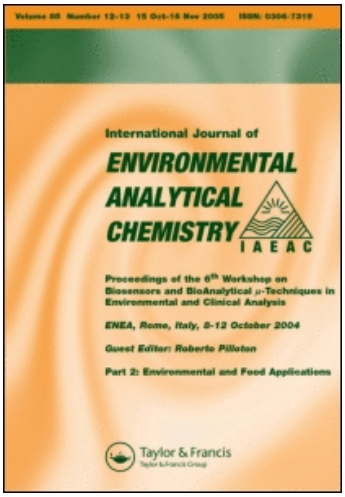

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Characterization of Mutagenic Coal Fly Ash and Extracts†

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Post-electrostatic precipitator (ESP) fly ash samples were collected from a coal-fired electric power generation plant under three modes of plant operation: normal operation, a low NO_x-emission mode of combustion, and operation with the ESP shorted-out. Results of chemical and physical characterization of the ashes were compared with bacterial mutagenicity bioassay to determine parameters or compounds correlating with bioactivity. The general physical properties, ultimate composition, and trace elemental and radiochemical species determined did not correlate with the mutagenicity. Only the presence of aromatic hydrocarbons and chemically derivatizable polar organic compounds appeared to be associated with mutagenicity of the fly ash.

KEY WORDS: Coal fly ash, chemical and physical analysis, mutagenicity bioassay.

INTRODUCTION

The chemical and physical nature and potential health/environmental effects of the potentially bioactive species present in coal fly ash is a major concern of the electric utility industry. This topic is the subject of much current investigation. Several recent studies[1–7] have demonstrated that solvent extracts of some coal fly ashes contain low levels of mutagenic activity. However, only the general nature of the mutagens has been deduced from indirect experiments. Both inorganic[7] and organic[2,3,5,6] mutagens have been hypothesized. More specific chemical information has

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been reported[5] by Fisher who suggests that polar, weakly acidic organic species, possibly nitroaromatics,[6] account for a significant portion of the mutagenicity.

This paper presents the results of a coupled chemical analysis-biological assay of coal fly ash and its solvent extracts. Comparison of results for three fly ashes collected under three different plant operation conditions suggests that aromatic species and also chemically derivatizable polar organics may be associated with fly ash extract mutagenicity.

EXPERIMENTAL

Samples

Three samples of fly ash were provided by Dr. Ralph Mitchell of the Battelle Columbus Laboratories, Columbus, OH, under an Electric Power Research Institute contract. The fly ash samples were collected downstream of the ESP of a 575 MW coal-fired power plant located in the eastern U.S. and fired with coal from Appalacian mines. Effluent from the ESP was drawn at 75 cfm through an insulated Teflon bag maintained above the dew point of sulfuric acid using the heat from the sampled aerosol. Approximately 215 g of fly ash was collected in 20 hours during normal plant operation (this sample will be referred to as the "normal" fly ash), 435 g in 24 hours during a low oxides of nitrogen emissions mode of plant operation ("low NO_x" fly ash), and approximately 5 kg in 120 hours during a period when the ESP was shorted out ("ESP-shorted" fly ash). Full details of the sample collection and plant operation was reported elsewhere.[8] The fly ashes were stored in the dark at −20°C in glass bottles prior to study.

Procedures

Chemical and physical characterizations The following bulk fly ash analyses were performed by the indicated standard ASTM procedures:[9] carbon and hydrogen (ASTM-D-3178-73), nitrogen (ASTM-D-3179-73), ash (D-3174-73), moisture (D-3173-73), and oxygen (D-3176-74). Sulfur was measured with a LECO Corporation Model IR32 analyzer, using the manufacturer's procedure. Organic plus elemental carbon was estimated by subtacting inorganic carbon from total carbon. Inorganic carbon was measured using an Oceanography International Model 524 Total Carbon Analyzer instrument and the EPA procedure[10] for carbon determination in water and wastes.

