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Mutagenicity in Emissions from Coal- and Oil-Fired Boilers

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The mutagenicity of emission samples from three oil-fired and four coal-fired boilers have been compared by using the Salmonella/microsome assay. Very little or no mutagenic activity was observed in samples from five of these boilers. The sample from one oil-fired boiler showed mutagenic activity of about 500 revertants/MJ, and the sample from a coal-fired fluidized bed combustor had an activity of 58.000 revertants/MJ measured with strain TA 98 in the absence of metabolic activation. All samples contained substances that were cytotoxic to the test bacteria, thus making it difficult to obtain linear dose-response curves. Mutagenic activity at low levels may remain undetected due to this toxicity of the samples.

Samples with mutagenic activity below the detection limit in the Salmonella test have also been tested for forward mutations at the HGPRT locus in V79 hamster cells. Weak mutagenic effects were detected in two of the samples, whereas the sample from one oil-fired boiler remained negative. In this test, as well as in the Salmonella test, a strong cytotoxic effect could be observed with all samples.

Introduction

Emission of mutagenic substances from coal combustion was first reported by Chrisp and coworkers in 1978 (*1*). They found that serum extracts of fly ash particles collected at 95–100°C downstream from an electrostatic precipitator of a large, coal-fired power plant contained direct mutagens when tested in the Ames Salmonella assay. Bacterial strain TA 1538 was found to be the most sensitive, whereas no mutagenic activity could be found with strain TA 1535, indicating the presence of frame-shift mutagens. They suggested that most of the mutagenic activity was caused by organic compounds. Later studies showed that most mutagens were associated with the finest and most respirable size fractions of the fly ash and that the activity depended upon the sampling temperature (*2*). No activity could be found in extracts from a similar-sized fraction of particles from the electrostatic precipitator of the plant operating at 104-107°C (*2,3*).

Particles emitted from three experimental fluidized bed combustors have been tested for mutagenicity in the Salmonella assay (*4-6*). In all cases the particles were sampled at temperatures above 100°C, and some of the extracts elicited considerable mutagenic activity without metabolic activation. Further studies on one of these combustors have shown that the mutagenic activity of particles collected under steady-state operation of the combustor was considerably reduced compared to that of particles collected during start-up conditions (*7,8*).

Results from testing of fly ash extracts in the *E. coli* Pol A+/A- test have indicated that inorganic mutagens may be present on such particles as well (*9*). Furthermore, fly ash has been found to be mutagenic in a test with Paramecium, a protozoan that ingests nonnutritive particles (*10*).

With the strong demand for more energy in the world today and the expected increase of the utilization of coal for heating and electricity production, there has been a concern over the impact of coal-burning emission on environment and health.

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Such concern has initiated the Swedish Coal-Health-Environment Project. An objective of this project is to quantify and compare the emissions of certain inorganic and organic compounds as well as mutagens from boilers burning coal and oil and utilizing different modes of combustion.

The emission measurement program within the Coal-Health-Environment project includes twelve coal- and three oil-fired boilers. Results from the mutagenicity testing and some of the chemical analysis from the first seven plants of the program are presented in this paper.

Materials and Methods

Sampling

Emission samples have been collected from boilers at seven different plants. Boilers at plants A to C used oil as a fuel, whereas the remaining boilers, D-J, burn low sulfur coal.

Plant A was a steam boiler (600 tons/hr steam) equipped with an electrostatic precipitator. During the tests the load was 54% (·12 MW_{th}). The boiler load was depending on the energy required for district heating.

Plant B was a hot water boiler without any flue gas cleaning system. The maximal load was 50 MW_{th}. During the tests the capacity was limited to 68% (37 MW_{th}).

Plant C was a small hot water boiler with a capacity of 5.8 MW_{th}. It was operated almost at maximal load during the tests. The boiler was not equipped with any flue gas cleaning system.

Plant D was a pulverized coal-fired power plant operating at full load producing 270 MW_e (600 MW_{th}). The boiler was equipped with a high efficiency electrostatic precipitator.

Plant F was a coal-fired industrial steam boiler (40 tons/hr steam). The low sulfur (0.9%) coal was fired on a moving grate (wanderrost). During the tests the load was 78% (25 MW_{th}). The boiler was equipped with multiple cyclones.

Plant G was a coal-fired steam boiler. The steam was heat exchanged and used for district heating. The low sulfur coal (0.7%) was fired on a moving grate similar to that of plant F. During the tests the boiler load was 90% (29 MW_{th}). The samples were collected in a flue gas stream cleaned in a bag house filter installed for test purposes.

Plant J was a small fluidized bed coal combustor arranged mainly for demonstration but also producing hot water for district heating. The capacity was 4.7 MW_{th}, and the samples were collected at 87% load. The FBC was equipped with a bag house filter.

