV-absorbing or fluorescent species were introduced during the extraction procedure. In this and the other sets of chromatograms, the pen recording the fluorescence chromatogram is slightly ahead of the pen recording the UV chromatogram. The horizontal scale drawn in at the lower edge of the chromatograms identifies the injection point, 100% acetonitrile point and the end point for each of the fluorescence and UV chromatograms, always with the fluorescence mark to the left (ahead) of the double marks.

Table 16. HPLC Chromatograms of Benzene/Methanol Extracts

Sample	Total Number of Chromatograms Obtained (UV + FL)	Representative Chromatograms Illustrated in Figure	Amount of Fly Ash Extracted for each Chromatogram*	% of Total Extract/ Detector**
Coarse oil fly ash	2	8-a	100 mg	10.00
Control	2	8-b	--	10.00
Fine oil fly ash + filter	2	8-c(i) 8-c(ii)	∿0.1 mg ∿3.85 mg	0.013 0.50
Control filter	2	8-d(i) 8-d(ii)	-- --	0.02 10.00

*Each chromatogram set (UV + FL) represents the benzene/methanol extract from this weight of oil fly ash.

**Each chromatogram set (UV + FL) represents this percentage of the total benzene extract

Figure 8-a shows the HPLC chromatograms for the polar components extracted from the coarse oil fly ash. Essentially no fluorescent response could be detected, and very little UV absorbing material was detected other than the large peak at the beginning of the chromatogram. As this chromatogram was produced from a reverse-phase column, this large peak represents extremely polar material, possibly inorganic species which had been extracted into the methanol of the extracting mixture and eluting with the aqueous solvent front in the chromatographic process. These chromatograms were obtained from the polar extract from 100 mg of coarse oil fly ash, or 10% of the total benzene/methanol extract.

Fig. 8-a. HPLC chromatograms of the benzene/methanol extract of coarse oil fly ash.
First (lower) trace: fluorescence detector
Second (upper) trace: UV detector
Each chromatogram represents the benzene/methanol extract from 100 mg of coarse oil fly ash (10% of total benzene/methanol extract)

