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VETERINARY AND HUMAN TOXICOLOGY, 1988, 30(6), 513-517
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Reprinted from Veterinary and Human Toxicology, Vol. 30, No. 6, December 1988, pp. 513-517
Indicators of Lead, Zinc and Cadmium Exposure in Cattle: I. Results in a Polluted Area

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(Received February 2, 1988; Revision Received June 21, 1988; Accepted June 24, 1988)

ABSTRACT. Dairy cattle on a farm located in the vicinity of a lead and zinc-ore processing factory were studied over 21 mo and compared with cattle on a control farm. Mean daily intakes or lead from the diet were 4.3 mg/kg body weight, with great variations; mean daily zinc intakes were 5.6 mg/kg body weight; and mean daily cadmium intakes were 0.064 mg/kg body weight. The 3 major indicators of contamination were blood lead concentrations, with mean values of 50 µg/100 ml of blood, zinc protoporphyrin with mean values of 165 µg/100 ml blood, and lead concentrations in hair which averaged 10 µg/g. Blood zinc concentrations and zinc concentrations were not significantly increased. One cow developed fatal post-partum paralysis. Liver, kidney and bone lead concentrations and kidney cadmium concentrations were good "post-mortem" indicators of exposure.

Since 1971, studies have been conducted on cattle in the vicinity of a lead and zinc-ore processing factory in northern France. Blood samples have been collected from live animals to determine concentrations of lead, cadmium, calcium, magnesium, zinc protoporphyrin (ZPP) and hemoglobin. Earlier urine samplings to determine delta aminolevulinic acid proved difficult to carry out and were stopped. Most of the affected cows and sheep were slaughtered. Necropsies were performed and tissues were analysed for lead, zinc and cadmium; anatomopathological, bacteriological, and virological analyses were performed. The first results were published previously (1,2).

Data on lead storage in bovine hair (3) prompted new research. A 2-year study was conducted on the cattle of the most polluted farm to ascertain whether metal storage in tail hair was a significant indicator of exposure. The purpose of this communication is to report the results of this investigation. Further investigation was conducted on 4 animals to determine the relationship between ingestion of contaminated feed and the 3 main parameters of exposure -- blood lead concentrations, zinc protoporphyrin and lead concentrations in hair -- and also to study the decrease of these parameters when feed was no longer contaminated.

MATERIALS AND METHODS

Animals

The test farm was located 1 km 6 north-north east of a lead and zinc-ore processing factory. The control animals belonged to the National Agronomic Institute located in an area free of contamination. The test farm herd consisted of 20 Friesan cows and 2 heifers born on the farm. The adult cows were bought at about 5 years of age. The animals were fed corn silage in winter and fresh natural grasses from pastures in summer (zero-grazing). Mineral salts and supplemented feed were added. Corn and natural grasses came from various sites located 0.5 km to 2 km from the factory.

Sample Collection

The test program was conducted from October 1, 1984 to April 16, 1986. Lead concentrations at each site were determined when grasses were harvested, which permitted us to assess the amount of lead ingested by the animals over the period of testing. Samples of blood and hair were collected 3 times -- on June 17, 1985, on November 29, 1985 and on April 16, 1986.

Only 9 animals (7 cows and 2 heifers) were included in all the 3 sample-collections on account of herd renewals and exclusion of
cows about to calve. The first sample collected 18 animals (16 cows and 2 heifers); the second sampling had 19 animals (including 12 from the first group), and the third sampling comprised 17 animals (all from the second group). All the adult cows on the farm were slaughtered, and liver, kidney and bone samples were collected for toxicology and histopathology.

For each sample-collection 15 control dairy cows (Friesian cattle in the same age groups as the test cows) were chosen at random among 132 animals. Blood and hair samples were collected on January 10, 1986, June 28, 1986, and January 15, 1987. Ten animals were submitted for all the 3 samplings. No control animals were slaughtered.

Blood samples were collected from the subcaudal vein in 3 heparinised evacuated blood collection tubes (Becton Dickinson Vacutainer Systemes, Europe BP 37 38241, Meylan, Cedex, France). One tube was centrifuged at high speed to obtain nonhemolysed plasma. Hair was clipped for 30 cm from the median part of the tail, above the end tuft, and placed in a polyethylene bag. The hair had never been clipped before. The second and third hair samplings were from hair which had grown again.

Analyses

Blood lead. Determination of lead was by anodizing stripping voltammetry (ESA 3010 A, Esa Inc, 45 Wiggins Avenue, Bedford) on a 100 μl sample (detection limit was 2 μg/100 ml).

Zinc and copper. Determination of Zn and Cu in plasma was by a flame absorption spectrophotometer (Perkin Elmer 303) on a 1 ml sample diluted 1:5 (4) (detection limit was 10 μg/100 ml).

Cadmium. Determination of Cd was by an atomic absorption spectrophotometer equipped with a graphite furnace (Perkin Elmer HGA 72) after calcination and extraction by 5N UNO₃ (5) (detection limit was 0.1 μg/100 ml).

