Application of blood cadmium analysis to industry using an atomic fluorescence method

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ABSTRACT A flame photometric method using atomic fluorescence (AFS) for cadmium (Cd) estimation is described and applied to the measurement of Cd in blood. The AFS system employs a modified, low cost atomic absorption spectrophotometer, with a high intensity Cd light source provided by an electrodeless discharge tube, excited in a thermostatted microwave cavity. The analytical sensitivity of the technique is sufficient to permit dilution (1 in 5) of blood, before aspiration into the air-hydrogen flame. The method is rapid (25 duplicates/hour), and gives acceptable precision (coefficient of variation within batch 2.5%, between batch 8.1%). The method was applied to a reference population and to three different groups of industrial workers. The reference population had a mean blood concentration of 35.1 nmol Cd/litre. Group 1 (general categories of worker) had 65.2 nmol Cd/l, Group 2 (demolition workers), 137.9 nmol Cd/l, and Group 3 (shipbreakers), 105.9 nmol Cd/l. There was a relationship between increases in blood Cd and in blood Pb which was statistically significant for Group 3.

The health hazards of increased cadmium absorption from industrial and environmental pollution are well recognised (Friberg *et al.*, 1971). Absorbed Cd enters the circulating blood and is then deposited in various tissues such as kidney and liver, where it is bound to protein. The metal is very slowly excreted and continued exposure results in a build up of tissue levels leading eventually to renal damage and other toxic effects.

Present screening procedures are based upon the detection of proteinuria and of an increased urinary excretion of Cd. It is known, however, that such tests will be positive only when sufficient Cd has been absorbed to cause some degree of renal impairment. Blood Cd concentration does not reflect the body burden of tissue-bound Cd (Harvey *et al.*, 1975), but will, if increased, give an indication of recent absorption of the toxic metal. Routine blood Cd analysis could therefore allow the selection of individual workers for further clinical and biochemical examination (Lauwerys *et al.*, 1974) and give an early indication that improvement in

ventilation, and other industrial hygiene measures, are required.

Until recently the routine measurement of blood Cd was difficult, due in part to the low concentration of the metal found in blood, and to technical problems of blood Cd assay by commonly available techniques. Various normal ranges have been reported for blood Cd in non-exposed subjects (Friberg *et al.*, 1971), but it is now generally agreed that normally less than 45 nmol Cd/l (5 µg Cd/l) will be found, although somewhat increased concentrations may be expected in the blood of cigarette smokers (Nandi *et al.*, 1969).

Cernik and Sayers (1975) have recently described a practicable method based upon a carbon cup, atomic absorption procedure, which was applied to blood samples obtained from men in cadmium-using industry. We now report our investigations using an atomic fluorescence method, which has the advantages of the speed and simplicity of other flame photometric techniques, yet has adequate sensitivity. The procedure was used on blood samples supplied in a routine manner by Employment Medical Advisers, and taken from men in industry who were known to be at risk of increased lead absorption but who were not directly employed in cadmium-using processes.

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Methods

APPARATUS

The instrument used for all measurements was a Perkin-Elmer 290B atomic absorption spectrometer and this was coupled to a Honeywell Elektronik 19 strip chart recorder.

The light source used to generate the atomic fluorescence was a Cd electrodeless discharge tube (EDT), manufactured by EMI Limited, and operated in a Broida 210L microwave cavity, powered by a Microtron Mark I microwave generator.

An air-hydrogen flame was used and the standard long slot burner was replaced by one specially manufactured. A stainless steel tube of 1" (25.4 mm) outer diameter, 1/32" (0.71 mm) wall thickness, which fitted into the normal burner slot, had a burner head of the same material welded to it. This burner head had two rows of nine holes (drill size no. 52).

As atomic fluorescence signals are similar to normal flame emission signals, the spectrometer was coupled to the flame emission accessory available from the manufacturer (part no. 290-0200).

The EDT light source was mounted in front of the spectrometer so that its light path was at right angles to the optical measurement path of the instrument. The emission chopper (mechanical type) was then mounted between the EDT and the air-hydrogen flame so that the light source was modulated at the same frequency and in phase with the amplifier-detector system of the spectrometer. Using this system, fluorescence signals generated in the flame by the intense light source could be measured without interference from thermal emissions from the flame. The light source and chopper were mounted on a single gantry which could be removed and replaced in a reproducible position when required. The EDT source was maintained at a constant temperature by passing nitrogen gas at an elevated temperature (about 300°C) through the open end of the microwave cavity.

ANALYTICAL PROCEDURE

Reagents All reagents were of the highest purity available, and each batch was checked for Cd content. Water from a specially designed deionisation unit which contained five different resins was used for the preparation of all solutions. All glassware was acid-washed and rinsed in deionised water before use.

Cadmium standards Aqueous Cd standards were found to be suitable, thus obviating the addition of Cd to pooled blood required by other procedures (Cernik and Sayers, 1975). A stock Cd solution was made by dissolving CdO (analytical reagent grade)

in hydrochloric acid. By dilution with hydrochloric acid (0.01 mol/l) working standards in the range 10 to 100 nmol Cd/l were prepared daily.

Blood samples Blood (5 ml) was collected by venepuncture, using disposable syringes, needles and plastic sample tubes containing anticoagulant (potassium EDTA or lithium heparin). Negligible amounts of Cd were found during checks of contamination of the blood collecting procedure.

Stability of blood Cd Venous blood collected as described above was stored for more than a month either at 4 to 10°C, or at -10 to -20°C. No significant change in Cd concentration was noted for blood containing about 45 nmol Cd/l or 250 nmol Cd/l. It was found best to store whole blood samples in the frozen state as the resultant haemolysis gave a more homogeneous solution.