Fig. 6a

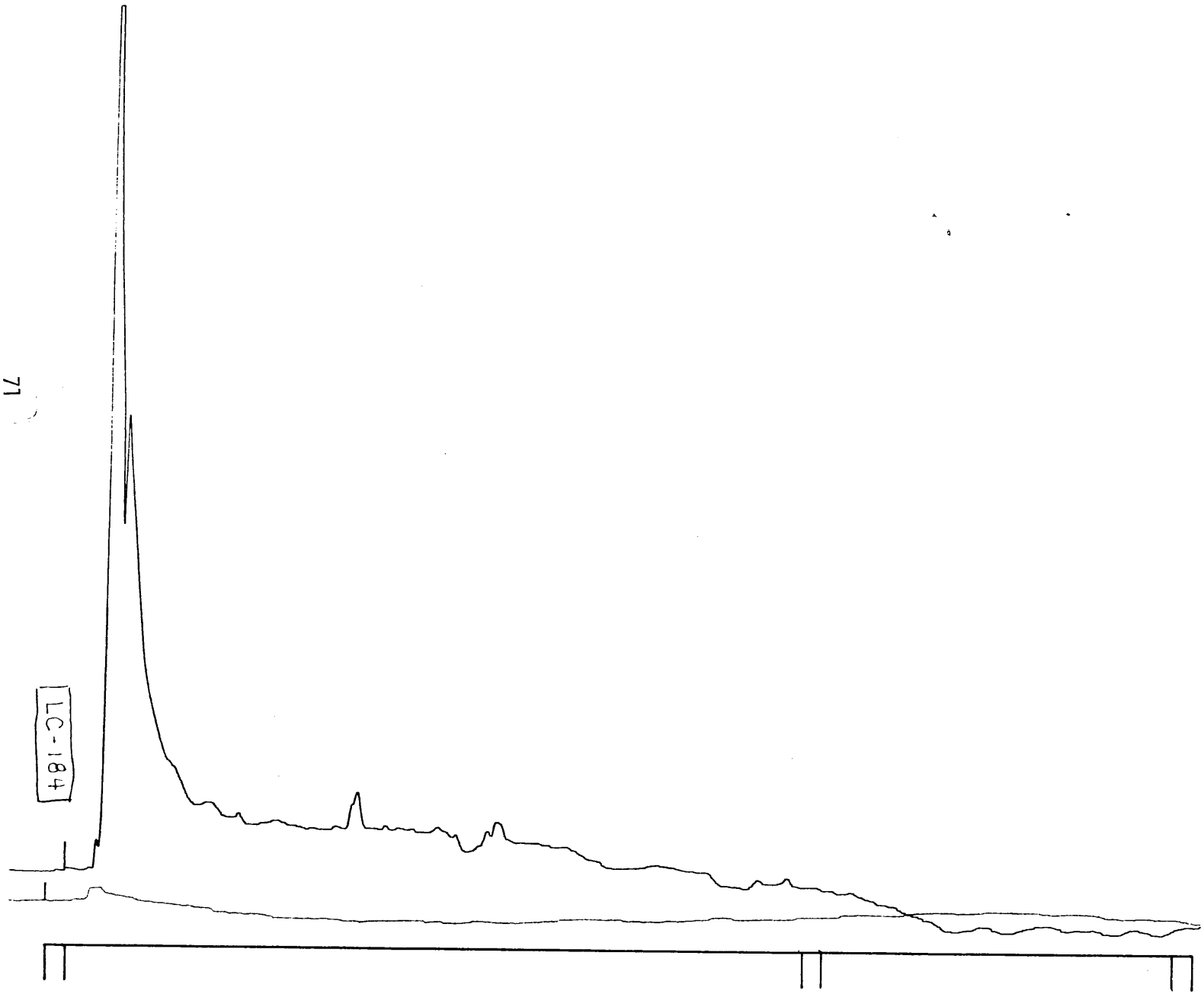


Fig. 8-b. HPLC chromatograms of the benzene/methanol extract of control sample.
First (lower) trace: fluorescence detector
Second (upper) trace: UV detector
Each chromatogram represents 10% of the total benzene/methanol extract.

Fig. 8b

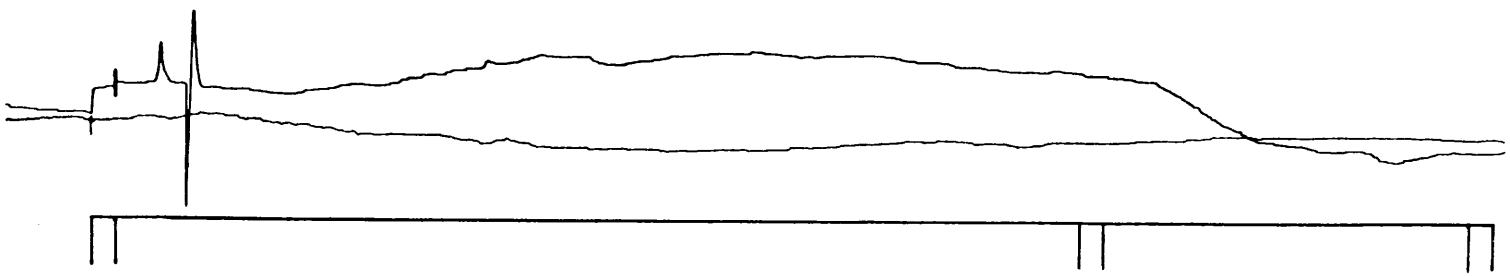


Fig. 8-c. HPLC chromatograms of the benzene/methanol extract of fine oil fly ash.

First (lower) trace: fluorescence detector

Second (upper) trace: UV detector

(i) Each chromatogram represents the benzene/methanol extract from ~0.1 mg of fine oil fly ash (0.013% of total benzene/methanol extract).

8c (i)

