Transfer of Cadmium from Feed to Ewe Food Products: Variations in Transfer Induced by Lead and Zinc

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We investigated the transfer of cadmium to ewe food products after oral administration of cadmium, and the variations in transfer induced by lead and zinc. We used a control group comprising 4 ewes and 3 groups each comprising 5 ewes exposed to cadmium, cadmium and lead and cadmium, lead and zinc. The cadmium group received cadmium oral administrations at a dosage of 2.5 mg/kg/d for 21 d (1st period) and 1.25 mg/kg/d for 31 d (2nd period). The cadmium-lead group received, in addition to cadmium given at the same dosage as the cadmium group, 2.3 mg/kg/d lead for 52 d. The cadmium-lead-zinc group received, in addition to cadmium and lead given at the same dosage as the cadmium-lead group, 3.5 mg/kg/d zinc for 52 d. The administration period began and ended with the lactation period. In the cadmium group, the cadmium concentration in milk reached 3.0 ± 1.0 µg/l during the 2nd period and was higher than that in blood (1.8 ± 0.6 µg/l). Eighty-one days after the end of the exposure period, the highest cadmium concentrations were observed in the kidneys (16.6 ± 4.5 mg/kg or 0.08% of the total dose given orally) and in the liver (8.6 ± 2.3 mg/kg or 0.10% of the total dose). The cadmium concentration in muscle remained low. When lead was coadministered with cadmium, the cadmium concentrations increased in all tissues and in milk. The transfer of cadmium to ewe food products may be increased in areas polluted with both lead and cadmium. When zinc was coadministered with lead and cadmium, the cadmium concentrations decreased in all tissues except kidney.
1. Introduction

The more cadmium contaminates the agricultural environment the more it contaminates the food chain and endangers human consumers' health. Its levels in soils and plants are increased by the application of phosphate fertilizers and sewage sludge.\(^1\) It also contaminates tissues, mainly the liver and the kidneys\(^2\) and, to a lesser extent, the milk of grazing animals. Therefore humans who are mainly exposed to cadmium through their food intake, will become increasingly exposed to it as cadmium pollution increases.\(^1\) The experts at the Food and Agricultural Organization of the United Nations (FAO) and the World Health Organization (WHO) estimated the provisional tolerable cadmium daily intake limit to be 1 \(\mu\)g/kg/d for humans.\(^3,4\) At present, daily cadmium intake is estimated to be 10–40 \(\mu\)g/d in non-polluted areas.\(^1\) In the USA, it was estimated to be 1.38 \(\mu\)g/kg/d for babies and 1.13 \(\mu\)g/kg/d for infants of which 59\% and 31\% respectively was provided by milk and dairy products.\(^5\)

Because many cadmium contaminated environments contain high levels of lead and zinc\(^6-8\) and because interactions have been described between the pathways involved in cadmium, lead and zinc metabolism,\(^9-11\) the present study aimed to investigate the transfer of cadmium to ewe food products after daily oral administration of cadmium and to study the variations of this transfer induced by daily lead and zinc administrations. The transfer of lead to ewe food products was studied simultaneously and will be the subject of another report. Ewes were selected as a model farm animal because humans consume their meat and milk.

2. Materials and Methods

2.1 Animals

Nineteen Prealpes lactating ewes from 2 to 6 years old were used. Throughout the study, their mean weight was 62 ± 4.6 kg and they remained in good health. They had had their lambs 7 to 8 d before the start of the study.

The animals were maintained in the sheepfold of the experimental farm at Brouessy (INRA\(^1\), France). Every morning they received 1.2 kg of hay, 0.4 kg dehydrated luzern, 0.6 kg of barley and 0.03 kg of mineral additive. They were given ad libitum access to water. The concentrations of cadmium, lead, zinc, calcium and phosphorus in the diet and the water were determined.

2.2 Experimental design

The ewes were divided into four groups, cadmium, cadmium-lead and cadmium-lead-zinc groups each containing 5 animals and one control group of 4 animals.

