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A Follow-up Study of Lead Absorption in Cows as an Indicator of Environmental Lead Pollution

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In the previous study (Karačić et al. 1984) lead absorption in cows grazing in the vicinity of a lead smeltery and in a control area was presented for two periods. The first period was in 1976, two years before the installation of an effective gas cleaning system in the lead smeltery, and the second period was in 1982, four years after the sanitation. The results obtained in the lead smeltery area revealed a highly significant improvement of all biochemical indicators of lead absorption intensity in cows during 1982 in comparison with the same findings during 1976. However, in spite of the lower lead absorption in 1982, blood lead concentration and δ-aminolevulinic acid dehydratase activity in 1982 were still highly significantly different from the same findings in cows from the control area. Consequently the study was continued in 1984 and 1988 under identical conditions. Having in mind the same conception, i.e. that a follow-up of lead absorption in cows could be a useful indicator of environmental pollution, the results are presented in this paper for all periods. The main object of the paper is a critical evaluation of the results obtained in relation to environmental monitoring in the same lead smeltery area.

MATERIALS AND METHODS

In each period the examined cows were different, but they were from the same pastures in the lead smeltery area and in the control area during the entire study. Seasonal variations were also respected since the cows were always examined in the spring (from the middle of May to the middle of June).

The details with regard to the lead smeltery, emissions and depositions of air pollutants before and after bag filter introduction are available elsewhere (Fugaš et al. 1984). The annual mean of airborne and deposited lead in the control area was low, 0.09 $\mu g\ m^{-3}$ and 77 $\mu g\ m^{-2}\ d^{-1}$ respectively.

In the lead contaminated area the number of cows was 30 for each period while in the control area this varied (22 in 1976, 15 in 1982, 20 in 1984 and in 1988).

In each cow from both areas blood lead (Pb-B), erythrocyte δ-aminolevulinic acid dehydratase (ALAD), and erythrocyte protoporphyrin (EP) were determined. The methods applied were always the same for the exposed and control groups as follows: electrothermal atomic

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absorption spectrometry (ET-AAS) for Pb-B (in 1976 and 1982: Fernandez 1975; in 1984 and 1988: Stoeppler et al. 1978), spectrophotometric for ALAD (Berlin and Schaller, 1974) and spectrofluorometric for EP (Chisolm and Brown 1975). Both methods for Pb-B (CV $\leqslant$ 5%) were verified by participation in the British QCS and CEC QC programmes, while EP (CV $\leqslant$ 3%) was verified by participation in Erythrocyte Protoporphyrin Proficiency Testing, CDC, Atlanta (USA). Determination of ALAD was also accurately performed (CV $\leqslant$ 2%).

Due to a skewed distribution, the results in each group of cows and in each period are presented as median (M) and range (R) values. The differences in biochemical indicators of lead absorption between the exposed and control groups were tested by means of the nonparametric median test (Siegel 1956).

RESULTS AND DISCUSSION

Table 1 shows median and range values for Pb-B, ALAD and EP in cows from the lead smeltery area and Table 2 the same findings in cows from the control area. In cows from the lead smeltery area there was a trend towards normalisation following Pb-B and EP decrease, and ALAD increase after bag filter installation. The Pb-B, ALAD and EP variations in four periods of the study in cows from the control area can be explained by the large range of "normal" values in cows regardless of breed or age of animals (Ruhr 1984).

Table 1. Median (M) and range (R) values of biochemical indicators of lead absorption in cows from the lead smeltery area before and after bag filter installation

Year of examination	Statistical parameter	Biochemical indicators		
		Pb-B μM L^{-1}	ALAD U L^{-1} Ercs	EP μM L^{-1} Ercs
1976	M	3.09	0.6	14.55
	R	1.21-5.45	0.0-1.8	3.88-58.22
	N	30	30	30
1978	Bag filter installation			
1982	M	1.44	1.5	3.47
	R	0.82-3.40	0.6-5.9	1.68-12.38
	N	30	30	30
1984	M	0.75	2.7	3.48
	R	0.24-1.81	1.3-7.0	1.62-19.54
	N	30	30	30
1988	M	0.66	3.4	2.66
	R	0.27-1.66	1.4-12.1	1.41-15.91
	N	30	29	30