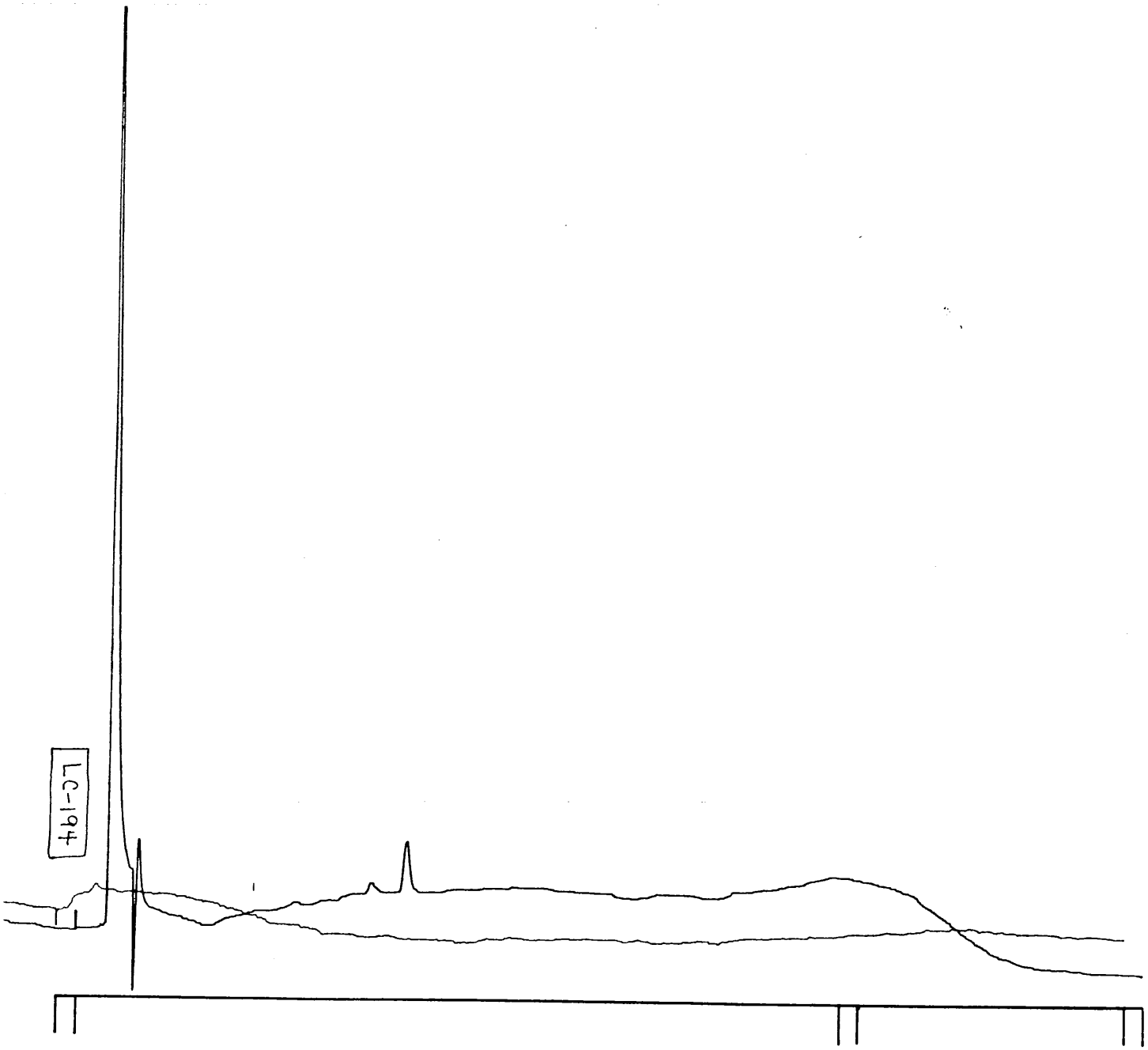
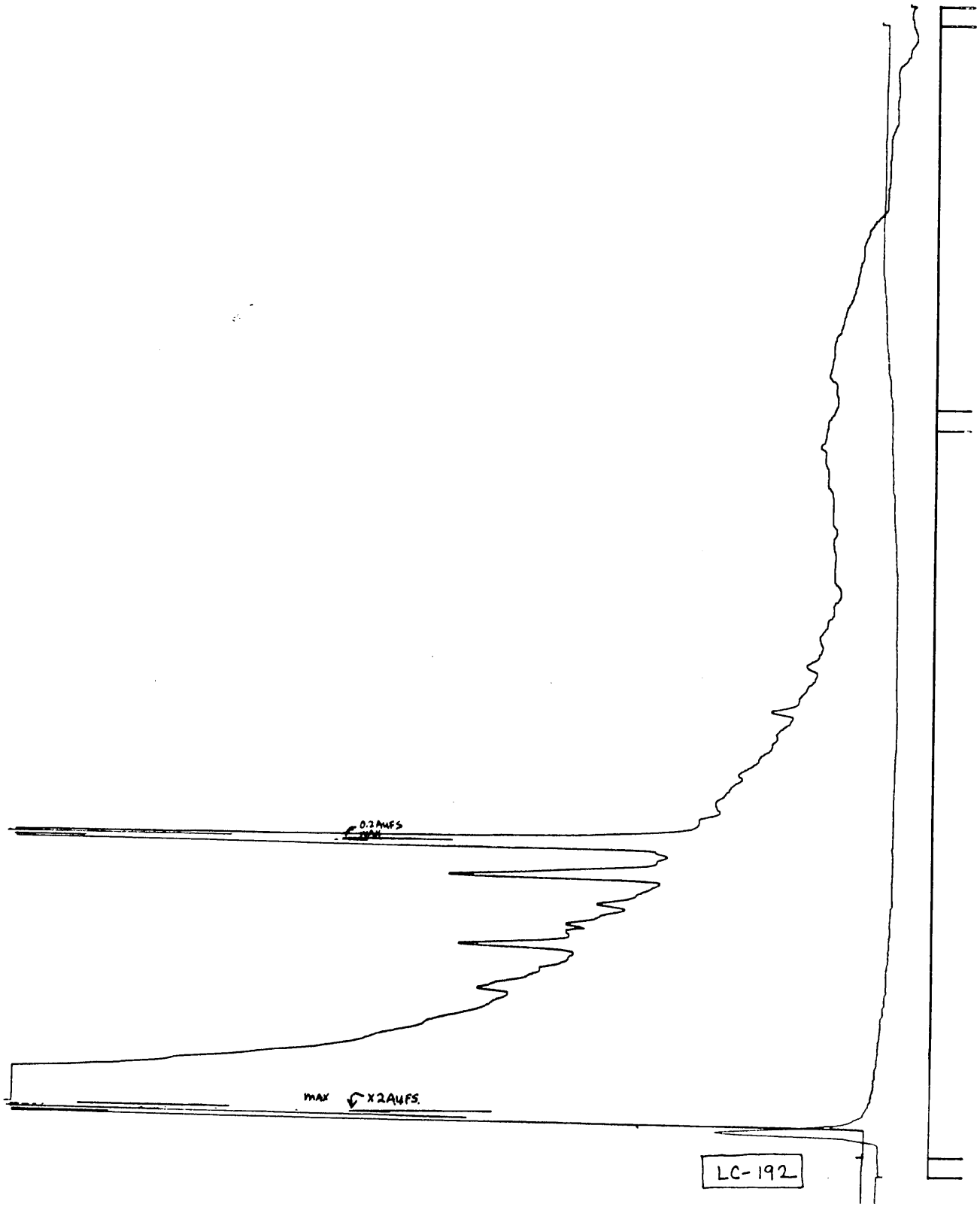


