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# Lead Levels in Wool as an Indication of Lead in Blood of Sheep Exposed to Automotive Emissions

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Automotive emissions are by far the most serious source of environmental lead pollution because of their ubiquitous nature. Their effects on humans have been extensively studied during the past decade and have been reviewed by BROOKS (1977). Blood lead levels are generally considered to be the best indices of the lead burden of humans (LUDWIG et al. 1965) and of domestic animals (BLOOM et al. 1976, WARD et al. 1977).

Because of sampling problems, human hair has sometimes been considered as an alternative to use of blood for assessing lead burdens in humans (REEVES et al. 1976, SMYTHE 1977) though there are conflicting opinions about its reliability as an alternative. For example SMYTHE (1977) considered that only the distal (outer) segments of human hair had a lead level related to the environment of the subject.

Work on lead poisoning in domestic animals has tended to concentrate on experiments involving artificial feeding with lead salts (BLAXTER 1950) and comparatively few studies have been carried out *in situ* along the verges of busy highways. Recently however, WARD *et al.* (1978a, 1978b) have studied the effect of lead pollution upon sheep grazed in such locations and have shown that the lead content of the blood of these animals is a reliable indication of traffic densities and is also a reflection of the lead burden of body organs.

Because of the analogy with work already carried out on humans, it is of obvious interest to establish to what extent the lead content of the wool of sheep exposed to automotive emissions is indicative of the lead content of whole blood. The results of such experiments are presented in this report.

## MATERIALS AND METHODS

Animals Studied: A flock of 100 Romney sheep of both sexes and of ages ranging from 1 to 5 years (60% were 2 years old) were studied. This flock had been maintained for 136 days in a paddock situated 3-50 m from a major highway near the township of Bulls, New Zealand. The traffic density was about 5000 vehicles in 24 hr.

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Sampling Procedures: A length of wool was taken from the middle section and left side of each animal. Each sample was about 1 cm diameter when compressed, was about 10 cm long and was cut as close as possible to the skin. Blood samples (10 mℓ) were taken from the neck of each animal and placed immediately in standard heparinized vacutainers.

Preparation of Wool for Analysis: Wool specimens from nine selected sheep were divided into 2 cm segments and each segment was further divided into 5 subsamples for 5 different washing procedures: deionized water; deionized water with 0.2% detergent; absolute ethanol; acetone; methyl isobutyl ketone; 2M HCl. When the optimum washing conditions had been established, a further 90 specimens were treated by this one method. Samples (0.5 - 2.0 g) were placed in polypropylene bottles and shaken for 30 min with 150 mℓ of washing agent, they were then filtered via a Büchner funnel and washed with a further 8 portions of 100 mℓ of deionized water. After drying at 110° for 3 hr, the samples were ashed overnight at 450 °C and the wash was dissolved in 100 times its weight of 2M HCl prepared from redistilled constant-boiling reagent.

Analytical Methods: Lead levels in whole blood were determined by use of a Varian Techtron Model 63 carbon rod atomizer attachment for a Varian Techtron Model AA5 atomic absorption spectrophotometer. Standard curves were prepared by fortifying whole blood (containing about 0.20 μg/mℓ lead) with incremental amounts of lead and then using the method of addition to calculate the original content in the standards. Beer's Law was obeyed in the range 0.1 to 2.0 μg/mℓ. To avoid interference from matrix effects, Triton X-100 (a detergent) was added in the ratio of 1 volume to 2 volumes of blood before analysis. Corrections for non-atomic absorption were made with a Model BC-6 automatic background corrector. The spectral line at 217.0 nm was used for all lead determinations. The precision of replicates containing about 0.20 μg/mℓ was about ±5%.

Solutions of wool samples were analyzed by conventional flame atomic absorption spectrophotometry using the same spectral line and the Model AA5 instrument.

## RESULTS AND DISCUSSION

Distribution of Lead in Wool Samples: Wool samples from nine representative individuals (including those with minimum, median and maximum blood lead levels) were divided into five 2-cm segments and washed with 0.2% detergent before analysis for lead. In all cases there was an increase in the lead content along the direction from the skin to the distal (outer) portions of the wool. This increase was quite marked in the outer 2 cm portions and particularly for those individuals with the highest blood lead levels. This is illustrated in Figure 1. For the sake of clarity only four of the nine specimens are shown in the figure.

