Bull. Environ. Contam. Toxicol. (2005) 74:526–536

DOI: 10.1007/s00128-005-0616-0

Bulletin of Environmental Contamination and Toxicology

Heavy Metals in Tissue Samples of Finnish Moose, *Alces alces*

E.-R. Venäläinen,[1] M. Anttila,[2] K. Peltonen[1]

[1] Department of Chemistry, National Veterinary and Food Research Institute, Post Office Box 45 (Hämeentie 57), FIN-00581 Helsinki, Finland

[2] Department of Pathology, National Veterinary and Food Researach Institute, Post Office Box 45, (Hämeentie 57), FIN-00581 Helsinki, Finland

Received: 15 September 2004/Accepted: 4 December 2004

The level of heavy metals in wild animals has been the focus of some studies (Treble et a., 1998, Lusky et al. 1997). These studies were initiated by the fact that in many areas wild animals form a significant part of the traditional diet. For example, moose, caribou and whitefish are the most important sources of food for the Canadian Artic Indigenous Peoples. The cadmium exposure resulting from consumption of traditional food in Fort Resolution, Northwest Territories in Canada has been estimated and the highest levels of cadmium were found in the liver and kidney of caribou and moose (Aim et al. 1998). Levels of Hg, Cd, Pb, Cu, Ni, Zn and Fe has been analyzed in moose, reindeer, brown bear, wild boar and squirrel samples obtained in Karelia, Russia (Medvedev 1999). The results pointed to a widespread heavy metal contamination in that area with samples from moose, reindeer and brown bear containing the highest levels of heavy metals. The wild boar samples had the lowest concentrations of the animals studied.

The estimated Finnish moose meat consumption in 2000 was 8.3 million kg, in other words an annual consumption of 1.6 kg of moose meat per capita, but also liver and kidneys may be consumed in some cases (Finnish Game and … 2001). However, the estimated consumption of liver and kidney is much lower than the meat consumption because Finnish legislation prohibits the use of moose liver and kidneys of animals older than one year to avoid excessive exposure to cadmium (Ministry of Agriculture 1997). However, should these organs be extensively used, they may represent a significant source of cadmium, and one group of people at elevated exposure risk are hunters and their families (Vahteristo et. al 2003). Heavy metals in moose tissues are regularly monitored in the annual national residue control programme and some high cadmium levels in liver and kidneys are occasionally detected, however heavy metal levels in muscle are low. (Venäläinen et al.1999).

Cadmium is widely distributed throughout the environment and human activities have had an important role in its dispersion to the biosphere. The natural sources of cadmium are volcanic eruptions and old granite rocks, which are an important geochemical source. Anthropogenic sources of cadmium are related mainly to mining and rock processing industry, fertilizers, atmospheric deposition, animal manures and to smaller extent to liming agents, sewage sludge and other wastes (Nordic Council of Ministers 1992, European Commission 1996). Cadmium accumulates mostly in kidney and liver and has a biological half-time of 10-20 years in humans. Long-term

Correspondence to: E.-R. Venäläinen

exposure to cadmium results in an irreversible tubular nephropathy which may develop into renal insufficiency. (Jarup et al.1998).

Historically leaded gasoline has been the most important source of atmospheric lead. However, most countries have now prohibited the use of leaded gasoline. This action has greatly reduced emissions of lead into the atmosphere. Anthropogenic sources of lead other than traffic are typically stationary fossil fuel combustion, non-ferrous metal production and iron and steel production (AMAP 2002). The toxicology of lead is well known and exposure to lead may cause altered behaviour since lead can affect neuronal cells in the brain and periphery resulting in characteristic symptoms known as lead poisoning. Lead also affects the function of many enzymes, most notably those associated with the production of haemoglobin and cytochromes. Other effects include kidney damage and dysfunction, anaemia, intestinal dysfunction, and reproductive problems including abnormal growth and development (Nielsen et al. 2000).

Zinc and copper are important constituents in a number of different enzyme functions in man and animals. Zinc and copper accumulate mostly in muscle and in liver. They reach the environment by industrial releases from production and refining of metals (Melanen et al. 1999).

Our investigations into the concentrations of lead, cadmium, copper and zinc in tissues of moose were performed for the first time in 1980. The study included more than 130 animals originating from three different locations in Finland. The second study was done in 1990 by analyzing moose samples (100 animals) collected from the same three game management areas as the1980 samples (Niemi et al. 1993). The data we are presenting in this communication is based on samples collected in 1999 and the sample collection was still from the same game management areas as in the two earlier studies. In addition, a new game management area, south eastern Finland was also included. Muscle, liver and kidney from 100 moose of different ages were collected. These follow up studies aim to provide a comprehensive picture of the levels of heavy metals in game animals since the level in these animal tissues reflects the general heavy metal pollution of the environment in that particular area. An important aspect is also to monitor consumer/hunter exposure to the toxic metals.

MATERIALS AND METHODS

Moose samples were collected during the autumn of 1999 after the Hunters Central Organisation granted permissions that we could collect samples from animals culled in the game management areas of south western Finland, southern Finland, central Finland and south Eastern Finland. The hunters were informed on how to conduct the sampling and told how to pack samples. We provided all the materials needed for shipping the samples to the laboratory. Muscle, liver, kidney and tooth samples were packed separately, frozen and sent to the laboratory in temperature controlled chambers. In the laboratory, the samples were stored at –18 °C until analyzed.