Fig. 8-c. HPLC chromatograms of the benzene/methanol extract of fine oil fly ash.

First (lower) trace: fluorescence detector

Second (upper) trace: UV detector

(ii) Each chromatogram represents the benzene/methanol extract from ~3.85 mg of fine oil fly ash (0.5% of total benzene/methanol extract). Note peak maxima at 2 and 0.2 absorption units full scale (AUFS) (cf. rest of chromatogram obtained at 0.05 AUFS).



8c (ii)

Fig. 8-d. HPLC chromatograms of the benzene/methanol extract of control filter sample.

First (lower) trace: fluorescence detector

Second (upper) trace: UV detector

(ii) Each chromatogram represents 10% of the total benzene/methanol extract. Note that the attenuations used for the UV chromatogram are 1.0, 0.05, 0.1, 0.05, and 0.2 AUFS, respectively, as marked on the chromatogram.

8d (i)

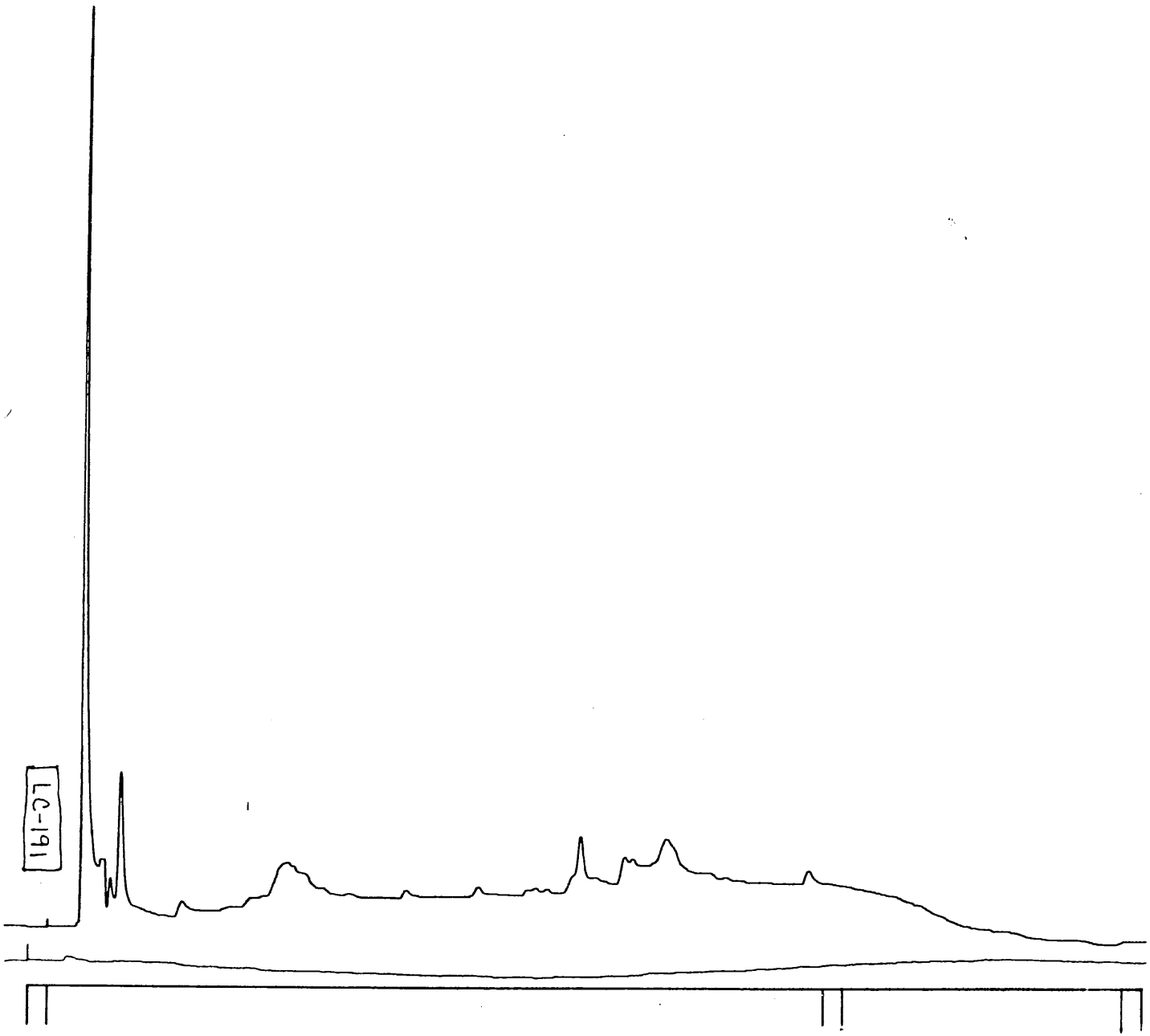
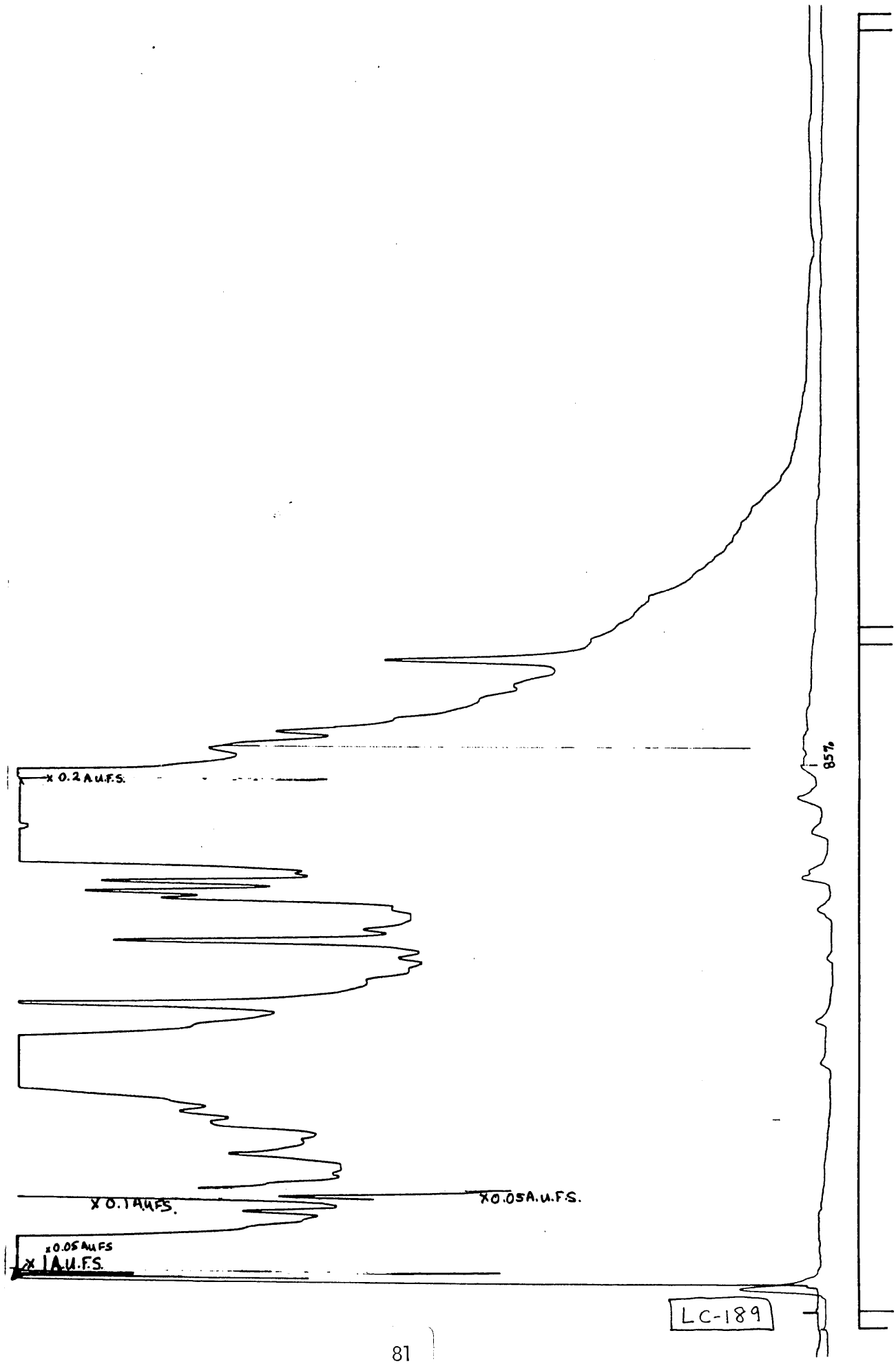


Fig. 8-d. HPLC chromatograms of the benzene/methanol extract of control filter sample.