Table 2. Median (M) and range (R) values of biochemical indicators of lead absorption in cows from the control area

Year of examination	Statistical parameter	Biochemical indicators		
		Pb-B $\mu M\ L^{-1}$	ALAD $U\ L^{-1}$ Ercs	EP $\mu M\ L^{-1}$ Ercs
1976	M	0.29	4.3	1.45
	R	0.24-0.53	0.9-14.0	1.14-2.15
	N	22	22	22
1982	M	0.27	5.0	2.22
	R	0.09-0.60	1.6-8.8	1.70-3.27
	N	15	15	15
1984	M	0.14	9.2	1.98
	R	0.12-0.27	3.5-13.3	1.52-3.61
	N	20	20	20
1988	M	0.11	6.5	2.17
	R	0.07-0.23	4.1-13.4	1.17-2.81
	N	20	20	19

In cows from the lead smeltery area the most pronounced difference before and after bag filter installation was found in EP values in 1982. In later periods (1984 and 1988) year to year differences were more pronounced for Pb-B and ALAD than for EP, which was expected since Pb-B is a more specific and ALAD a more sensitive indicator for lead absorption than EP. Table 3 shows the statistical significance of the differences in Pb-B, ALAD and EP between periods. The superiority of Pb-B and ALAD over EP has been confirmed.

The statistical significance of the differences in biochemical indicators of lead absorption in cows from the lead smeltery and control area in each year of the examination is presented in Table 4. The highly significant ($p<0.001$) difference in Pb-B and ALAD was still evident ten years after the introduction of the effective emission control system, indicating that the process of normalization in cows is a rather slow one. The same was established in the inhabitants living in the vicinity of the lead smeltery (Prpić-Majić et al. 1988).

The major reason for the slow normalization of biochemical indicators of lead absorption in cows after bag filter installation is presumed to be the animal feeds grown in lead contaminated pastures. In addition to pasture plants, which absorb lead via the roots from the soil and via leaves from the atmosphere, ingested soil constitutes an important source of lead absorption in grazing animals (Thornton and Abrahams 1983). In the vicinity (900-7500 m) of the same lead smeltery, environmental studies performed in 1981-1985 (Hršak 1987) demonstrated the increased lead content in the first 10 cm soil layer ranging on average from 162 to 1398 $\mu g\ g^{-1}$ (control area: 47 $\mu g\ g^{-1}$).

Table 3. Statistical significance of differences in biochemical indicators of lead absorption in cows from the lead smeltery area according to the year of examination

Years comparable	Statistical parameter	Biochemical indicators		
		Pb-B $\mu M\ L^{-1}$	ALAD $U\ L^{-1}$ Ercs	EP $\mu M\ L^{-1}$ Ercs
1982-1976	χ^2	13.066	17.066	26.666
	p	$<$ 0.001	$<$ 0.001	$<$ 0.001
1984-1976	χ^2	38.400	41.666	26.666
	p	$<$ 0.001	$<$ 0.001	$<$ 0.001
1988-1976	χ^2	22.533	49.438	26.666
	p	$<$ 0.001	$<$ 0.001	$<$ 0.001
1984-1982	χ^2	21.600	13.066	0.00
	p	$<$ 0.001	$<$ 0.001	$>$ 0.50
1988-1982	χ^2	32.266	12.840	1.067
	p	$<$ 0.001	$<$ 0.001	$>$ 0.10
1988-1984	χ^2	0.267	1.067	1.067
	p	$>$ 0.50	$>$ 0.10	$>$ 0.10

1976 - before bag filter installation in the lead smeltery
1982, 1984, 1988 - after bag filter installation in the lead smeltery