The 3 exposed groups were administered cadmium daily as cadmium chloride at a dose of 2.5 mg Cd/kg/d for 21 d and 1.25 mg Cd/kg/d for 31 d. The cadmium-lead group were also administered lead daily as lead chloride at a dose of 2.3 mg Pb/kg/d for 52 d.

\(^1\)Institut National de la Recherche Scientifique, or the French Institute of Agricultural Research
cadmium-lead-zinc group received cadmium, lead and also zinc as zinc oxide at a daily dose of 3.5 mg Zn/kg/d for 52 d. The administration period began and ended with the lactation period. Cadmium, lead and zinc were enclosed in a gelatin capsule which was placed at the base of the tongue. The capsule was immediately swallowed.

The ewes were slaughtered 81 d after the end of the exposure period.

2.3 Sampling

Blood samples (5 ml) were collected from the left jugular vein into vacutainer tubes guaranteed free of any heavy metals (ref 367735, Becton Dickinson, Maylan, France). These were then stored at 4°C until their cadmium and zinc contents were determined. Blood was collected the morning before the metal administration 0, 7, 14, 21, 24, 28, 31, 35, 38, 42, 45, 49, 51, 52, 56, 59, 63, 66, 70, 77, 91, 105, 119, and 133 days after the beginning of the exposure, except for cadmium-lead-zinc group where the final sequence was 77, 84, 98, and 112 days after the beginning of the exposure.

Milk was collected the morning before metal administration 0, 7, 14, 21, 28, 35, 42 and 49 days after the beginning of the exposure. Milk was homogenized and samples (150 ml) were collected in polyethylene flasks. The samples were stored at 4°C. Their cadmium content was determined on the same day as the sampling.

After the ewes were slaughtered, liver, kidney, muscle, abomasum, small intestine and udder samples were collected and stored at −20°C until analysis.

2.4 Metal measurement

The blood and milk samples were first diluted 1:10 for blood and 1:5 for milk in 0.05 N nitric acid in the presence of 0.1% Triton X. The cadmium concentrations in blood and milk were then directly measured by graphite furnace atomic absorption spectrometry (AAS) with a 1100B Perkin Elmer AGA 700 spectrophotometer according to the technique recommended by Lauwerys et al. The decomposition temperature was 550°C for blood, and 600°C for milk; the atomization temperature was 1450°C. The limit of detection was estimated to be 0.3 µg/l and the limit of quantification 0.8 µg/l. The intraday coefficient of variation was 25% at 0.8 µg/l, 13% at 2 µg/l and 2% at 5 µg/l in blood and 6% at 1 µg/l and 5% at 5 µg/l in milk.

Community Bureau of Reference lyophilized blood (BCR n°194 and 195) and lyophilized milk (CRM n°063 and 150) were analyzed to validate the method. Mean cadmium concentrations in the standard blood samples were determined to be 0.5 ± 0.4 µg/l and 5.4 ± 0.5 µg/l respectively versus the certified concentrations of 0.5 ± 0.1 µg/l and 5.37 ± 0.24 µg/l. Mean cadmium concentrations in the standard milk samples were 2.8 ± 1.2 ng/l and 21.8 ± 1.6 ng/l respectively versus the certified concentrations of 2.9 ± 1.2 ng/l and 21.8 ± 1.4 ng/l.

Zinc concentration in plasma was measured using a flame AAS as previously described by Lamand.

The tissue samples were dry-ashed at 450°C. The white ashes were dissolved in 5 ml (5 N) nitric acid and diluted with distilled water to a final volume of 50 or 100 ml. After

\(^2\text{Commission of European Communities, Community Bureau of Reference, Brussels, Belgium}\)
appropriate dilution, the cadmium concentration was measured by graphite furnace AAS. The decomposition temperature was 300°C and the atomization temperature was 1400°C. The limit of detection was estimated to be 4 μg/kg dry matter and the limit of quantification 15 μg/kg dry matter. The intraday coefficient of variation was 4% at 100 μg/kg and 2% at 200 μg/kg dry matter. National Institute of Standards and Technology bovine liver (NIST 3 n°1577b) and Community Bureau of Reference pig kidney (BCR n°0675) were analyzed to validate the method. We measured zinc concentrations in tissues using the same method that we used for plasma.