First (lower) trace: fluorescence detector

Second (upper) trace: UV detector

(i) Each chromatogram represents 0.5% of the total benzene/methanol extract.



8a (ii)

Figure 8-c shows the chromatograms obtained for the polar extract of the fine oil fly ash + filter sample. The set labeled (i) was obtained from the extract from about 0.1 mg of ash or about 0.013% of the total polar fraction. The set labeled (ii) was obtained from the extract from about 3.85 mg of ash or about 0.5% of the total polar fraction. Note that in this second set of chromatograms, two UV peak maxima are marked on the scales of 2.0 and 0.2 AUFS, respectively, with the remainder of the chromatogram run at 0.05 AUFS as for all of the other UV traces. The chromatograms in Figure 8-c(ii) show only a small amount of fluorescent material eluting with the solvent front and a few UV-absorbing components as well as the main UV-absorbing material eluting with solvent front.

Figure 8-d shows the chromatograms obtained for the polar extract of the control filter sample. The set labeled (i) was obtained from 0.5% of the total polar extract, whereas the set labeled (ii) was obtained from 10% of the total polar extract. Note that the UV-trace in this latter set was recorded at several attenuations: 1.0, 0.05, 0.1, 0.05, and 0.2 AUFS in order of retention time. The chromatograms labeled 8-d(i) show essentially no fluorescent components but a fairly complex mixture of UV-absorbing species with, again, the major component eluting with the solvent front. The chromatograms labeled 8-d(ii) do show a small amount of fluorescent material including one or more species which elute with the solvent front. The UV trace shows an extremely complex mixture of UV-absorbing components extending well into the nonpolar region. Presumably the previous three extractions with benzene were insufficient to remove all of the nonpolar material, or the column may have been overloaded, causing band broadening.

Comparison of the UV traces in Figure 8-c(ii) and 8-d(i), representing the polar fractions from the fine oil fly ash + filter and the control filter, is instructive in that they were both obtained from 0.5% of the total polar extracts and were both obtained at an attenuation of 0.05 AUFS. This comparison shows that the chromatograms are qualitatively different but that quantitatively [using the similarity between the UV traces 8-c(i) and 8-d(i)], the UV trace in Figure 8-c(ii) contains approximately 40 times as much UV-absorbing material as the UV trace in Figure 8-d(i). It can, therefore, be concluded for the polar fractions that the control filter is not as extensively contaminated with respect to the fine oil fly ash + filter, as was observed for the benzene extracts.

4. Summary of Organic Characterization Studies

An overall evaluation of the organic characterization studies for the oil fly ash samples can be made by comparison of the gas and liquid chromatograms for three out of the four samples processed, since in both types of analyses, the data for the procedural control sample indicate that the laboratory manipulations had been successfully accomplished without the introduction of contaminants or artifacts.

The relevant chromatograms which will be evaluated here are summarized in Table 17. It should be noted first that neither the GC chromatograms nor the LC chromatograms are very similar for the three samples, except perhaps for the FID traces for samples 1 (coarse ash) and 2 (fine ash), which both appear to contain a high molecular weight homologous series. In the absence of contaminants due to the different sampling methods for these ash samples, it would be expected that, qualitatively, the two oil fly ash samples would appear to be very similar, if not identical; the only difference would be quantitative, relating to the surface area/mass considerations for the two particle ranges.

Table 17. Summary of GC and LC Characterization of Oil Fly Ash Samples

Sample No.	Description	GC			LC		
		Figure	A	B	Figure	A	B
1	Coarse oil fly ash	7-a	25 mg	2.5	8-a	100 mg	10
2	Fine oil fly ash + filter	7-c	∿3 mg	0.4	8-c(ii)	∿3.85 mg	0.5
3	Control filter	7-d	--	0.02	8-d(i)	--	0.5

A. Amount of fly ash extracted for each chromatogram

B. Percent of total extract/detector trace

Second, it should be noted that the data obtained from the control filter sample (Sample 3) indicate that this supposed "blank" sample is significantly contaminated--particularly with respect to the nonpolar material analyzed by gas chromatography. Since the laboratory blank sample is, indeed, blank, the contamination must have occurred sometime prior to the laboratory analysis, probably the above mentioned crayon marks on the Teflon fabric filters. We might have verified this with samples 3 and 4 (Table 2) collected on different filters, but the Air Resources Board required that they be sent to another lab, and were therefore lost for follow-up.

The chromatograms for samples 2 and 3 do not, however, appear particularly similar which might be expected if a significant fraction of organic material in Sample 2 was derived from a contaminated filter as seen in Sample 3. Two possible explanations of these features might be that the initial contaminants on the filter used for sampling the fine oil fly ash were removed by vaporization to some unknown extent by the temperature and gas flow to which it was subjected, or there was less on the sample filter initially. Additional organic components would then be contributed by the deposition of the fine oil fly ash and its associated surface-deposited materials. Further resolution of this problem would require detailed analyses of these samples by GC/MS to identify and quantitate individual compounds in the three samples.