Table 4. Statistical significance of differences in biochemical indicators of lead absorption in cows from the lead smeltery and control area according to the year of examination

Year of examination	Statictical parameter	Biochemical indicators		
		Pb-B $\mu M\ L^{-1}$	ALAD $U\ L^{-1}$ Ercs	EP $\mu M\ L^{-1}$ Ercs
1976	χ^2	38.133	25.527	38.133
	p	$<$ 0.001	$<$ 0.001	$<$ 0.001
1982	χ^2	22.500	16.899	3.600
	p	$<$ 0.001	$<$ 0.001	$>$ 0.05
1984	χ^2	33.333	16.333	3.000
	p	$<$ 0.001	$<$ 0.001	$>$ 0.05
1988	χ^2	33.333	16.558	1.666
	p	$<$ 0.001	$<$ 0.001	$>$ 0.10

1976 - before bag filter installation in the lead smeltery
1982, 1984, 1988 - after bag filter installation in the lead smeltery

An important point of this work is that the cow could be a model animal to follow up the residual amounts of lead in different environmental mediae. The environmental monitoring performed together with biological monitoring in a human study (Prpić-Majić et al. 1988) revealed that after bag filter installation airborne lead was reduced to $\leqslant 2.0$ µg m^{-3} and continued to be close to this value in all periods of the study. On the other hand lead in deposited particulates first showed a tendency to decrease and later to increase, while lead concentration in household dust varied significantly not only from house to house, but also within the same house. This indicates that neither of these parameters were representative enough to show the total environmental residual lead contamination which may be a potentional risk for normal life. Cows grazing in the pastures, i.e. untreated feed, are in direct link with the environmental lead contamination from different mediae (air, water and soil) and therefore can much better represent the level of the actual lead pollution. In this work, by regularly analysing in cows characteristic indicators showing the level and effect of lead which is integrated into one internal load from different environmental mediae, it was possible to establish that even 10 years after effective technical sanitation, the environment was still polluted by lead.

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REFERENCES

Berlin A, Schaller KH (1974) European standardized method for the determination of δ-aminolevulinic acid dehydratase activity in blood. Z Klin Chem Klin Biochem 12:389-390

Chisolm JJ, Brown DH (1975) Micro-scale photofluorometric determination of "free" erythrocyte porphyrin (protoporphyrin IX). Clin Chem 21:1669-1682

Fernandez FJ (1975) Micro-method for lead determination in whole blood by atomic absorption, with use of the graphite furnace. Clin Chem 21:558-561

Fugaš M, Hršak J, Souvent P (1984) The effect of a new emission control system on lead, zinc and cadmium concentrations in the environment of a lead smeltery. Staub-Reinhalt Luft 44:273-275

Hršak J (1987) Emission and transformation of lead, zinc and cadmium aerosols in the environment of a lead smeltery (in Croatian). Ph D Thesis, University of Zagreb

Karačić V, Prpić-Majić D, Skender Lj (1984) Lead absorption in cows: Biological indicators of ambient lead exposure. Bull Environ Contam Toxicol 32:290-294

Prpić-Majić D, Hršak J, Skender Lj (1988) Biological and environmental monitoring in a lead smelter community. In: Astruc M, Lester JN (eds) Heavy Metals in the Hydrological Cycle, Publications Division, Selper Ltd, London, p 19

Ruhr LP (1984) Blood lead, delta-aminolevulinic acid dehydratase and free erythrocyte porphyrins in normal cattle. Vet Hum Toxicol 26:105-107

Siegel S (1956) Nonparametric statistics for the behavioural sciences. The extension of the median test. Mc Graw-Hill, New York
Stoeppler M, Brandt K, Rains TC (1978) Contributions to automated trace analysis. Part II Rapid method for the automated determination of lead in whole blood by electrothermal atomic-absorption spectrophotometry. Analyst 103:714-722
Thornton I, Abrahams P (1983) Soil ingestion - a major pathway of heavy metals into livestock grazing contaminated land. Sci Total Environ 28:287-294.