Several combustion parameters were monitored continuously during sampling and kept as close to steady-state conditions as possible. The flue gas conditions at the different boilers are given in Table 1. The numbers in the table are mean values, and the variation in these parameters was rather low, with standard deviation usually less than 10%.

The O_2 content in the flue gases indicates the amount of excess air present at the sampling point in the stack. In boilers A, F and G, there was a significant leakage of air into the flue gas in the air preheater and the precipitators.

There is a marked difference in the combustion conditions of plant J compared to the other boilers as shown by the CO content of the flue gases. Also, particulate emission was exceptionally high from plant J caused by problems in the bag house filter.

Sample Collection

The samples were collected isokinetically downstream of the plant's cleaning device by a modified version of the SASS train developed by Studsvik

Table 1. Flue gas conditions during tests.

Parameter	Plant A	Plant B	Plant C	Plant D	Plant F	Plant G	Plant J
Fuel input, kg/sec	7.7	0.90	0.14	24	1.0	1.1	0.2
Fuel input, MW	312	37	6.0	600	25	29	4.8
Flue gas conditions at the sampling point							
Temperature, °C	158	151	220	140	155	118	164
Gas flow, m^3/sec^a	96.7	10.7	1.7	210	12.8	13.3	1.7
Gas flow, m^3/MJ^a	0.31	0.29	0.29	0.35	0.51	0.46	0.35
Moisture, vol-%	11	11	11	7	5	5	7
O_2, vol-%	3.0	2.4	2.8	5.6	10	10	6.7
CO_2, vol-%	13.0	13.7	13.6	14.1	9.3	10.5	12.9
CO, ppm	40	3	16	<1	15	8	2000
SO_2, ppm	1050	520	—	603	394	377	333
NO_x, ppm	190	255	150	479	49	132	130
Particulates, mg/m^{3a}	10	10	154	80	78	1	2200

[a]Calculated as standard cubic meters dry gas and MJ heat input with the fuel.

(*11*). The sampling device was all glass and is shown schematically in Figure 1.

The interchangeable probe extended into an oven module which was kept at 160°C during the sampling. The oven contained a variable range cyclone and a high purity tissue quartz thimble filter (Grycksbo AB). At a flow rate of 4 m^3/hr (standard, dry), the cut size of the cyclone was adjusted to 2 μm. After leaving the filter the flue gas was drawn through a high efficiency cooler, a condensate collector and an XAD-2 sorbent trap. The coolant was maintained at a temperature below -10°C so that the temperature of gas entering the XAD-2 adsorbent bed did not exceed 5-10°C. After sampling, the probe and the cooler were washed with acetone, which then was treated as a part of the sample. The flue gas volumes in each sample varied from 15 to 30 m^3 (standard, dry) for the Salmonella test and about 100 m^3 (standard, dry) for the V79 forward mutation test.

Sample Preparation

The larger particles (aerodynamic equivalent diameter > 2 μm) were extracted twice with acetone by using ultrasonication, whereas the tissue quartz filters were Soxhlet-extracted for 16 hr with acetone. The extracts were concentrated to near dryness by evaporation at reduced pressure. Dimethyl sulfoxide (DMSO) was added, and the remaining acetone was removed under a stream of nitrogen.

For analytical purpose and for testing of the most mutagenic sample, several successive extractions were performed using solvents of increasing polarity. The extraction methods were the same as described above. The condensates were extracted twice with dichloromethane (DCM), first at pH 2 and thereafter at pH 11. The two extracts were usually combined, evaporated and redissolved in DMSO.

The adsorbent columns were Soxhlet-extracted with acetone as described for the filters for the Salmonella test. For testing in V79 cells, the XAD-2 beds were extracted with DCM. The samples were all transferred to DMSO before testing in the Salmonella test and to acetone before testing in the V79 test.

When subjected to chemical analysis, the extracts from the condensate and the adsorbent columns were fractionated into an acidic, a basic and a neutral fraction as described previously (*12*). This procedure was also followed for the most mutagenic of the stack gas samples.