2.5 Statistical analysis
We performed statistical analysis using ANOVA. The Student’s Newman-Keuls test was used for means classification.

3. Results

3.1 Diet composition
Diet composition and total mineral intake are shown in Table 1. Cadmium and lead intake from feed were low, 8 and 25 μg/kg/d, respectively. Zinc feed intake reached 3.7 mg/kg/d which was very close to the zinc oral dose (3.5 mg/kg/d). Calcium intake was as high as 28 g/d when requirements were 15 g/d in the best lactating ewe.

3.2 Cadmium in blood and milk
The cadmium concentrations in blood and milk of the control ewes were below the limit of quantification during the entire study.

Table 1
Average composition of the diet (mg/kg feed) and total mineral intake for each ewe (mg/d) during the study.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hay (mg/kg)</th>
<th>Dehydrated luzern (mg/kg)</th>
<th>Barley (mg/kg)</th>
<th>Mineral additive (mg/kg)</th>
<th>Water (mg/kg)</th>
<th>Total intake (mg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorus</td>
<td>1800</td>
<td>2500</td>
<td>2700</td>
<td>70000</td>
<td>0.27</td>
<td>7000</td>
</tr>
<tr>
<td>Calcium</td>
<td>11000</td>
<td>25300</td>
<td>500</td>
<td>150000</td>
<td>120</td>
<td>28000</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.36</td>
<td>0.075</td>
<td>0.01</td>
<td>0.66</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>Lead</td>
<td>0.63</td>
<td>0.7</td>
<td>0.34</td>
<td>8</td>
<td>0.001</td>
<td>1.5</td>
</tr>
<tr>
<td>Zinc</td>
<td>31</td>
<td>19.5</td>
<td>24</td>
<td>5600</td>
<td>0.038</td>
<td>228</td>
</tr>
</tbody>
</table>

US Department of Commerce, National Institute of Standards and Technology, Gaithersburg MD, USA
The cadmium concentrations in blood (Fig. 1) increased up to a plateau concentration during the first 21-d period of metal administration in the 3 exposed groups. The concentrations reached 1.8 ± 0.6 μg/l in the cadmium group, 2.6 ± 1.6 μg/l in the cadmium-lead group, and 2.3 ± 1.2 μg/l in the cadmium-lead-zinc group. They remained almost constant over the following 30 d. Because the ewes had very different blood cadmium concentrations within the same group, the group mean was not significantly different among the groups (p>0.05). The cadmium concentrations in blood decreased very slowly after the end of the exposure. The blood concentrations were still above the initial values 81 d after the last cadmium administration (1.8 ± 1.0 μg/l).

The cadmium concentrations in milk (Fig. 2) increased during the first 21-d period of metal administration, then remained almost constant during the following 30 d. The mean concentrations reached 3.0 ± 1.0 μg/l in the cadmium group, 4.2 ± 2.4 μg/l in the cadmium-lead group and 4.1 ± 1.9 μg/l in the cadmium-lead-zinc group.

The cadmium milk to blood concentration ratios did not vary among the groups and were 1.8 ± 0.7, 1.8 ± 0.8 and 2.0 ± 0.6, respectively in the cadmium group, the cadmium-lead group and the cadmium-lead-zinc group during the plateau concentration, from day 21 to 51. About 0.002 to 0.004% of the total cadmium given orally was excreted through milk during the entire lactation period. The percentage did not vary substantially among the groups.