There is little doubt, however, that some degree of contamination of the fine oil fly ash sample did occur prior to analysis in the laboratory, and probably during preparation of the fabric filters. Therefore <u>all</u> experimental data which might reflect the surface-coated organic material on the fine oil fly ash particles must be viewed with appropriate caution.

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APPENDIX FOR ORGANIC CHARACTERIZATION STUDIES

For all experimental procedures, extreme care was taken to avoid introducing contaminants and to minimize the production of artifacts. All transfers between containers was done quantitatively with multiple rinsings. The samples were kept in the dark as far as possible to minimize any photooxidative effects and they were stored in a refrigerator between various steps in the procedures. All glassware was cleaned with chromic acid, rinsed with distilled water, then rinsed with double-distilled water which had been passed through a Millipore "Milli-Q" system, before drying in an oven.

A1. Preparation of Total Organic Extracts for Mutagenicity Testing (See Table 13 for Summary)

a. Coarse ash and control samples.

An aliquot of the coarse oil fly ash (306 mg) was weighed into a centrifuge tube fitted with a Teflon-lined screw cap. A control sample was similarly prepared except that no fly ash was actually placed in the tube. Both samples were then processed identically.

Extraction was carried out with 3 x 10 ml of azeotropic benzene/methanol (60:40 by weight; both Mallinckrodt Nanograde) using an ultrasonic probe for agitation. In order to avoid any overheating effects, the probe was switched on for 15 seconds every minute for 10 minutes for each extraction. The samples were then centrifuged at 2,000 rpm for 15 minutes, and the supernatants pipetted off and filtered through a pure Teflon filter (Millipore, 0.2 μm pore size, special order) held in a stainless steel filter holder (Gelman, model 11101) and using Teflon-coated O-rings (Vanway, VW-030). Filtration was accomplished by forcing the solution through the filter with approximately 25 psig of nitrogen (Matheson, Zero gas) purified by passing through a filter of molecular sieve and Drierite. The resulting 3 filtrates were combined in a glass screw cap jar and concentrated to about 3 ml by a stream of dry, purified nitrogen (N_2, see above). The solutions were then transferred to 5 ml pointed vials, blown down just to dryness with N_2, and 6 ml of DMSO (Schwarz/Mann, spectrophotometric grade) was added for the mutagenicity assay.

b. Fine ash + filter, and control filter samples

The fine oil fly ash was imbedded in the Teflon felt filter material and so could only be extracted together with the filter material. A similar Teflon felt filter was therefore used as a control sample for this procedure.

In view of the small increase in weight of the filter due to the ash (2.42 g, see Table 13) and the large weights of the filters themselves (78.08 g and 86.56 g), the weight of fine oil fly ash could only be estimated as follows. For this experiment, approximately 1/8 of the total filters were cut out and weighed. This procedure gave samples of 12% and 13% of the total ash + filter and control filter, respectively (cf. 12.5% for exactly 1/8). At this point it was necessary to assume that the fine ash was evenly deposited over the Teflon felt filter. It was known, however, that this was not the case as the filter used for sampling had strips about 1/2" wide at the top and bottom for mounting in the filter holder and which did not come into contact with the gas stream flowing through the sampler. Also the felt filters had a vertical seam about 3/4" wide which would contribute excessively to the weight of the filter but not to the surface area over which the ash could be collected. As the total weight of the fine ash collected was 2.42 g, the amount used for this experiment was estimated to be about 2.42 g x 0.12 or about 290 mg.

The 1/8 filter pieces were carefully cut up into small pieces and placed in a centrifuge bottle for extraction. The procedure used was identical to that described above except for the amount of solvent used. The first extraction was carried out using 60 ml of the solvent. However, after the centrifugation step only 35 ml could be removed so for the second and third extractions only an additional 35 ml of solvent was added each time.

For the fine oil fly ash + filter sample, an extra control sample had to be included due to a problem encountered during evaporation to dryness. During the concentration procedure, when the volume of the benzene/methanol extract had been reduced to about 730 µl, it was found that further concentration could not be accomplished due to the presence of moisture. The additional control sample was added at this stage and consisted of 730 µl of water (Baker, HPLC-grade). Using additional amounts of benzene and methanol, the two samples could finally be evaporated to dryness, prior to the addition of DMSO.

Preparation of Benzene Extracts for Gas Chromatography

Benzene extracts of the samples were prepared for analysis by capillary gas chromatography (GC) by the methods described above in Appendix Section A except for the following procedures. An aliquot of the coarse fly ash weighing 0.999 g was extracted, together with the control sample, using 3 x 10 ml of benzene.