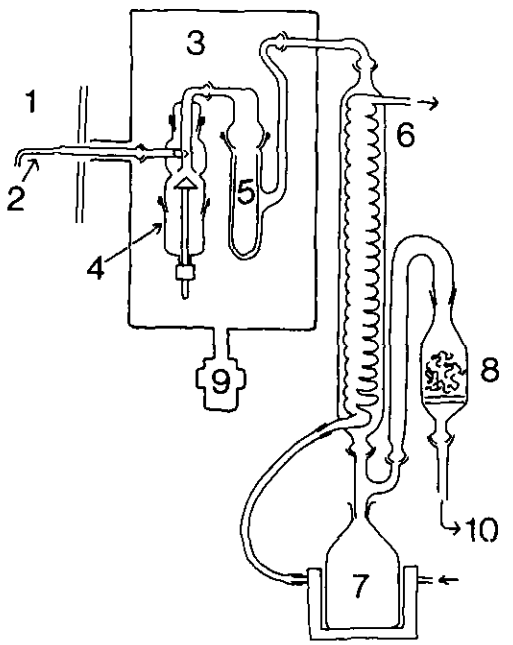

FIGURE 1. Studsvik sampling system: (1) stack; (2) glass probe; (3) heated oven, 160°C; (4) glass cyclone; (5) quartz fiber thimble; (6) cooler; (7) condensate collector; (8) XAD-2 adsorbent bed; (9) heater; (10) to the pump and volume meter.

Mutagenicity Testing

Salmonella/Microsome Test. The samples were tested for mutagenicity in the Ames Salmonella microsome assay (*13*) using the two bacterial strains TA 98 and TA 100 kindly supplied by B. N. Ames.

The microsomal fraction (S9 mix) contained rat liver supernatant prepared from Aroclor 1254-induced male Wistar rats and the necessary cofactors. An amount of 50 μL, corresponding to 2.5 mg protein, was added per plate.

The testing was done with duplicate plates, and the extracts were tested in three to six doses. Most of the extracts were toxic to the bacteria, as revealed by inhibition of the background growth, and the dose range of testing for these samples was therefore limited. All assays included tests with positive control compounds, benzo(a)pyrene (BaP) and 1-nitropyrene (1NP) as well as blank extracts of filters and XAD-2 controls.

In the presence of S9, 5 μg BaP gave 320–620 net revertants per plate with strain TA 98 and approximately 1000 net revertants with strain TA 100. In the absence of S9, 100 ng INP resulted in 280–500 net revertants per plate with TA 98.

TLC-Ames Test. Concentrated extracts in acetone were applied on a thin layer chromatography (TLC) silica-layered plate (10 × 10 cm). Two-dimensional chromatography for separation of components in the extracts was performed with two solvent systems: (I) chloroform:acetic acid (10:0.1)

or (II) chloroform:benzene:ethyl acetate:methanol: aqueous ammonia (4:1:3:2:0.2) as described previously (*14*).

After development, the mutagenicity testing was performed directly on the TLC plate by a modification of the Salmonella assay. Minimal agar was added directly on top of the TLC plates and the bacteria with S9 mix added in the top agar (three times regular amounts).

Forward Mutation at the HGPRT Locus in V79 Hamster Cells

The screening for mutagenicity and toxicity in V79 Chinese hamster cells was performed according to the optimal protocol described by van Zeeland and Simons (*15*). A detailed description of the test procedure is given elsewhere (*16*).

Materials

Technical grade cyclohexane and acetone were purified by distillation. Dichloromethane (Rathburne HPLC Grade) was used without further purification. Potassium hydroxide (EKA pa) was used without purification. Quartz fiber filter was purified by heating to 530°C. Amberlite XAD-2 (Rohm-Haas) was purified by successive washing with water, methanol and acetone. The resin was then packed in the sampling ampoule and Soxhlet-extracted with acetone and dichloromethane for 48 hr with intermediate changes of the solvent. All glassware used for sampling was carefully rinsed and then heated to 450°C overnight.

Benzo(a)pyrene was purchased from Sigma and 1-nitropyrene (labeled 3-nitropyrene) from Koch-Light.

Results and Discussion

Salmonella/Microsome Assay

The extracts from the two particle fractions, the condensate and the adsorption columns for all samples were tested separately and in combination. Combined extracts were tested with strains TA 98 and TA 100 with and without metabolic activation. For most samples, the activity was most pronounced in TA 98 without S9. The results obtained under these test conditions are summarized in Table 2. As can be seen from Table 2, mutagenic activity was detected in extracts of stack gas samples from four of the seven boilers investigated so far. Three of these were coal-fired, whereas one was oil-fired. For two of the coal-fired plants, D and G, the mutagenic activity was very low, just above the detection limit.

No mutagenic activity was observed in the blank extracts. However, some of the blank XAD-2 extracts were slightly toxic to the bacteria. All the samples contained relatively high concentrations of compounds that were toxic to the test bacteria, thus making it difficult to obtain linear dose-response curves. Dose-response curves for the most mutagenic sample, J, with strain TA 98 are shown in Figure 2.

The numbers for revertants per m^3 (MJ, kg fuel) in Table 2 were calculated from the linear parts of the dose-response curves when linearity was obtained.

