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Lack of Mutagenicity of Extracts and Filtrates from "Hog-fuel" Fly Ash

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CHRISP et al. (1978) have shown that fly ash collected from a power plant burning low sulfur, high ash coal contains mutagenic material which can be extracted into horse serum, which is used as a model for lung alveolar fluid. This result caused concern that similar material might be present in the fly ash released from burning bark and wood-chips ("hog-fuel") in the boilers of various mills of a large forest products corporation. Using five different strains of histidine-requiring auxotrophs of Salmonella typhimurium, we have found that none of the samples from six different plants, in various parts of the United States, contains serum-extractable mutagens, and that only one of these contains mutagens extractable with hot benzene. Another sample, taken from a different boiler run at the same plant which produced the mutagenic sample, was without mutagenic activity.

Fly ash samples were collected downstream from the electrostatic precipitator at mills using different varieties of wood: (A) Douglas fir, (B) Southern pine, (C) Douglas fir stored in salt water ponds, (D) hemlock, (E) Douglas fir, and (F) Douglas fir and ponderosa pine. Samples (400 mg) were extracted with benzene in a Soxhlet extractor for 19 h; extracts were reduced to dryness under reduced pressure and redissolved in 4 mL of dimethyl sulfoxide. Other samples (2.0 g) were mixed with 20 mL of horse serum (Bio-Rad Lab.) incubated for 2 weeks at 37C in a shaker-incubator, centrifuged at 35,000 x g, and the supernatant was filtered through a disposable 0.45 µm membrane filter (Millipore).

Five strains of his⁻ auxotrophs of S. typhimurium were used. The testing methods and nature of the mutations have been described (AMES et al. 1975). N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG) (without activating enzymes), chrysene, and 2-acetamidofluorene (with microsomal enzymes) were used as positive controls. Enzyme preparations (microsomes + supernatant) for activation of promutagens were obtained from 160-170 g male Fischer rats treated with 500 mg/kg of Aroclor 1254 five days before sacrific. A single preparation was conducted with 25 g of liver; and the activating frac-

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tion obtained (S-9) was stored in 1-mL vials at -80°C. 0.1 mL of either DMSO solution or serum filtrate was added per 2 mL of the top agar before addition to the plates. Table 1 shows the response of the bacteria to the test samples and to controls. Only the benzene extract of ash sample D showed any activity. The highest dose used here was 26µg of extract residue/plate. Based on the mutant yield with TA100, this residue is of roughly the same potency as benzo[e]pyrene, which also is most active toward TA100 (MCCANN et al. 1975). The failure of horse serum to extract detectable mutagenic activity from any of the samples suggests that the actual hazard to the surrounding population from any of these mills, even mill D is minimal.

In fact, a second sample obtained from mill D some weeks after the first sample gave very little benzene-extractable residue, and the same amount of residue added to the bacteria yielded no detectable mutagenic activity (data not shown). The wood source was the same in both furnace runs, but records are inadequate to identify any differences in furnace conditions. Thin-layer separation of benzene extracts from both samples showed numerous substances in the mutagenic residue which were not present in the later sample. Separation of the mutagenic fraction into five fractions by preparative thin-layer chromatography yielded samples of roughly equal mutagenicity. An attempt to purify the most polar fraction, which was somewhat more active than the others, revealed at least 30 different components in this fraction. Because of this complexity and the observation that different burn conditions avoid mutagenic fly ash entirely, no further attempt was made to isolate pure fractions for screening or chemical analysis.

While the data overall suggest that the use of "hog-fuel" does not add any mutagenic load to the environment, the results obtained with the two samples from mill D indicate that monitoring of fly ash together with adequate records of burn conditions should be used to ensure that complete destruction of mutagens is taking place.

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Table 1. Mutagenicity of Fly Ash Extracts in Salmonella typhimurium[a]

Sample	Strain				
	TA 98	TA100	TA1535	TA1537	TA1538
Control	4	20	2	3	2
Control (Serum)	8	28	4	0	7
A (Benzene), 22 µg	4	13	2	0	3
A (Serum)	4	19	4	2	1
B (Benzene), 27 µg	6	16	1	0	1
B (Serum)	7	23	4	1	7
C (Benzene), 35 µg	9	20	3	1	4
C (Serum)	4	22	4	3	5
D (Benzene), 26 µg	24	90	1	19	12
D (Serum)	7	27	1	1	5
E (Benzene), 27 µg	3	22	2	4	2
E (Serum)	2	25	2	1	8
F (Benzene), 30 µg	7	18	2	2	4
F (Serum)	7	28	4	4	4
2-Acetamidofluorene, 4.5 µg	>500	>300	3	5	>500
Chrysene, 4.5 µg	6	156	4	4	5
MNNG, 1.1 µg	6	>600	>600	5	4

[a]Revertants/35 mm plate. All samples contained 2.0 mg S-9 protein/plate, except for MNNG. Each value is an average of at least two plates.