The aerodynamic particle size distribution was measured using a Bahco Microparticle Classifier which was calibrated with standard limestone

whiting according to American Society of Mechanical Engineers Power Test Code 28 (1965). Bulk density of the fly ash was determined with a Kimble No. 15123 pyncnometer, using water as the fluid. Surface areas were measured using the BET apparatus[11] with krypton. Inorganic and radiochemical analyses were performed by neutron activation analysis and gamma spectroscopy as described elsewhere.[12,13]

Extraction Solvent extraction was accomplished by packing approximately 40 g of each ash into a 1.1 cm ID × 62 cm stainless steel liquid chromatography column and pumping 100 ml each of the following redistilled solvents (in sequence) using a Milton-Roy model 190 pump: benzene, methylene chloride, ethyl acetate, ethanol. The solvent fractions were collected separately and concentrated to dryness with flowing nitrogen under reduced pressure and temperature. The fraction residues were weighed and redissolved in 0.3 ml of the same solvent as was used in the extraction. Small scale extractions were performed on 3 g of fly ash by ultrasonicating three times with 10 ml of Burdick and Jackson methylene chloride, using a 185 watt Branson ultrasonic cell disruptor. The extracts were concentrated to 0.3 ml. An aliquot of 0.1 ml was evaporated to dryness and heated at 70°C for 30 min with 0.1 ml of N-trimethylsilyl-N-methyl-trifluoroacetamide before analysis.

Chromatographic and spectral analysis Extract fractions of the fly ash obtained from the solvent pumping procedure were analyzed by gas chromatography (GC) on a Perkin-Elmer Sigma I instrument using a 3 m × 3 mm OD glass column packed with 3% Dexsil 400 on 100/120 mesh Supelcoport temperature programmed from 100°C (hold 5 min) to 320°C at 2.5°C/min. The small scale extracts were analyzed by GC on a Hewlett Packard Model 5880 GC using a 30 m SE-54 fused silica capillary column temperature programmed from 80°C (hold 3 min) to 270°C at 3°/min. Both instruments were equipped with flame ionization detectors. Mass spectra were recorded using a Hewlett Packard Model 5985 gas chromatograph/mass spectrometer in the electron impact mode at 70 eV ionizing voltage, under similar GC capillary column conditions.

Transmission infrared (IR) spectra of the extract fractions were recorded on a Digilabs Fourier transform IR spectrometer. An aliquot of each fraction containing 1–2 mg of extract was applied to 300 mg of finely powdered spectrograde KBr, and after the solvent evaporated, a pellet was pressed by the usual technique. One hundred scans were recorded at a resolution of 4 cm^{-1}.

Bacterial mutagenicity bioassay The Salmonella histidine reversion assay developed by Ames[14] was used to determine mutagenicity. Strains

designated to detect missense (TA-1535) and frameshift mutation (TA-1537, TA-98 and TA-100) were chosen for these studies. The experimental procedures described by Ames[14] were followed. Briefly, an overnight bacterial culture (approximately 2×10^8 cells/plate) was mixed with the test material in 2 ml of molten top agar and poured over previously prepared Vogel-Bonner[15] minimal medium plates. Metabolic activation was achieved by the addition of 0.5 ml of S-9 mix obtained from rat livers induced with either phenobarbital (ϕB) or Aroclor (Ar). The revertant colonies were counted after 2 days of incubation at 37°C.

The bacterial strain was periodically checked for its phenotypic characteristics such as histidine anxotrophy and the presence of mutation of *rfa*; *uvr* Band ampicillin resistance. The response to known mutagens was also determined concurrent to each experiment (e.g., for TA-98: 650 rev/50 μg 2-nitrofluorene, 350 rev/25 μg benzo(a)pyrene + Ar S-9 mix, and 1250 rev/25 μg 2-acetylaminofluorene + ϕB S-9 mix).

Mutagenic activities were determined independently at least three times for the fly ash extract sample in a non-toxic dose range. The specific activities were determined from the slopes of linear dose-response data and were calculated as revertants per microgram of extract.

RESULTS AND DISCUSSION

One research objective of the electric power utility industry is to determine the potential bioactivity of particulate emissions from coal-fired power plants and the chemical or physical factors associated with bioactivity. Examination of fly ashes collected at the same plant under different operation modes introduces an element of greater comparability from the common fuel and furnace, and facilitates comparative studies of fly ashes.