In cases of nonlinearity and when calculating detection limits, we used reversion frequencies twice the number of spontaneous revertants as criterium for positive response, as suggested by Ames (*13*). When linear dose-response curves could not be obtained, the results were calculated from the slope of the tangent to the dose-response curve at its lowest significant point. The problems caused by the toxicity of the samples imply that the uncertainty in the results and also the detection limits are greater than usual with this test system. As the uncertainty was even greater in TA 100 than in TA 98, the results obtained with this strain has been omitted from this report.

The mutagenicity data in Table 2 either represent a sum of the test results for the separate

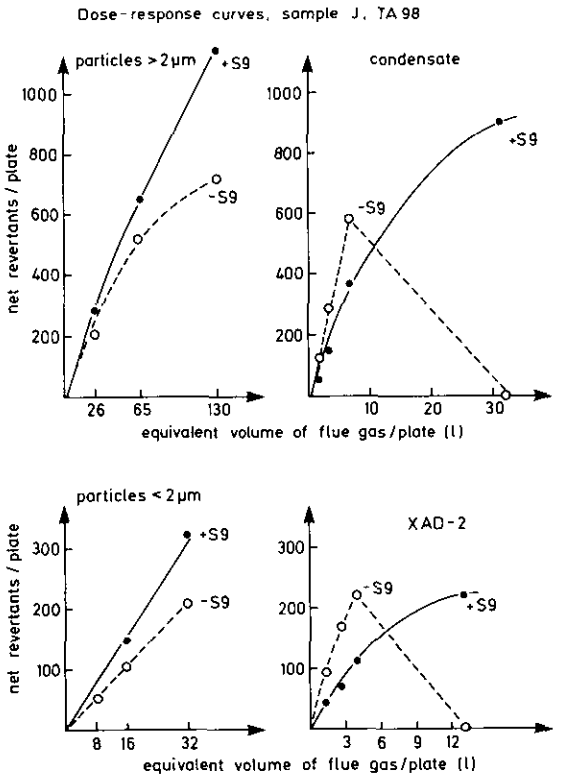

FIGURE 2. *Dose-response curves for separate fraction extracts* of emission samples from a coal-fired fluidized bed combustor; strain TA 98.

Table 2. Mutagenicity in emission samples from oil- and coal-fired boilers as measured in the Ames test with Salmonella strain TA 98.

Sample code	Fuel	Boiler description	Sample size, m^3 (standard, dry)	Gas flow rate, 10^3 m^3/hr	Mutagenicity		
					rev/m^3	rev/MJ	rev/kg fuel
A	Oil	312MW_{th}	29.2	350	< 160	< 50	< 2,000
B	Oil	37MW_{th}	28.2	39	1.700	490	20,200
C	Oil	5.8MW_{th}	26.9	6	< 250	< 70	< 2,600
D	Coal	Pulverized coal, 270MW_e	14.7	756	150	50	1,300
F	Coal	Moving grate, 25MW_{th}	30.6	46	< 150	< 80	< 1,900
G	Coal	Moving grate, 29MW_{th}	19.7	48	150	75	1,800
J	Coal	Fluidized bed, 4.7MW_{th}	32.5	6	165,000	57,750	1,386,000

fractions of a sample or the results from testing a combination of these fractions. The mutagenic activity of the extracts separately and in combination are given in Table 3 for the four samples with positive response. As seen from Table 3, testing of the combined fractions usually resulted in lower mutagenic activity than the sum of test results for the separate fractions. This was probably due to influence from the most toxic fractions, which in most cases were the XAD-2 fractions. For two of the samples in Table 3 (D and G), the toxicity of the XAD-2 extracts was so dominant that this extract had to be omitted from the combined sample in order to obtain positive net revertant counts.

As seen from Table 3, the sample from the fluidized bed combustor was the only sample showing muta-

Table 3. Mutagenicity of separate and combined fractions of emission samples.

Boiler	Fuel	Fraction	Mutagenicity with TA 98, rev/m^3		Effect on standard mutagens	
			-S9	+S9	Relative response of BaP[a]	Relative response of INP[a]
B 37MW_{th}	Oil	1. Cyclone	< 30	< 30	1	0.6
		2. Filter	440	150	0.8	0.7
		3. Condensate	450	210	1.3	1.0
		4. XAD-2	210	290	1.2	0.6
		Σ 1-4	1100	650		
		Combined sample of 1-4	1760	450		
D 270 MW_e, pulverized coal	Coal	1. Cyclone	< 30	< 40	1	n.t.[b]
		2. Filter	70	30	0.5	n.t.
		3. Condensate	< 30	< 40	1	n.t.
		4. XAD-2	n.d.[c]	n.d.[c]	0.5	n.t.
		Σ 1-4	70-130	30-110		
		Combined sample of 1+2+3	150	< 130		
G 29 MW_{th}, moving grate	Coal	2. Filter	90	50	< 0.5	1
		3. Condensate	60	50	1	1
		4. XAD-2	n.d.[c]	n.d.[c]	< 0.5	< 0.5
		Σ 2-4	150	100		
		Combined sample of 2+3	120	< 150		
J 4.7 MW_{th}, fluidized bed	Coal	1. Cyclone	8100	10000	0.7	n.t.[b]
		2. Filter	6500	10000	1.0	0.7
		3. Condensate	84000	45000	1.0	1.2
		4. XAD-2	66200	27000	1.1	0.6
		Σ 1-4	164800	92000		
		Combined sample of 1-4	131000	107500		