Calcium and magnesium. Colorimetry with Bio-Merieux reagents (ref 61 041 and 61 411, Marcy l'Etoile 69290 Craponne, France) was used.

Zinc protoporphyrin. Direct determination was performed on a blood drop with hemato-fluorometer AVIV (Biochemical Incorporation, Towbin Av 810, Box 994, Lakwoot NG).

Hemoglobin. Determination was with a Coultronics hemoglobinometer (29 avenue Georges Pompidou, 95580 Margency, France).

Hair, liver, kidney, bone. The hair was washed with 1% Triton X and demineralized water using a procedure derived from Ward (6). Proceeding in stages, the samples were mineralized at 450 C with sulphuric acid. The ashes were treated with concentrated nitric acid (15 N), and the dried extract was again mineralized at 150 C. The white ashes were dissolved in diluted nitric acid (5 N). Zinc and copper were determined by flame absorption spectrophotometry, while lead and cadmium were determined by atomic absorption spectrophotometry with furnace. Detection limits (ppm/dry wt) were:

<table>
<thead>
<tr>
<th>Element</th>
<th>Hair</th>
<th>Liver</th>
<th>Kidney</th>
<th>Bone</th>
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<tr>
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<td>0.4</td>
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<tr>
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<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Cadmium</td>
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<td>0.02</td>
<td>0.02</td>
<td>-</td>
</tr>
<tr>
<td>Copper</td>
<td>2.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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</table>

Statistical Analysis

Statistical calculations were performed using student's t-Test. Correlation analysis was carried out using statistical methods of simple correlation between lead and ZPP levels in the blood and lead concentrations in the hair.

RESULTS

The estimated intake of lead through feeding in the polluted area was 6 mg/kg/day between October 1, 1984 and January 15, 1985, and 4.8 mg/kg/day between January 16, 1985 and May 4, 1985. It varied from 3.2 mg/kg/day to 0.5 mg/kg/day between May 5, 1985 and June 19, 1985 (when the first samples were collected) (Fig 1). Between the first and the second sample-collections, the daily intake of lead was very variable with intake ranging from 2.5 to 3 mg/kg/day, separated by very high peaks (22.5 mg/kg/day from June 20 to June 24, 24.5 mg/kg/day from July 7 to July 12). After October 12 (48 days prior to the second sample-collection), the daily intake stabilized at 2.5 mg/kg/day and remained unchanged between the second and the third sample-collections.

Figure 1. Lead intake through feeding from October 10, 86 until April 16, 86.
Zinc and cadmium concentrations were not measured in all the samples, but many analyses conducted in the area showed that the concentrations of zinc in the forage amounted to about 130% of lead concentrations. The concentrations of cadmium amounted to about 1.5% of the lead concentrations.

The estimated intake of lead, zinc and cadmium by the animals on the control farm was 0.025 mg/kg/day of lead, 0.002 mg/kg/day of cadmium, 1.2 mg/kg/day of zinc.

Table 1 shows the mean values obtained in blood and hair from the 3 samplings on the control farm and from each sampling in the contaminated area, with the statistical significance between the results on the control farm and the contaminated farm (t-test). Table 2 shows the mean concentrations of these elements in the liver, kidney and bone of the animals reared in the contaminated area.

**DISCUSSION**

Figure 2 shows that 2 blood parameters, lead and ZPP levels were multiplied by a factor above 10. The same applied to lead concentrations in hair. In spite of significant statistical differences, the other parameters did not show such variations.

In spite of the high concentrations of zinc contained in the diet, there were insignificant differences between blood zinc concentrations in test and control animals in the first sampling. The concentrations were 3 times higher in the third sampling of test animals, but those samplings were of poor quality and hardly reliable. In the third sampling only the zinc concentrations in hair were significantly different between test and control animals. The zinc in kidney and liver were not above the reference values (7,8). This suggests that zinc absorption is decreased after body zinc status has been elevated by dietary means. This phenomenon has also been described in the rat (9).

In the first sampling, blood cadmium concentrations in test animals were lower than in control animals. Hair concentrations were higher but the difference was not significant on account of the high standard deviation. The concentrations in kidney and liver were markedly above what is considered normal by Dorn et al (10) — 1.04 and 0.24 ppm respectively — but were below the critical concentrations of cadmium in human renal cortex (210-260 ppm) reported by Nomiyama (11).

Blood and hair copper concentrations in the control animals were below the normal limits of deficiency suggested by Lamand et al (4), 70 μg/100 ml and 7 μg/g respectively. Concentrations in the first sampling of test animals were not significantly increased compared with controls, which confirms the absence of contamination by copper. The concentrations obtained in the other samplings were significantly different in various ways.

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**Table 1.**

Measured concentrations of lead, zinc and cadmium in blood and hair levels in test and control animals.

<table>
<thead>
<tr>
<th></th>
<th>Lead</th>
<th>Zinc</th>
<th>Cadmium</th>
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</tr>
<tr>
<td>mg/l</td>
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<td>n</td>
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</table>

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**Table 2.**

Cadmium levels in the liver, kidney and bone of the animals reared in the contaminated area.