Since there was an apparently higher correlation between blood lead levels and the outer 2 cm of wool in all nine individuals, this portion of the wool was used for all subsequent experiments. This build up of lead in the distal segments of wool rather than those adjacent to the skin, implies that lead is accumulated directly via airborne emissions rather than indirectly via the internal organs of the sheep, otherwise the pattern of lead distribution would have been reversed.

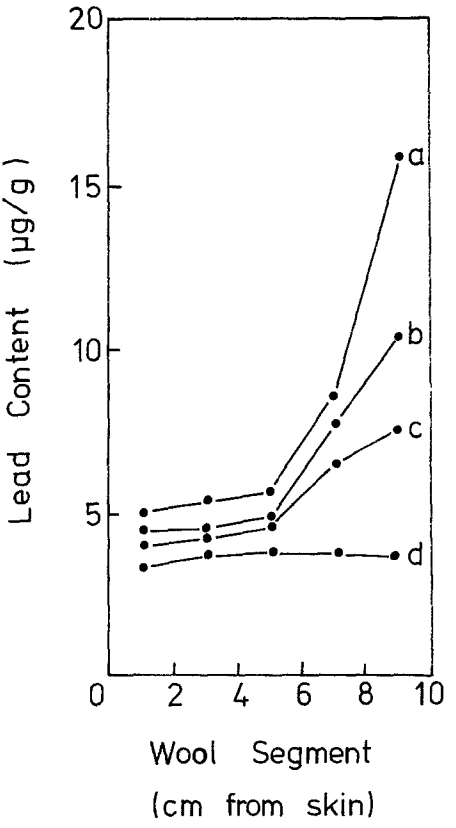


FIGURE 1. Lead content of wool segments of four sheep grazed near major highway.

(a) blood lead level 1.70 µg/mℓ

(b) blood lead level 1.00 µg/mℓ

(c) blood lead level 0.65 µg/mℓ

(d) blood lead level 0.16 µg/mℓ

The Effect of Various Washing Treatments upon the Lead Content of Wool: Table 1 shows the lead content of blood and wool for nine individual animals selected on the basis of giving a wide range of blood levels including maximum and minimum values. The least amounts of lead were removed by deionized water, ethanol, methyl isobutyl ketone and dilute HCl, whereas 0.2% detergent and acetone appeared to be the most effective in removal of lead. This difference in values probably represents the portion of the lead content of unwashed hair consisting of particulate matter embedded in lipid material. When lead levels in whole blood were correlated with lead concentrations in wool after various treatments, it was found that the correlation was in all cases very highly significant ($P < 0.001$). The acetone ($r = 0.952$) and detergent ($r = 0.988$) treatments which removed lipid-bound lead gave correlations very slightly less significant than those for other treatments. Because of this , the standard treatment used for subsequent work was washing in deionized water which had the further advantage of simplicity and freedom from contamination problems.

Table 1

Lead Content (µg/mℓ and µg/g) of Sheep Blood and of Sheep Wool (Outer 2 cm) After Various Treatments

| | Washing treatment for wool samples | | | | | |
|---|---|---|---|---|---|---|
| Whole blood | deionized water | 0.2% detergent | ethanol | acetone | MIBK | dilute HCl |
| 1.70 | 26.0 | 16.0 | 24.0 | 15.5 | 22.0 | 24.0 |
| 1.25 | 19.4 | 11.7 | 18.5 | 10.5 | 16.5 | 16.5 |
| 1.00 | 18.4 | 10.4 | 15.6 | 8.6 | 14.0 | 14.9 |
| 0.80 | 15.6 | 8.0 | 12.0 | 9.2 | 13.5 | 13.9 |
| 0.65 | 12.2 | 7.5 | 8.6 | 8.0 | 11.5 | 10.5 |
| 0.48 | 10.4 | 6.0 | 6.8 | 7.2 | 8.5 | 8.6 |
| 0.25 | 7.0 | 5.5 | 6.0 | 6.9 | 6.0 | 6.8 |
| 0.20 | 6.8 | 5.2 | 5.4 | 6.0 | 5.8 | 6.2 |
| 0.16 | 4.0 | 3.7 | 3.9 | 4.2 | 3.9 | 3.9 |
| Value of $r$ for correlation with whole blood | 0.990 | 0.988 | 0.991 | 0.952 | 0.991 | 0.990 |
| Value of P | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

## The Relationship between Blood Lead Levels in Sheep and the Lead Content of the Outer 2 cm Segment of the Wool

Figure 2 shows the relationship between the lead content of the outer 2 cm segment of wool and the blood lead level.