[a]Calculated as rev[(BaP or INP + sample) − rev (sample)]/rev (BaP or INP).
[b]Not tested because of limited sample.
[c]Not detected, but in contrast to the other extracts where no mutagenicity was detected, these extracts were so toxic that no detection limit could be calculated.

genic activity in the extract from the cyclone-collected particles. As mentioned, the bag house filter of this boiler did not function properly during sampling and this accomplished an exceptionally high concentration of particles greater than 2 μm in the stack gas.

The lack of mutagenicity in the other cyclone samples is in accordance with the findings of Fisher (*3*) that mutagenicity is mainly associated with the smallest particles. However, Fisher found a sampling temperature of 100°C or less to be critical for condensation of mutagens on particles (*3*).

In our studies, the samples were collected at 160°C which in most cases was close to the stack gas temperature (Table 1). The difference between the mutagenicity of cyclone and filter extracts may therefore partly be explained by the fact that the cyclone particles are removed from the flue gas at the moment of sampling, whereas particulate matter on filters is continually exposed to the flue gas stream, with a greater possibility of adsorbing volatilized organics. Continually exposure to the reactive combustion gases also increases the possibility of forming filter artifacts. However, there was no correlation between mutagenicity of the filter fractions and the concentrations of reactive gases as NO_x and SO_2 in the flue gas as would have been expected if the filter mutagens represented artifacts.

In Table 3 are also included the results from testing the samples in combination with the positive controls, BaP and 1NP. These tests were performed to see how the toxicity of the samples would influence the mutagenic activity of known mutagens. This testing was usually done with one dose of standard mutagen and two doses of the sample, and the numbers given represent the average from these tests. Most of the samples had a reducing effect on the mutagenic activity of the direct mutagen nitropyrene. This was also observed with the extracts from nonmutagenic samples (data not given). The reduction of mutagenicity was probably caused by the reduction of bacterial growth due to the presence of toxic compounds, even if the possibility of antagonistic effects cannot be disregarded without further studies. These results should only be taken as qualitative indications, and comparison of the numbers should be avoided, since the tests were performed at very few doses and the size of the doses have been different for the various samples and fractions.

The interpretation of the observed effects on the mutagenicity of BaP is even more complicated, since a possible effect on the S9 system has to be considered as well. For all samples, the toxicity as observed by microscopy or by the shape of the dose-response curves diminished in the presence of S9. Still, about half of the particle fractions and most of the XAD-2 fractions caused a reduction of the mutagenic activity of BaP, probably due to their toxic effect. More striking, however, is the observation that condensate extracts most often caused a slight increase of the mutagenicity. This was also observed with the nonmutagenic extracts where the relative response (defined in Table 3) varied from 1.1 to 1.3. Further studies are needed to find whether this enhancement was caused by the presence of substances acting as comutagens for BaP or possibly by changing the test mixture to yield more optimal concentrations of S9.

Samples that were found to be nonmutagenic were retested in a newly developed assay where the substances in a complex mixture are separated by thin layer chromatography and detected *in situ* by the Ames Salmonella test (*14*). After separation on the TLC plates with the chromatographic system described under methods, the mutagenicity assay was performed directly on the TLC plates. So far, this TLC/Ames test is insensitive to the most nonpolar organic compounds as unsubstituted PAH (*14*).

The rationale for using this test is that a separation of toxic compounds from mutagenic compounds may be obtained (if they are separable), thereby demasking the mutagenic effect.

Testing with this system confirmed the previously obtained negative results. Figure 3 shows the distribution of bacterial colonies on the TLC plate for a solvent blank and the condensate fraction of sample A compared to the filter fraction, and the condensate fraction of a positive sample, sample B. No difference can be seen between the plate with the solvent blank and the condensate fraction of sample A. The other fractions from sample A looked similar to the one given in the figure. The plates run with sample B show a clustering of bacterial colonies in two different areas. For the particle extract, the activity was concentrated in an area close to the application spot, indicating that the most mutagenic compounds are rather polar and probably acidic. The condensate extract, however, showed most of its activity in the upper right corner of the plate, in the same area of the plate, whereto derivatives of polycyclic aromatic hydrocarbons like nitro-PAH would migrate.

