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Determination of Fly Ash in Lung Tissue

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In studies of the toxicity of inhaled coal combustion fly ash, it is more desirable to determine the amount of material actually deposited in the lung rather than to only measure the concentration of the ash in the exposure chamber. Previous studies to determine lung deposition by neutron activation of fly ash (GRIFFIS et al. 1980) or coal dust (GIBB et al. 1975) prior to animal exposure have been reported. However a method which does not require neutron activation of the fly ash is needed.

The elemental chemistry of coal fly ash has been extensively studied and was reviewed by SMITH (1980). Major elemental constituents are oxygen, silicon, aluminum, calcium, iron and magnesium. A method to determine the fly ash content of lung tissue by conventional wet chemistry techniques is reported. Our approach to quantitation of fly ash in lung tissue was to use a single element as a tracer for the ash. Aluminium was chosen because it is present in low concentrations in biological tissue, it is present in high, uniform concentrations in fly ash and it can be determined in both fly ash and lung tissue.

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MATERIALS AND METHODS

Analytical Method

The amount of fly ash in lung tissue was determined by analyzing for aluminum in fly ash samples, lung tissue from control animals and lung tissue from animals exposed to a fly ash aerosol. Samples were stored frozen (-10°C) until analysis could be performed. Reagent grade chemicals were used except where noted. The samples (lung or fly ash) were thawed, dried at 120°C overnight and acid digested in 23-mL pressurized Teflon digestion vessels (Uniseal Decomposition Vessels, Columbia Organic Chemical, Columbia, SC) using the method of GREEN & MANAHAN (1978). Two mL of aqua regia and 0.1 mL HF were added to the sample, the digestion vessels were sealed and heated at 150°C for 2 h. After cooling to room temperature, 5 mL of 5.0% boric acid (ultrapure H_3BO_4, Mallinckrodt, St. Louis, MO) were added and the sample was then diluted to 50 mL with 0.5% HNO_3.

The concentration of Al in each sample was determined by atomic absorption spectroscopy (AAS) using an Instrumentation Laboratory (IL) Model 951 Atomic Absorption Spectrometer with a Model 555 Graphite Furnace and a Model 254 FASTAC auto sampler (IL, Wilmington, MA). Pyrolytic graphite coated tubes and nitrogen were used. The absorbance at 309.5 nm (1.0 nm slit width) was measured, using a 2.5 sec integration of the peak area. The temperature program is shown in Table 1. The IL Model 254 auto-

TABLE 1

Temperature Program

Step	Temperature(°C)	Time(sec)
1	150	0
2	150	5
3	500	5
4	1000	15
5	2500	0
6	2500	10

mated sampler was used with a delay of 10 sec and a deposit time of 7-12 sec (dependent upon Al concentration). A working curve was constructed using varying concentrations of Al standard solutions. The amount of fly ash in each lung was calculated by the following equation:

$$\mu g \text{ fly ash/lung} = \frac{(\mu g \text{ Al in lung}) - (\text{mean } \mu g \text{ Al in control lungs})}{\text{mean fraction Al in fly ash}}$$

Spiked Lungs

The method was validated by analyzing lungs from normal rats spiked with National Bureau of Standards' Standard Reference Material (SRM 1633) fly ash. Tissue from six Fischer-344 rats, males and females, 16-30 weeks old, dry lung weight 0.17 g (range 0.13-0.28 g) were used in these experiments. All animals were maintained in polycarbonate cages with aspen bedding and the cages were changed weekly. The animals were given food (Lab Blox, Allied Mills, Chicago, IL) and water ad libitum. Following excision, lungs were blotted free of liquid, were placed in plastic bags or bottles and were frozen until analyzed. Errors resulting from weighing and transfering μg quantities of fly ash were decreased by mixing the ash with cellulose (TLC Reagent Grade, Baker). About 1 mg of the fly ash:cellulose mixture (0-100% fly ash) was added to the lung. The analysis was conducted as if the spiked lung was a lung from an animal exposed via inhalation to the fly ash, except for subtracting the Al content of the cellulose (28 μg/g).

Lungs From Exposed Animals

Female Syrian hamsters [Sch:(SYR)], 10 to 16 weeks of age, were exposed by inhalation to fly ash in a 27-inch Laskin-type exposure chamber (DENICOLA et al. 1979). Fly ash used in this study was obtained from the fluidized bed combustion (FBC) of Montana Rosebud subbituminous coal. The ash was obtained from the baghouse, the last cleanup device before the stack. The combustor has been described previously (NEWTON et al. 1980). Animals were exposed for 6 h to fly ash having a mass median aerodynamic diameter of about 3 μm and a geometric standard deviation of 2.6. The aerosol concentration was 123 ± 7 mg/m^3 (mean ± standard deviation). Animals were sacrificed by exsanguination immediately after exposure. Lungs from four animals exposed to the ash and from five control animals were analyzed. The mean body weight for the hamsters was 100 g (range 108-118) and the mean dry lung weight was 0.14 g (range 0.11-0.15).