The chemical and physical parameters characterizing the nature of the three fly ash samples used in this study are shown in Table I and Figure 1. The three samples appear to be very similar. The only differences detected among the three ashes were the total carbon content, color (apparently affected by carbon content), and possibly the total nitrogen and oxygen content. The increased carbon content of the low NO_x fly ash appears to arise from the more fuel-rich combustion conditions employed to decrease oxidation of nitrogen species in the fuel. However, the elevated carbon content of the ESP-shorted fly ash was unexpected. A low NO_x combustion mode was not in effect during the collection of this sample. Total nitrogen was low for each sample so apparent differences in nitrogen content may not be significant. The observation that total oxygen

TABLE I

Physical and chemical characteristics of fly ash samples

Characteristic	Value for fly ash sample		
	Normal	Low NO_x	ESP-shorted
Color	Lt. Gray	Dk. Gray	Dk. Gray
Density, g/cc	2.37	2.31	2.31
Median aerodynamic Particle diameter, μm	6.3	7.7	7.3
Specific surface area, m^2/g	2.7	2.3	2.1
Total C, wgt. %	4.24	12.37	10.14
Org. + Ele. C,[a] wgt. %	0.11	0.15	0.22
Total H, wgt. %	0.64	0.49	0.33
Total N, wgt. %	0.03	0.09	0.01
Total O, wgt. %	9.28	3.75	3.43
Total S, wgt. %	4.55	2.36	2.04
Ash, wgt. %	81.26	80.94	83.96
Moisture, wgt. %	1.82	0.64	0.82

[a]Estimated organic plus elemental carbon.

appears to be highest in the ash with the lowest total carbon could indicate a more efficient combustion of carbon.

Only small differences were detected in the particle aerodynamic size distributions (Figure 1) and specific surface areas of the ashes. All three were quite similar in respirable ($\leqq 10\,\mu$m)[16] fine particle content. The ESP-shorted fly ash had a greater percentage of large particles which presumably would have been removed by the ESP if it had been in operation.

For mutagenicity bioassay preparation, each of the three fly ash samples were subjected to the high pressure solvent extraction. This procedure limits exposure of the fly ash to heat, light, air, and other oxidizing agents, and maximizes solvent contact with the fly ash. We believe that these factors minimize sample degradation and artifact formation. The four resulting solvent extract residues of each of the three fly ashes were then subjected to a mutagenicity bioassay utilizing the Ames *Salmonella typhimurium*/microsomal activation system.[14] We found that all four of the solvent extracts of both the normal and the low NO_x fly ashes lacked mutagenic activity detectable by any of the four tester strains with or without S-9 mix activation. This result does not exclude the possibility that these fly ashes contain unextracted mutagens. Indeed, we have

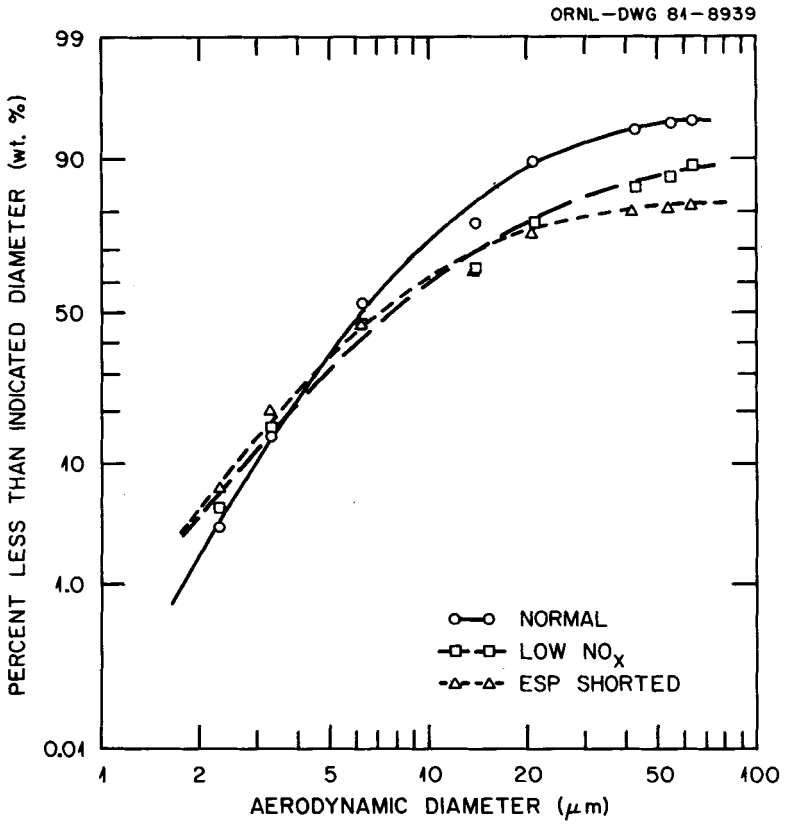

FIGURE 1 Aerodynamic particle size distribution of fly ash samples.

found[17] that the low NO_x ash is much more sorptive for benzo(a)pyrene, a known mutagen and carcinogen, than the other two ashes.