Parallel samples from all seven plants have been subjected to detailed chemical analysis. The analytical methods and the results are published elsewhere (*12,17*). The same types of organic compounds were found in the extracts from all the boilers. The concentrations did, however, vary over a wide range depending on the efficiency of the combustion. A comparison of the total amount of organics related to the heat production of the boiler is given in Table

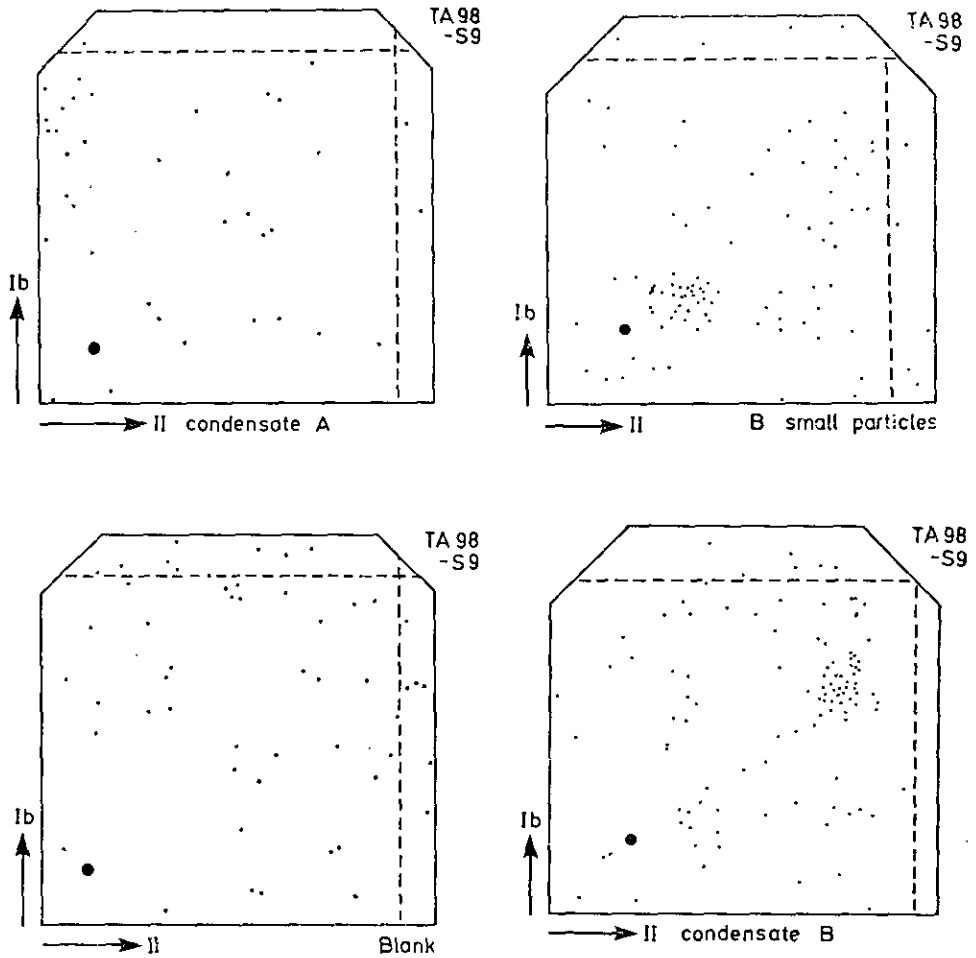

FIGURE 3. TLC/Ames test of emission samples from oil-fired boilers; TA 98, -S9.

4, together with the mutagenicity data and the content of carbon monoxide as an indicator for combustion efficiency. The total amount of organics was determined as the sum of total chromatographable material in the separate extracts for each sample (*17*).

It is obvious from Table 4 that for the low emission levels that seemed to be normal for conventional boilers operated under steady-state conditions there was no clear correlation between the parameters in the table. Emissions of both mutagens and total organics from the coal-fired FBC-boiler were several magnitudes of order higher than from the other boilers. This is also illustrated

Table 4. Emission of CO, mutagens and total organics (Σ total chromatographable matter) from different oil- and coal-fired boilers.