Approximately 1/3 of the same filters as used in the experiment described above were used for this extraction. Actual weights gave 32% and 31% of the total ash + filter and control filter, respectively (26.14 g/80.50 g total and 26.85 g/86.56 g total), giving the amount of fine ash used for this experiment estimated to be 0.77 g using the same assumptions. These 1/3 filter samples were cut up and extracted with 3 x 120 ml of benzene.

All of the benzene extracts were concentrated to about 2 ml, then transferred to a pointed vial and evaporated down to 100 µl. At this stage, the extracts were inspected visually. All four samples were then subjected to GC analysis as described below.

A3. Gas Chromatography of Benzene Extracts

Capillary gas chromatography was carried out using a modified Varian 3740 gas chromatograph fitted with a glass-lined capillary injector (Varian model 1070). The column was a 30 m x 0.25 mm i.d. wall-coated open tubular glass capillary column (J and W Scientific) coated with SE54. The end of the column was connected to a 2-way capillary effluent splitter made of glass-lined stainless steel tubing (Varian Model 2110, P/N 03-949576-00) which was connected to a flame ionization detector and a thermionic specific detector operated in the nitrogen mode. Each amplified detector output was recorded on a linear strip-chart recorder (chart speed 1 cm/min).

Helium (Matheson, Zero gas) was used as the carrier gas and the makeup gas. The column inlet pressure was 20.0 psig which gave a linear velocity of 36 cm/sec at 40°C. The makeup gas was added at the base of the effluent splitter at a flow rate of 60 ml/min to give the required 30 ml/min to each of the two detectors. The injector and the two detectors were maintained at 300°C; the column oven was initially stable at 40°C, then at the injection time was programmed linearly at 5°/min to 280°C and held at that temperature

until a significant time had elapsed after the last sample component eluted. All sample injections were carried out using the splitless solvent effect procedure with benzene (bp. 80°C) as the solvent; the injector splitter was opened 40 seconds after the injection. In all cases, a total volume of 2 μl of the benzene solution was injected and both detector signals were recorded at 8×10^{-12} amps fs.

A4. Preparation of Benzene/Methanol Extracts for Liquid Chromatography

Extracts of the polar components of each of the samples were prepared for analysis by high performance liquid chromatography (HPLC). This was achieved by extracting the samples with azeotropic benzene/methanol after they had been extracted with benzene to provide extracts for GC analysis. The method used was the same as that described above except for the following procedures.

The samples for this extraction were the benzene-extracted samples that were described in section A2; the procedures were the same as for the benzene extracts except for the use of azeotropic benzene/methanol (60:40 by weight) instead of benzene. After filtration, the extracts were evaporated under N_2. For the fine ash sample, when the volume of the solution reached about 2 ml, the solution appeared as two distinct layers--the lower one containing crystals and colored a light greenish-brown. The three other samples were inspected visually at a volume of 100 μl. The coarse ash sample was a greenish brown, the control sample was clear, and the control filter sample was brown. This latter observation indicates (as for the corresponding benzene extract) that considerable material could be extracted from the control filter. The samples were subsequently blown just to dryness with N_2 and the weights of the extracts were determined. To the fine ash sample (about a 1 ml volume of a light green solid weighing 801.8 mg) was added 2 ml of acetonitrile (Burdick and Jackson, Distilled-In-Glass, UV-grade) which produced a yellow-brown upper layer and an oily lower layer. Aliquots of the upper layer were taken for HPLC analysis. The remaining three samples were taken up in 100 μl of acetonitrile for HPLC analysis.

A5. Liquid Chromatography of Benzene/Methanol Extracts

High performance liquid chromatography was carried out using a Varian 5020 liquid chromatograph coupled in sequence to a variable ultraviolet-visible (UV/VIS) detector (Varian Varichrom) and a fluorescence detector

(Varian Fluorichrom). The injector was a Rheodyne model 7125 fitted with a 10 µl loop. The column (30 cm x 4 mm) was packed with a 10 µm octadecyl reverse phase material (Varian MCH-10) preceded with a precolumn packed with Vydac RP packing (The Separations Group). The column temperature was maintained at 35°C. The UV detector was operated at 254 nm and at an attenuation of 0.05 AUFS unless otherwise stated. The fluorescence detector was fitted with an excitation interference filter at 254 nm and an emission band filter at 410 nm; all the fluorescence chromatograms were obtained at an attenuation of x 20.

All chromatograms were run under identical solvent compositions. Solvent A was HPLC-grade water (Baker) + 2% acetonitrile (Burdick and Jackson, HPLC-grade). Solvent B was 100% acetonitrile (Burdick and Jackson, HPLC-grade). Samples were injected in 100% acetonitrile using the full 10 µl loop, after the system had equilibrated at A:B = 80:20 for 5 minutes. At the injection time, a solvent gradient was started from 20% B to 100% B in 20 minutes. The solvent was then held isocratically at 100%B for a significant time after the last sample component had eluted. The two detector outputs were recorded simultaneously on a linear dual pen chart recorder.

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