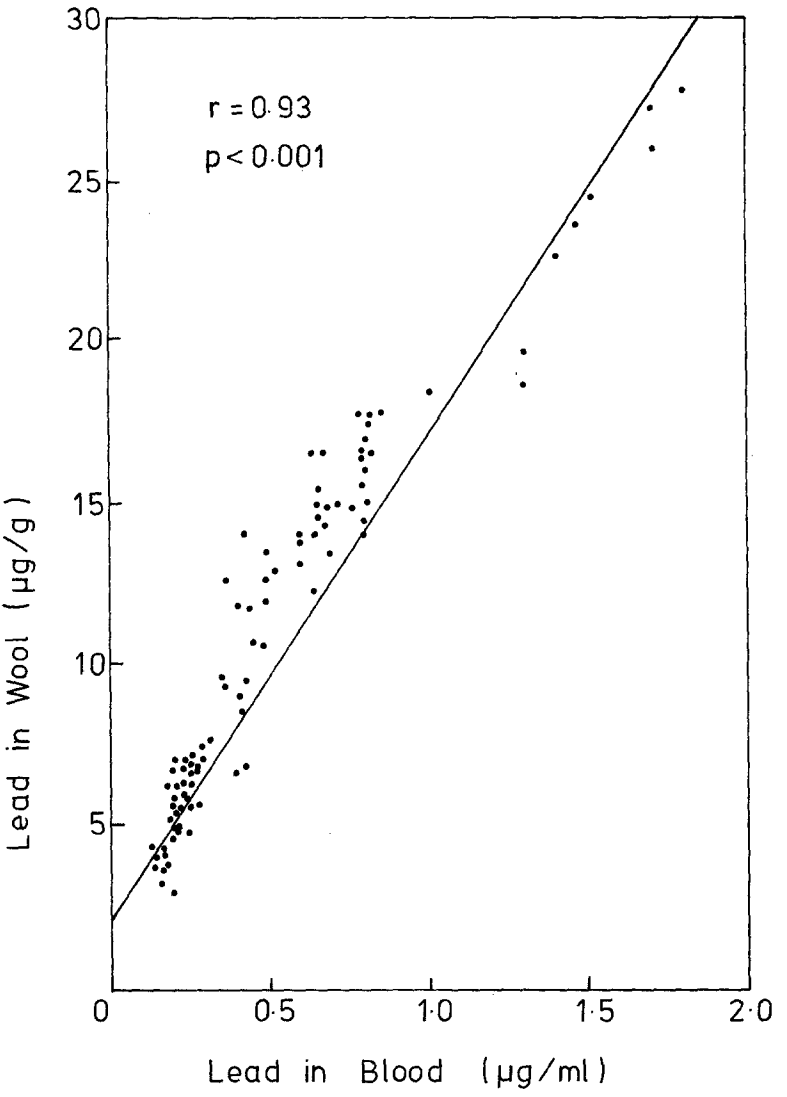


FIGURE 2. Lead content of outer 2 cm segment of fleece as a function of the lead content of whole blood.

It is clear from this figure that there is a very highly significant ($P < 0.001$) relationship between the two variables. The Pearson Product Moment correlation coefficient does of course depend on the data being normally distributed but a logarithmic transformation of the data produced essentially the same value of r.

The correlation is much superior to that normally found for human subjects where any inherent relationship can easily be masked by occupational factors and use of different types of hair dressings (REEVES et al. 1975).

The relationship between lead levels in wool and blood is so marked that it would seem that wool sampling is a possible alternative to blood sampling and has the great advantage of speed, simplicity and ease of analysis. In spite of these observations, it must be remembered that this study has involved a single population of one breed of sheep grazed in a single locality. The blood-wool conversion factor may well be different for other breeds of sheep and should be investigated before making a final decision as to the suitability of wool samples as an alternative to blood samples in assessing the lead burden of sheep exposed to automotive emissions.

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