Boiler	Fuel	CO, mg/MJ	Total organics, mg/MJ	Mutagencity, (TA 98, –S9) rev/MJ
A	Oil	15	1	< 50
B	Oil	1	0.08	490
C	Oil	6	0.08	< 70
D	Coal	< 1	0.1	50
F	Coal	10	0.05	< 80
G	Coal	6	0.07	75
J	Coal	900	9	57750

by the more detailed analytical results given in Table 5. As seen by the CO concentration, this boiler was operating under deficient combustion conditions. During sampling several problems occurred with the regulation of the load and the combustion efficiency of this boiler. However, relatively high concentrations of mutagens have previously been reported in emissions from fluidized bed combustors (*4–8*). This may be a typical phenomenon caused by the low combustion temperature (800–950°C) used in such boilers. Further investigations are needed to find whether the emission of mutagens from fluidized bed combustors can be reduced to the level of conventional boilers.

Because of its high mutagenicity, the relation between the mutagenic activity and the results from chemical analysis were studied more closely for the FBC sample. The extracts from the particles and from the XAD-2 were separated in subfractions in the same manner for analysis and for mutagenicity testing. The detailed results from the testing of these subfractions have been published elsewhere (*18*).

The analytical results (*17*) showed that the sum of identified PAH compounds was 3.5 times higher in the cyclone sample than on the filters. However, it has been shown by Ames et al. (*19*) that mainly high-boiling PAH compounds (four rings or more) are mutagenic and the amount of such high-boiling PAH was about equal in the two particulate fractions. This may explain why the mutagenicity with S9 was the same for the two fractions (Table 3). The amount of BaP in the samples could only account for less than 10% of the mutagenicity in the presence of S9. The high mutagenicity in the absence of S9 in all fractions indicates that this activity is caused by compounds different from the conventional PAH.

The particulate fractions contained organic acids and phenols which are toxic to the Salmonella bacteria. These compounds are probably the reasons for the difficulties in obtaining linear dose-response curves with these samples in high doses.

The condensate and XAD-2 extracts showed considerably higher mutagenic activity than the particulate fractions, and, in contrast to the particle extracts, these extracts showed the highest mutagenic activity in the absence of S9. The analysis showed that the amount of polycyclic organic mat-

Table 5. Results from chemical analysis of the mutagenic samples.[a]

Compound	Content of compound, μg/m³			
	Heavy fuel oil, Plant B	Pulverized coal, Plant D	Moving grate, Plant G	Coal, inefficient combustion, Plant J
Ethylene	100	180	5	5
Benzene	0.5	5	5	2000
Toluene	0.5	10	10	600
Naphthalene	0.5	~ 1	5	7000
Acenaphthene	<	<	<	270
Acenaphthylene	<	<	<	1400
Fluorene	<	<	<	200
Phenanthrene	<	0.2	0.2	600
Anthracene	<	<	<	100
Pyrene	<	<	0.07	200
Fluoranthene	<	0.1	0.07	200
Benz(a)anthracene	<	<	<	30
Chrysene	<	<	<	10
Benzo(ghi)fluoranthene	<	<	<	20
Benzo(b and k)fluoranthene	<	<	<	25
Benzo(a)pyrene	<	<	<	12
Indeno(1,2,3-cd)pyrene	<	<	<	5
Dibenz(a,h)anthracene	<	<	<	1
Benzo(ghi)perylene	<	<	<	4
Phenol	< 1	4	< 0.1	700
2-Chlorophenol	<	<	<	10
4-Chlorophenol	<	<	<	10
2,4- and 2,6-chlorophenol	<	<	<	3
Acetic acid	160	200	100	200
Benzoic acid	12	80	25	40
Benzaldehyde	1	2	1	750
Acetophenone	1	5	3	—
Benzonitrile	<	<	<	500
Quinoline	<	<	<	200

[a] < denotes below the detection limit, which varied between 1 and 10 ng/m³.

ter (POM) compounds was correspondingly higher in these samples. The sum of chromatographable matter was 20 mg/m^3 for the two combined fractions of condensate and XAD-2 and 6 mg/m^{3}for the two particle fractions. The concentration of BaP was however below the detection limit in the condensate as well as in the XAD-2 extract.

Results from testing the subfractions of the XAD-2 extract are given in Table 6. In the presence of S9, 80% of the activity was found in the neutral fraction, 15% in the acidic fraction, and only 5% in the basic fraction. Without S9 all the mutagenic activity was found in the neutral and the acidic fractions. This is in good agreement with the results published by Fisher (*3*) who suggested that a significant portion of the mutagenic activity in coal fly ash can be accounted for by the presence of weak organic acids. In the presence of S9 the sum of activity of the individual fractions was equal to the activity of the original XAD-2 extract. However, in the absence of S9 nearly 80% of the activity was lost during fractionation, indicating that these mutagens were labile and degraded by the acid/base treatment.