3.3 Zinc in plasma

The zinc concentrations in plasma of the control ewe group were within the normal values. They varied from 0.8 to 1.2 mg/l. The zinc concentrations in plasma of the 3 exposed ewe groups decreased during the first 2-week period of metal administration and...
remained low until the end of the exposure period (Fig. 3). They were significantly lower than those observed in the control ewes ($p<0.05$). After metal administration ceased, they increased and returned to their initial values.

3.4 Cadmium in tissues

The highest cadmium concentrations were observed in the kidneys followed by the liver in the 4 groups. The cadmium-lead-zinc group showed the highest average kidney cadmium concentration, 21.8 ± 6.8 mg/kg fresh matter, and the cadmium-lead group showed the highest average liver cadmium concentration, 17.2 ± 13.8 mg/kg (Fig. 4). The lowest average kidney and liver cadmium concentrations were observed in the control group, 0.8 ± 0.2 mg/kg and 0.3 ± 0.1 mg/kg respectively, followed by the cadmium group, 16.6 ± 4.5 and 8.6 ± 2.3 mg/kg respectively. The average liver to kidney cadmium concentration ratio was highest in the cadmium-lead group. At the end of the experiment, 81 d after the last metal administration, the total cadmium amount in the liver and the kidneys reached 16.9 mg in the cadmium-lead group, 14.1 mg in the cadmium-lead-zinc group and only 10.5 mg in the cadmium group.

The cadmium concentration in the muscle remained low in the 4 groups and was approximately 0.04 mg/kg. In the small intestine, the abomasum and the udder, the cadmium concentrations followed the same kind of pattern as in the liver. The lowest concentrations were observed in the cadmium group, the highest concentrations in the cadmium-lead group, and the cadmium-lead-zinc group showed intermediate concentrations (Fig. 5). Even 81 d after the last metal administration, the cadmium concentrations in the abomasum of the exposed groups, 0.76 ± 0.1, 0.42 ± 0.24 and 0.40 ± 0.13 mg/kg in
Fig. 3. Plasma zinc concentrations in ewes after daily administration of cadmium, cadmium and lead, or cadmium, lead and zinc for 52 d (*different from control group p<0.05).
3.4 Zinc in the urine of ewes exposed to cadmium, cadmium-lead and cadmium-lead-zinc groups, respectively, and in the udder of the exposed groups, 0.54 ± 0.19, 0.91 ± 0.51 and 0.79 ± 0.41 mg/kg in cadmium, cadmium-lead and cadmium-lead-zinc groups, respectively, remained higher than those of the control group, 0.02 ± 0.01 mg/kg in the abomasum and the udder (p<0.05).

Fig. 4. (a) Average liver (■) and kidney (□) cadmium concentrations, (b) average ratio between liver and kidney cadmium concentrations of ewes 81 d after the end of daily administration of cadmium at 2.5 mg/kg for 21 d and 1.25 mg/kg for 31 d (*different from control p<0.05).
3.5 Zinc in tissues

Tissue zinc concentrations in the exposed groups were close to those in the control group (Fig. 6). The cadmium group showed a lower zinc concentration in the muscle than the other groups ($p<0.05$) and the cadmium-lead-zinc group showed a higher zinc concentration in the kidneys than the other groups ($p<0.05$).

4. Discussion

Because several diet components are known to affect cadmium bioavailability, the composition of the diet given to the ewes in this study was controlled. Calcium and zinc deficiencies increased the gastrointestinal availability of cadmium. The diet provided a good balance of minerals except for calcium the intake of which was higher than the daily requirement. Its cadmium and lead content were low. During this study, the ewes daily ingested 8 and 24 μg/kg/d cadmium and lead respectively, and they came from a non-contaminated area with respect to these two metals. Indeed, cadmium concentrations in blood and milk of all the ewes before the first metal administration and of the control ewes during the entire study were low, much lower than our quantification level (0.8 μg/l). Zinc intake from food was high (3.7 mg/kg/d) compared to the oral doses of zinc (3.5 mg/kg/d).