In contrast to the results for the other two ashes, the first three solvent extracts of the ESP-shorted fly ash contained low levels of mutagenicity detectable by three of the four tester strains, as shown in Table II. The TA-1535 strain was unable to detect mutagenic activity in any of the extracts of the ESP-shorted fly ash. The response to the other tester strains indicates the presence of mutagens acting by a frameshift mechanism, as we and other investigators have reported previously.[1–7] The benzene extract contained both indirect and direct-acting mutagens, while the methylene chloride and ethyl acetate extracts contained only indirect-acting mutagens. Interestingly, the methylene chloride extract required a phenobarbital induced S-9 mix to revert the TA-100 strain,

TABLE II

Results of mutagenicity testing of ESP-shorted fly ash extracts

	Rev/μg by strain[a]					
	TA-1537		TA-98		TA-100	
Solvent extract	+S-9	−S-9	+S-9	−S-9	+S-9	−S-9
Benzene	0.26	0.68	0.40	0.72	1.6	0
Methylene chloride	0	0	0.6	0	3.0[b]	0
Ethyl acetate	0	0	0.6	0	1.0	0

[a]With and without Arochlor S-9 activation.
[b]Activation with Phenobarbital S-9.

suggestive[18] of the presence of amines or aromatic nitro compounds. This observation is consistent with Fisher's positive Griess Test result[6] with a fly ash extract. The total mutagenic activity of the material extracted from the ESP-shorted fly ash, estimated by adding the weighted activities of the solvent fractions and assuming no synergistic or antagonistic effects, corresponds to approximately 10^3 revertants per gram of fly ash. This result is considerably lower than the approximately 3×10^4 revertants per gram estimated from the application[2] of the TA-1538 strain to filtered serum extracts of a 2.2 μm particle size fraction of a different coal fly ash.

None of the fly ash characteristics listed in Table I correlate with mutagenicity. Likewise, the results of inorganic and radiochemical analyses (Tables III) of the fly ashes do not provide many correlating results. For example, chromium, iron, manganese, and selenium compounds are reported (see discussion and citations in Reference 2) to be mutagenic to Salmonella, yet there is no apparent relation between their concentrations in the bulk fly ash and the fly ash extract mutagenicity. Only the halogens appear to parallel the mutagenic activity.

Results from the characterization of the solvent extracts of the fly ashes by both gas chromatography–mass spectrometry (GC–MS) and Fourier-transform infrared spectroscopy (FT–IR) appear to correlate with the observed mutagenicity. Consistent with their similar, but negative, mutagenicity bioassay responses, the corresponding solvent fractions of the normal and low NO_x fly ashes were found to be nearly identical in chromatographically and spectroscopically-definable content. GC–MS and FT–IR analysis showed that the benzene fractions were mainly aliphatic hydrocarbons with a trace level of aromatic hydrocarbons. The methylene

TABLE III

Results of inorganic and radiochemical analyses of fly ash samples.

Element	Concentration (μg/g) in fly ash[a]			Element	Concentration (μg/g) in fly ash[a]		
	Normal	Low NO_x	ESP-shorted		Normal	Low NO_x	ESP-shorted
Al(%)	12.5	13.0	13.3	La	61.4	64.9	69.5
As	329	278	263	Mg(%)	1.3	1.3	1.4
Au	0.13	0.05	0.18	Mn	152	123	123
Ba	859	783	706	Mo	50	41	38
Br	12.9	22.1	94.1	Na(%)	0.2	0.18	0.18
Ca(%)	0.86	0.86	0.90	^{222}Rn(Bq/g)	0.12	0.08	0.09
Ce	118	124	135	Rb	152	141	94
Cl	72	42	251	Sb	10.4	8.7	8.7
Co	36.1	36.7	36	Sc	33.8	34.9	36.6
Cr	600	221	225	Se	335	108	218
Cs	10.5	10.7	10.9	Sm	12.8	13.6	14.3
Cu	481	—	193	Sr	578	579	613
Eu	2.8	2.8	3.2	Ta	1.59	1.50	1.94
Fe(%)	5.0	5.39	4.68	Tb	1.43	1.79	2.47
Ga	89	80	87	^{232}Th	21.2	21.3	22.2
Hf	5.2	5.9	5.7	^{228}Th(Bq/g)	0.09	0.06	0.08
I	4.3	3.8	15.5	Ti(%)	0.66	0.70	0.71
In	0.32	0.31	0.27	U[b]	12.8	11.5	11.3
^{39}K(%)	1.86	1.85	1.92	V	353	342	327
^{40}K(Bq/g)	0.63	0.57	0.61	W	9.3	8.3	8.2
				Yb	6.1	5.3	6.2