Blood sample preparation Blood (1 ml) was taken from the thawed samples after ensuring thorough mixing, added to a clean 10 ml centrifuge tube, and diluted to 5 ml with hydrochloric acid. Because Cd in blood is mainly bound to erythrocytes (Nordberg *et al.*, 1971) the acid diluent contained 0.5% v/v Triton X, a detergent which ensures complete haemolysis. The diluted blood sample was then centrifuged (30 s, 3000 rev/min) to remove cellular debris, and aspirated directly into the air-hydrogen flame.

Operation of the instrument The EDT source was operated at a microwave power of 25 W, a nitrogen flow rate of 10 l/min, and a temperature of 288°C. The spectrometer was operated at a wavelength of 228.8 nm and a spectral band width of 2.0 nm. The air flow meter allowed a flow of 14.00 l/min at a feed pressure of 241.5 kPa (35 lbf/in²); the hydrogen flow meter allowed a flow of 14.20 l/min at a feed pressure of 55.2 kPa (8 lbf/in²).

Calibration All working standards (0, 10, 20, 40, 80, 100 nmol Cd/l), were aspirated and followed by the diluted blood samples. Their cadmium content was determined from a calibration graph of recorder signal peak height versus Cd concentration. Blank solutions gave no significant readings.

Analytical performance The detection limit was less than 1 nmol Cd/l and the useful working range 5 to 100 nmol Cd/l. The precision was 2.5% within batch, and 8.1% between batch. There was satisfactory recovery of Cd added to blood, and the method had an analytical rate of 25 duplicate analyses per hour.

Blood lead Blood Pb was determined by the Delves microsampling technique, using an IL251 atomic absorption spectrophotometer with continuous background correction.

POPULATIONS STUDIED

Reference populations Blood (1 ml) was taken from

Table Blood cadmium concentration in a reference population and three groups of workers

	Reference population	All lead workers Group 1	Demolition workers Group 2	Shipbreakers Group 3
n	105	100	44	39
Mean Cd concentration	35.1 (3.95)	65.2 (7.33)	137.9 (15.51)	105.9 (11.9)
S.D.	10.5 (1.11)	35.8 (4.02)	45.0 (5.06)	29.7 (3.34)
Range	17.0-62.0 (2.0-7.0)	16.0-231.0 (2.8-26.0)	62.0-222.0 (7.0-25.0)	31.0-178.0 (3.5-20.0)

Concentrations shown as nmol Cd/l and ($\mu\text{g Cd/l}$).

samples (lithium heparin tubes) submitted to the hospital laboratory for routine analysis. This blood, surplus to requirement, was stored frozen until required. These samples served as a reference population of persons known not to be industrially exposed to Cd at the time of blood collection.

Industrial workers Blood (1 ml) was taken from samples submitted for routine analysis of blood Pb as part of health checks by Employment Medical Advisors. Group 1 comprised 100 men drawn from all categories of industrial worker known to be at risk of increased Pb exposure. The 44 subjects in Group 2 were demolition workers engaged in metal cutting operations, and the third group studied comprised 39 men engaged in shipbreaking industry. The values obtained for these populations are shown in the table.

Discussion

The average level of blood Cd in the reference population, 35.1 nmol Cd/l, is similar to the control value, 35.6 nmol Cd/l, reported by Cernik and Sayers (1975) and to other published values for non-exposed subjects (Friberg *et al.*, 1971). The blood lead concentrations for the reference population are plotted against blood Cd concentration as shown in Figure 1, which indicates the approximate upper limits of normal.

All the industrial groups investigated showed increased levels of blood Cd, the greatest increases occurring in Groups 2 and 3. However these changes are moderate when compared with those values reported for men in Cd-using industry. For example, in pigment workers exposed to Cd dust, Cernik and Sayers (1975) found a mean value of 237 nmol Cd/l, and men in the smelting industry had mean blood Cd levels of 463 nmol Cd/l after exposure to Cd fumes.

It must be emphasised again that the increases in blood Cd do not reflect the extent of tissue accumulation of Cd, which can be directly measured by an *in vivo* neutron activation technique (Harvey *et al.*, 1975). Even moderate increases in blood Cd, however, provide evidence of recent absorption and, if

Fig. 1 Reference population, relationship between blood Pb ($\mu\text{mol/l}$) and blood Cd (nmol/l).

Approximate upper limits of normal indicated by dashed lines.

this is part of a continuous exposure period, considerable Cd tissue deposition could well occur.

Throughout the study we noted a tendency for men with raised blood Pb levels to have increased blood Cd concentrations. This was most obvious in Groups 2 and 3. In the shipbreakers (Group 3) the relationship was particularly striking (Figure 2) and was statistically significant ($r = 0.9$). Ingestion of metal fumes produced during metal cutting may be the common source of both metals, as Pb and Cd are commonly used in anticorrosion metal finishes.

The adverse health effects associated with chronic Cd exposure include hypertension and renal impairment, so that health statistics being collected for lead workers could be influenced by such unsuspected Cd absorption. In addition, it should be borne in mind that the oral chelating agent penicillamine, used to treat severe lead intoxication, may well be contra-

Fig. 2 Group 3, shipbreakers. Relationship between blood Pb ($\mu\text{mol/l}$) and blood Cd (nmol/l). Approximate upper limit of normal indicated by dashed lines. ($r = 0.9$).

indicated in cases where Cd absorption can be proved (Lyle *et al.*, 1968).

Although the long-term health effects of chronic exposure to Cd are not yet clear, (Buell, 1975), future epidemiological studies should include blood Cd analysis. This will help to define the extent of industrial and environmental exposure to this potentially dangerous metal.

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