In a previous study, cows fed with a polluted hay harvested from a site near a lead- and zinc-ore processing factory ingested 2.9 mg/kg/d zinc and 2.3 mg/kg/d lead. We chose the lead and zinc dosages on this basis. The oral zinc dosages should have been higher, to enhance the difference between zinc intakes of the cadmium-lead-zinc group and the other
groups. The cadmium oral dosages (2.5–1.25 mg/kg/d) were equivalent to the amount of cadmium consumed in 2 kg of hay contaminated by 75–38 mg Cd/kg. Although the cadmium dosage used provided a higher cadmium content than the usual levels in the diet of sheep, cadmium in leaves of plants grown on soil supplemented with 40 mg/kg ranged from 18.5 to 264.7 mg Cd/kg. In some industrial and mining areas, plants contained from 5 to 80 mg Cd/kg on a dry weight basis, and winter forage hay contained up to 15 mg Cd/kg. Therefore, our dosages and repeated oral administrations simulated cadmium exposure of sheep in very contaminated areas.

The cadmium concentrations in milk, 3 to 4.2 μg/L, were close to those observed in the milk of ewes in polluted areas. Average cadmium concentrations in milk collected from ewes in zones around a metal works ranged from 1.6 to 12 μg/L. Surprisingly, in ewes, cadmium concentrations in milk were higher than in blood, whereas in mice cadmium concentrations in milk are lower than in blood. Kirova observed that ewe milk contained up to 1.5 times more cadmium than cow milk collected at the same location. The transfer of cadmium from the digestive tract to milk remained low and during the lactation period, approximately 0.002 to 0.004% of the total cadmium dose was excreted through milk.

In the cadmium group, 81 d after the last administration, approximately 0.10% of the total cadmium administered orally still remained in the liver and 0.08% in the kidneys. In a previous study, ewes retained 0.19 and 0.08% of the cadmium administered orally in their liver and their kidneys, but they were slaughtered the day after the last administration. Goats retained 0.17 and 0.08% of a single oral dose of cadmium in the liver and the kidney.
Thus, the kidneys can store large amounts of cadmium for a longer time than the liver, in accordance with the cadmium half-lives calculated for kidney and liver in humans, 12 to 50 and 7 years, respectively. Lead administration seems to increase the transfer of cadmium to ewe food products. When lead was coadministered with cadmium, the cadmium concentrations increased in all the tissues and in milk. Information on lead interactions with cadmium are limited. Cadmium administration alleviates certain symptoms of lead toxicity but enhances other toxic lead effects. When zinc was coadministered with lead and cadmium, the cadmium concentrations decreased in all the tissues except the kidneys but they remained higher than those observed when cadmium was administered alone. Zinc pretreatment increases the deposition of cadmium in the kidneys of rats and decreases the deposition of cadmium in the liver of rats.

Cadmium administration decreased zinc concentrations in plasma and in muscle. Zinc metabolism is normally controlled by homeostatic mechanisms. When the intake of zinc is low, exposure to cadmium increases renal and hepatic zinc concentrations and therefore decreases zinc concentrations in other tissues. The zinc intake was not low in this study, and the cadmium-lead-zinc group also exhibited a low zinc concentration in plasma. In rats exposed to cadmium, zinc concentrations in plasma are decreased and the liver accumulates more zinc because metallothionein synthesis is increased in the presence of cadmium and metallothionein binds both cadmium and zinc.

Cadmium transfer was greater to the liver and the kidneys than to other tissues. For an adult, 50 g of the liver or 20 g of the kidney of one of these ewes would be enough to supply the tolerable cadmium intake limit of 7 μg/kg/week. A 15-kg child could drink up to 25–35 l of the milk of these ewes to reach the tolerable cadmium weekly intake limit. The transfer of cadmium to ewe food products may be increased in areas polluted with both lead and cadmium. The coadministration of cadmium, lead and zinc may reduce this increase.

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References