[a]Concentration in μg/g except where indicated as % or Bq/g.
[b]99.3%^{238}U, 0.7%^{235}U.

chloride extracts also exhibited aliphatic character but displayed in addition some carbonyl and aryl C–O functionality. The IR spectra showed that the even more polar ethyl acetate extracts were mainly sulfates with a small level of carbonyl and aryl C–O functionality. The ethanol extracts were almost completely sulfates.

The corresponding but weakly active extracts from the ESP-shorted fly ash differed from those of the other two ashes in two respects. First, the solvent-extractable masses from the benzene and methylene chloride solvents were greater by factors of 2 to 10 than those of the other two ashes. Also, and probably more significant, is the finding that the ESP-shorted fly ash extracts were quite different in their chromatographic and spectral characteristics than those of the other two fly ashes. GC–MS

examination of the benzene extract revealed that the chromatographable constituents were predominantly C_2- and C_3-alkyl substituted three and four ring polycyclic aromatic hydrocarbons. This observation could account for the mutagenicity exhibited by the benzene extract of the ESP-shorted fly ash in comparison to those of the other two fly ashes.

Infrared spectroscopic characterization indicated that the ESP-shorted fly ash extracts (particularly the methylene chloride and ethyl acetate extracts) displayed a much more pronounced carbonyl, aryl C–O, and –OH functionality. Figure 2 compares the IR spectra of the methylene chloride extracts of (A) the mutagenic ESP-shorted fly ash and (B) the non-mutagenic low-NO_x fly ash. The polar functionality displayed in the spectrum of the formar suggests that mutagenicity of the ESP-shorted fly ash solvent extracts is associated with organics having polar functional groups.

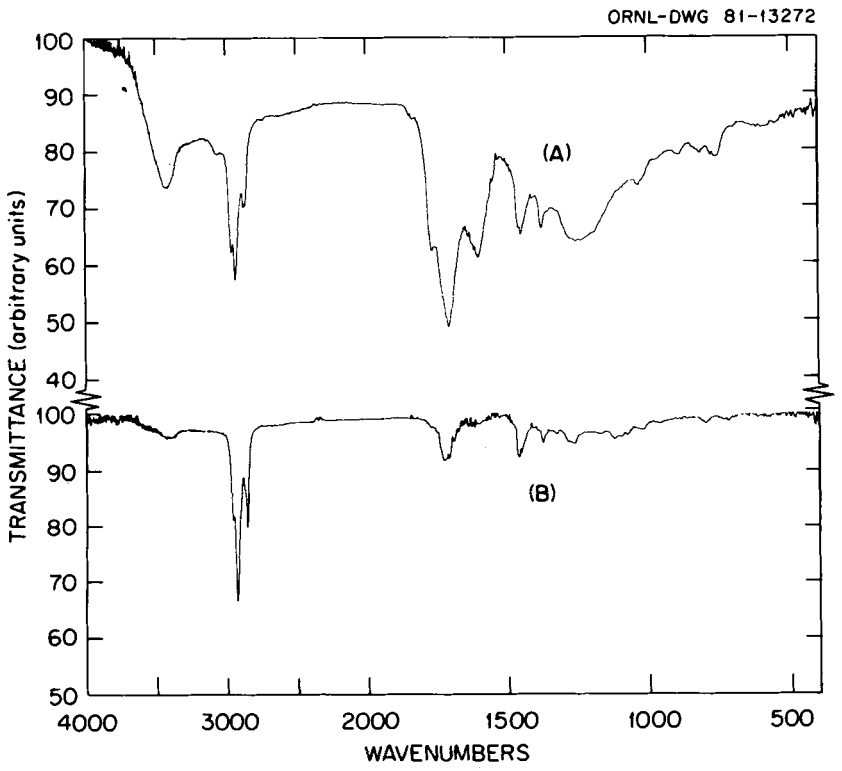

FIGURE 2 IR spectra of methylene chloride extracts of (A) ESP-shorted fly ash and (B) low NO_x fly ash.