<table>
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<tr>
<th></th>
<th>Lead</th>
<th>Zinc</th>
<th>Cadmium</th>
</tr>
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<td>n</td>
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</tbody>
</table>

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**Figure 2.** Blood parameters in control cows (—) and test cows (first sampling: ——).
Blood calcium concentrations in test and control animals were slightly below the normal values (97-124 mg/l) suggested by Kaneko (12). However, magnesium and hemoglobin concentrations were within normal limits (18-23 mg/l, 8-14 g/100 ml, respectively).

What can be inferred from the great variance of the 3 parameters, lead and ZPP concentrations in the blood and lead concentrations in the hair? Fig 3 shows the relationship between lead in blood and in hair for each animal and in each series of samples. In the first sampling, blood lead concentrations were comparatively low in all animals and unrelated to the lead in the hair, where mean concentrations were the highest. The cows that spent an average of 19.3 ± 9.6 months (range 6 to 41 mo) in sheds, accumulated high amounts of lead in the hair (which can be considered an organ of excretion) because lead intakes were high during the several months before sample-collection. Reversely, in the weeks which preceded this sampling, lead intakes were particularly low (Fig 1) and blood lead concentrations were only 34.3 ± 12.7 μg/100 ml whereas they were 50.8 ± 22.5 μg/100 ml 6 mo before (December 7, 1984). At the time of the second sampling, blood lead concentrations were higher and in very low correlation with the lead in the hair (r=0.34). At the time of the third sampling, mean blood lead concentrations were not very different but the correlation was much greater (r=0.89).

This closer correlation appeared due to the fact that lead intake remained unchanged (2.5 mg/kg/day) between October 12, 1985 and April 16, 1986 (ie, during the 48 days before the second sampling and over the 135 days which separated the second and the third samplings) (Fig 1).

Fig 4 shows the relationship between lead and ZPP in the blood of each animal and in each series of samples. Correlation is strong in the results of the first sampling (r=0.92), and lower in the second (r=0.73) and third samplings (r=0.61). It is statistically significant at p < 0.01 in all 3 cases. When an animal starts ingesting lead daily, blood lead increases more rapidly than ZPP (13). In the present study, when the first samples were collected the animals had been on the farm long enough for ZPP to reach fairly elevated and stable values. In the 9 animals that were submitted to all the 3 samplings, the mean ZPP concentrations were slightly lower at the second sampling but markedly higher at the third (respectively, 177 ± 131, 165 ± 91, and 258 ± 159 μg ZPP/100 ml). In 4 animals, progression was constant, but in the other 5, the concentrations were lower at the second sampling. These findings are difficult to interpret.

When the animals were slaughtered after an average of 28.1 ± 15 mo on the farm, the lead concentrations in kidney, liver and bone were moderate (Table 2). In liver and kidney, they were higher than normal, but they were within limits which do not manifest clinical signs.

The individual results show that when the sample and criteria are considered in some animals, concentrations are always above mean, whereas in others they are always below. One cow that had been 42 mo on the farm calved in the morning of April 9, 1986 and was found paralyzed and died immediately after a magnesium and calcium perfusion.

Exposure indicators were relatively high — 60 μg/100 ml lead in the blood in December 1984 and 46 μg/100 ml on November 29, 1985; ZPP concentrations were 63 μg/100 ml in December 24 and 160 μg on November 29, 1985; lead in the hair was 12.2 μg/g on June 19, 1985 and 7.8 on November 29, 1985. Necropsy revealed abundant percarditic liquid containing blood and a mild diffuse hepatic steatosis. Lead concentrations were 4.2 ppm (wet) in the liver, 5.5 ppm (wet) in the kidney and 50 ppm (dry) in the bone. The latter figure is surprising, given the time of exposure and the blood lead concentrations.

**CONCLUSION**

As the 3 indicators of lead exposure did not have the same significance and did not vary in exactly the same way, it was interest-
ing to use them to evaluate risks. Monitoring the time the animals spend in a contaminated area and the amounts of lead ingested daily were useful to interpret these 3 parameters. On the other hand, the concentration of zinc and cadmium in the blood and in the hair was of little interest. In the liver and kidneys, lead and cadmium concentrations were good indicators of exposure; zinc concentration was a fair indicator; and the bone was a very good indicator of lead exposure.

ACKNOWLEDGEMENTS

We thank la Societe Penarroya, l’Institut National de la Recherche Agronomique (INRA), l’Institut National Agronomique de Paris-Grignon, les services d’Anatomie-Pathologique, d’Alimentation et de Pathologie du Bétail de l’Ecole Nationale Vétérinaire d’Alfort, M. JP Bertrand, le Docteur Hathot, les Docteurs-Vétérinaires Caron et Barras, Madame Pupin, for their help in conducting this research. The assistance and cooperation of Mademoiselle Eliane Charles, Assistant Ingenieur Inra au Laboratoire de Pharmacie et Toxicologie de l’Ecole Nationale Vétérinaire d’Alfort, are also gratefully acknowledged.

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