RESULTS

The Al content of the control lungs was 0.88 ± 1.2 μg (mean ± std. dev., n = 6) for the rat and 0.22 ± 0.19 μg (n = 5) for the hamster. Concentrations of Al in fly ash were 14 ± 2% for NBS ash and 4.7 ± 1.2% for the FBC ash.

Fly ash was quantitatively recovered from spiked rat lung tissue (Figure 1). Overall recovery was 98 ± 10% (mean ± std. dev.). Fly ash was not detected at the lowest amount of fly ash added (10.6 μg), because the Al content of the spiked lung and the control lungs was the same. Two lungs were both spiked with 100% cellulose and the aluminum content for these lungs was the same as for the control lungs. The mean amount of fly ash in the lung tissue from exposed hamsters was 212 ± 39 μg/lung (range 190-270, n = 4).

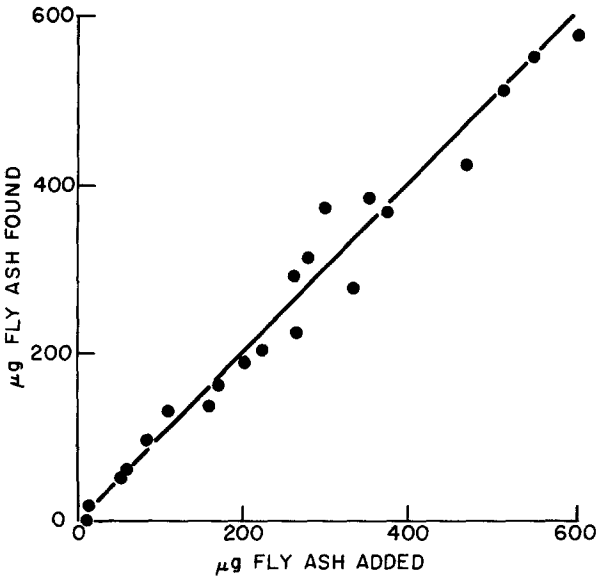

Figure 1. Recovery of fly ash in spiked lung samples. Points represent individual samples. The line shows 100% recovery.

DISCUSSION

In comparison with the previous method of determining lung deposition by exposure to neutron activated fly ash, this method has the advantage that fly ash can be used directly without the use of radioactivation. This not only simplifies the procedure but also avoids possible artifacts due to radiation damage to the particles or to the lung tissue. Since Al is a major component of the fly ash matrix, this method can also be used to follow the clearance of fly ash from the lung.

The main factors which influence the lower limit of detection are the amount and variability of Al in normal lung tissue and in the fly ash. The detection limit for fly ash in rat lung tissue is 24 μg per lung, assuming fly ash Al content of a constant 10% and using the measured Al content in background rat lung of 0.88 ± 1.2 μg. The detection limit would be 3.8 μg fly ash per lung for hamster lung tissue using the measured Al content in background lungs of 0.22 ± 0.19 μg and 10% as the average Al content of fly ash.

Silicon might have been a better choice for the tracer element, since there is more Si in fly ash than Al. The Si content of NBS 1633 fly ash has been reported to be 21% (ONDOV et al. 1975). However, using Si as the tracer for fly ash instead of Al, the detection limit would be higher because the background Si content of rat lungs (~ 0.14 mg, range 0.05-0.30 mg) is much higher than the Al content (MORRIS et al. 1974). Assuming a Si content of fly ash of 20% and a 20% variation in Si content of control lung tissue, the detection limit would be 280 μg of fly

ash per lung. Therefore, Al provided greater sensitivity than Si as a tracer for the fly ash.

A fractional lung deposition of 0.096 was calculated using a minute volume for Syrian hamsters of 50 mL/min (MAUDERLY et al. 1979). The deposition fractions obtained in this study were similar to the 0.097 calculated by THOMAS and RAABE (1978) for Syrian hamsters given nose-only exposure to a radiolabelled fused aluminosilicate aerosol with activity median aerodynamic diameter of 1.9 μm. This report demonstrates that the amount of fly ash deposited in lung tissue can be determined by analyzing for Al in the tissue, and in the ash.

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