Formation of mutagenic reaction products between XAD-2 and nitrogen oxides has also been reported (*20*). Some of the tentatively identified decomposition products from the reaction between XAD-2 and NO_x, as benzoic acid, ethylbenzaldehyde and nitrophenol, have been detected in the extracts from the XAD-2 fractions. However, benzoic acid has also been found in particle and condensate extracts and may originate from the combustion. Anyhow, the published breakdown products (*20*) contain several substances that would be toxic to the bacteria. Hence, inhibition of bacteria growth as well as an increase of mutagenicity could be expected as a result of XAD-2 breakdown. As mentioned, the XAD-2 fraction was in most cases the most toxic one, but its contribution to the total mutagenicity was in no case more than 40%.

Table 6. Mutagenicity testing of fractionated XAD-2 extracts from the coal-burning fluidized bed combustor, J.

Fraction	Revertants/m^3	
	with S9	without S9
Acid	3850	5640
Basic	1000	
Neutral	20100	9130
Σ each fraction	24950	14770
XAD-2 extract (from Table 4)	26920	66150

Forward Mutation in V79 Hamster Cells

Samples from three of the plants, A, C and F, have been tested for toxicity and mutagenicity in V79 cells without metabolic activation. The results are given in Table 7. Extracts from the cyclone-collected particles and the condensate from the coal-fired boiler F were found to have weak, but clearly significant mutagenic activity. The small particles, collected on filter from the oil-fired boiler C, were also found to contain mutagens. In contrast to the results from the Salmonella test showing the XAD-2 extracts to be the most toxic, the extracts from the small particles were the most cytotoxic to the V79 cells. As the XAD-2 beds were extracted with different solvents, acetone for the Salmonella test and DCM for the V79 cells, this difference may be explained by a different chemical composition as well as by a variation in the sensitivity to toxic and

Table 7. Results from testing of toxicity and mutagenicity of extracts from coal- and oil-fired boilers with V79 hamster cells.

Boiler	Fuel	Fraction	Highest nontoxic dose, m^3	Induced mutations per m^3 and 10^6 cells
A	Oil	Cyclone	[a]	[a]
		Filter	n.t.[b]	n.t.[b]
		Condensate	0.4	0
		XAD-2	0.8	3
C	Oil	Cyclone	0.6	0
		Filter	0.5	34[c]
		Condensate	0.8	2
		XAD-2	0.6	0
F	Coal	Cyclone	1	28[c]
		Filter	0.05	0
		Condensate	1.2	31[d]
		XAD-2	0.5	13

[a]The amount of particles > 2 μ was so small that this fraction was combined with the condensate.
[b]Not tested because of limited material.
[c]Significant at $p < 0.05$.
[d]Highly significant at $p < 0.01$. The data were subjected to combined probability analysis (*22*).

mutagenic compounds between the bacteria and the hamster cells.

Conclusions

Our results indicate that emissions of mutagens from coal- and oil-fired boilers are low when the boilers are operated at optimal conditions. For samples from three of the plants investigated, no mutagenicity could be observed with the Ames Salmonella/ microsome test. Only one of these remained negative when tested for mutagenicity in V79 hamster cells. This apparent discrepancy probably only expresses a difference in the detection levels for the two test methods under the test conditions used. As mentioned, the detection limit for the Salmonella assay was unusually high due to the toxicity of the samples. Furthermore, it depended on the size of the sample, which was larger for the V79 test. A third and parallel sample from plant F has been tested in the Salmonella test recently, and the preliminary results indicate that this sample has a mutagenic activity slightly above the detection limit.

For samples from one of the oil-fired boilers, a mutagenic activity of 500 revertants/MJ was found with the Salmonella test. Neither the combustion parameters nor the results from the chemical analysis explains why this sample differs from the samples from the other two oil-fired boilers.

Emissions of mutagens from the FBC boiler were several magnitudes of order higher than from the other boilers, 58,000 revertants/MJ. This was consistent with higher concentrations of CO as well as considerably higher concentrations of organic compounds in the stack gas of this boiler compared to the other. Furthermore, it was clear that at least part of this increased emission was due to problems with regulation of the combustion efficiency of this boiler. It is highly probable that the emission from the burning of coal and oil will vary with the combustion efficiency, as has been shown for wood combustion (*21*). It is quite likely that shorter periods of operational disturbances may occur during normal operation of boilers, and that such periods may be accompanied by relatively high emissions of mutagens. For evaluation of the impact on health and environment, one would therefore need an estimate on the variation in the emission of mutagens during normal operation. Such investigations should be performed. Some information on the matter may be obtained from ongoing studies within the Coal-Health-Environment Project, where 1-hr samples of particulate matter have been collected continuously during 2 weeks in the stack of a coal-fired power plant and are now being tested for mutagenicity with the Ames Salmonella test.

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