GC characterization of unfrationated, trimethylsilylated extracts of the three fly ashes also appears to correlate with mutagenicity. As can be seen from the glass capillary colum gas chromatographic profiles in Figure 3, the extract from the mutagenic, ESP-shorted fly ash is more complex and concentrated than those of the other two non-mutagenic fly ashes. GC–MS indicated that most of the species were trimethylsilylated, and hence contained polar functional groups with replaceable hydrogen. Of the many GC peaks observed, only the trimethylsilyl esters of dodecanoic through octadecanoic acids were tentatively identified by GC co-chromatography and by mass spectra. The other peaks remain unidentified at the present. These monocarboxylic acids also were observed in the other fly ash

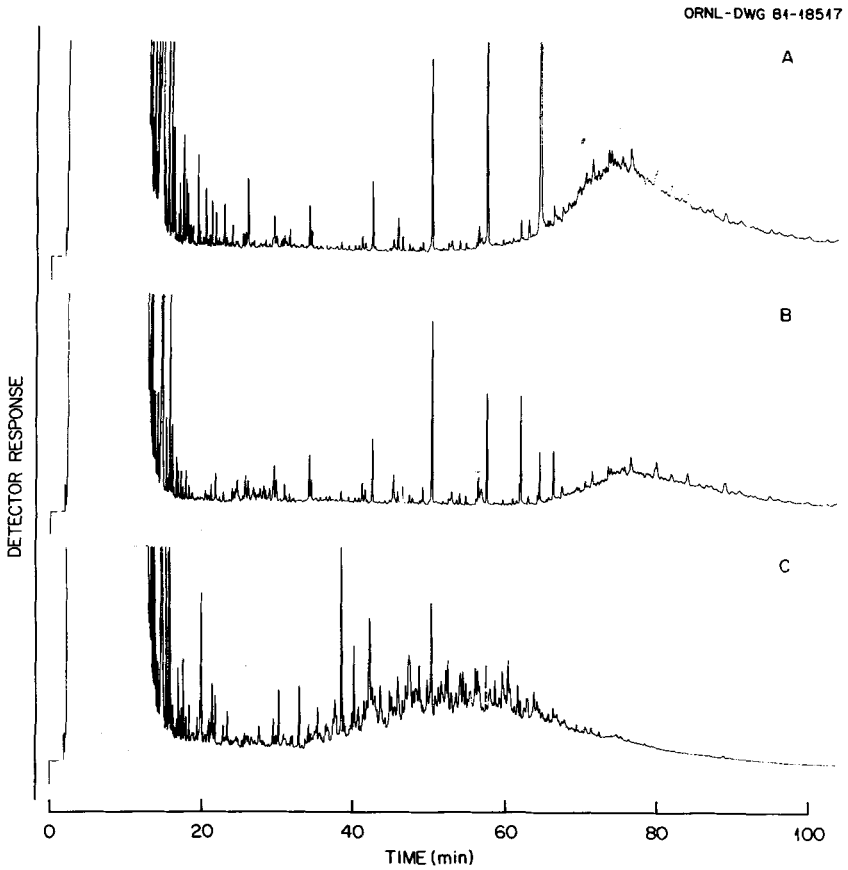

FIGURE 3 Capillary-column GC profiles of trimethylsilylated extracts of (A) normal, (B) low NO_x, and (C) ESP-shorted fly ashes.

extracts. However, the hexadecanoic and octadecanoic acid esters were found in the analytical blank. Thus, the assignment of these latter two carboxylic acids to this fly ash is not positive at the present. Succinic acid was identified only in the ESP-shorted fly ash extract.

CONCLUSION

Our finding of mutagenic activity in extracts from the ESP-shorted fly ash is consistent with Fisher's hypothesis[5] that a significant portion of coal fly ash mutagenicity arises from weak organic acids and other compounds[6] soluble in base but not acid solution. However, we are unaware of any reports of the mutagenic activity of carboxylic acids. We suggest instead that the mutagenic activity of this fly ash arises from the complex mixture of aromatic compounds and also the presently unidentified, but polar organics which may include carboxylic acids. These same polar species may be those appearing in Fisher's acid fraction. There is no indication that the trace elements or general chemical/physical properties determined in this study relate to mutagenicity.

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