Muscle (10 g), liver (5 g) and the cortex samples of kidneys (5 g) were homogenized and subsequently weighed into quartz dishes and dried in a water bath. Dried samples were dry-ashed in a thermostat controlled muffle furnace at 450 °C overnight. The ashed samples were allowed to cool down to room temperature, after which deionised water (2-3 ml) and concentrated nitric acid (1ml) were added and the samples were evaporated and heated up to 450 °C in a muffle-furnace and kept at that temperature overnight. The ash was dissolved in concentrated nitric acid (0.5 ml) and the sample volume was adjusted to 20 ml (liver, kidneys) or 10 ml (muscle) with deionised water (Niemi et al. 1991, Jorhem 1993).

Cadmium and lead were measured with a graphite furnace atomic absorption spectrometer applying pyrolytic THGA-tubes (transverse heated graphite atomiser tubes) with Zeeman furnace module (Perkin-Elmer, Singapore). Cadmium was measured at 228.8 nm and lead at 283,3 nm with hollow cathode lamps. Copper and zinc were measured with the same instrumentation except that we used an air-acetylene flame technique at 324,8 nm and 213,9 nm wavelength. All data was collected with a Perkin Elmer 5100 PC and with an AAnalyst 800 atomic absorption spectrophotometer.

Quantification was carried out by using standard solutions in 0.1 M nitric acid (Reagecon). The determination limit for cadmium was 0.001mg/kg wet weight and for lead 0.01mg/kg wet weight. The determination limits of copper and zinc were 0. 2 mg/kg wet weight.

Standard reference materials (BCR 184 bovine muscle, BCR 185R, bovine liver and BCR 186 pig kidney) were included in the sample series and all samples were analyzed two times. The reference material data was ± 10% from the certified mean values. Calibration of analyses was monitored using standards with different concentrations and instrument contamination was monitored by analysing control samples in the sample series. The recoveries of cadmium, lead, copper and zinc were determined by adding a known amount of a particular standard solution into the samples. The quantities of standards used were close to the amounts normally detected in samples. The recovery varied from 80 to 110%. Laboratory participates annually in proficiency tests (Food Analysis Performance Assessment Scheme =FAPAS, Istituto Superiore di Sanita= ISS) in which good results with a z-score of ± 2 is regularly achieved. The method used to analyze lead, cadmium, copper and zinc is accredited by the Centre of Metrology and Accreditation in Finland (EELA 8104 flex: Determination of metals in animal tissues with graphite furnace and flame atomic absorption spectrometry).

Hunters were asked to send a half of a mandible of the moose without breaking off the root tip. These samples were used to determine the age of each particular moose at Matson's Laboratory (Matson's laboratory, Milltown, USA). Before the teeth were delivered to Matson's laboratory they were cleaned in the pathology laboratory of EELA according to the method recommended by Matson's Laboratory. Shortly the periodontal membrane was softened in hot water (temperature below than 80 °C) and then the teeth were cleaned with a nylon mesh material. Teeth were extracted from the skulls and mandibles and finally they were packed carefully in paper envelopes, placed in a very sturdy cardboard box and mailed to Matson's Laboratory.

RESULTS AND DISCUSSION

The detection limit of cadmium was 0.001 mg/kg. Cadmium clearly accumulates in kidney (4.9-6.2 mg/kg) and a statistically significant difference was observed between kidney and liver levels as well as between kidney and muscle samples.

Tablel. The levels of heavy metals in various moose samples collected in 1999 (mg/kg wet weight), n=number of samples.

	South Western Finland		Southern Finland		Central Finland		South Eastern Finland	
Muscle								
Pb	0.02	n=23	0.03	n=28	0.03	n=22	0.02	n=28
Cd	0.003	n=23	0.004	n=28	0.004	n=24	0.004	n=27
Zn[ab]	56.9	n=23	60.5	n=28	60.2	n=24	62.1	n=28
Cu	1.07	n=23	1.01	n=28	1.06	n=25	0.97	n=28
Liver								
Pb	0.05	n=23	0.07	n=32	0.05	n=25	0.04	n=23
Cd	1.28	n=21	0.71	n=32	1.13	n=25	0.895	n=27
Zn	23.7	n=23	25.7	n=32	25.5	n=25	32.1	n=27
Cu[cd]	33.0	n=22	44.4	n=32	48.8	n=25	50.5	n=27
Kidney								
Pb	0.07	n=23	0.07	n=32	0.06	n=24	0.05	n=27
Cd[ef]	5.96	n=23	4.95	n=33	6.18	n=25	5.58	n=28
Zn	31.7	n=23	30	n=30	32.4	n=25	31.1	n=28
Cu	3.8	n=23	3.64	n=33	3.66	n=25	3.57	n=28

a= statistically significantly higher than the liver concentration
b= statistically significantly higher than the kidney concentration
c= statistically significantly higher than the muscle concentration
d= statistically significantly higher than the kidney concentration
e= statistically significantly higher than the muscle concentration
f= statistically significantly higher than the liver concentration

(p=0.0099; p=0.0051). Cadmium levels were not statistically significantly different in the various hunting areas. Cadmium seems to accumulate to kidney and also to liver as a function of the age of the animals. The accumulation was linear in kidney r=0.97 and in liver r=0.97 (Figure 1 and 2). However, the accumulation in muscle was not dependent on age, higher cadmium concentrations were observed only in moose which were over six years old.
The cadmium data in muscle, liver and kidney are summarized in table 1.

Although the atmospheric emission of cadmium has decreased by about 80% from 1990 to 1997, paradoxically the cadmium concentration in game animals has increased considerably over the same time period (Melanen et al. 1999). It is unclear how cadmium ends up to the food chain in Finland, but one of the reasons may be the low pH of the Finnish soil (Melanen et. al 1999). The pH of Finnish cultivated soil is close to 5.8 which is mainly due to the high proportion of organic matter in the soil. The slow decomposition rate of organic matter is a result of the cold and humid

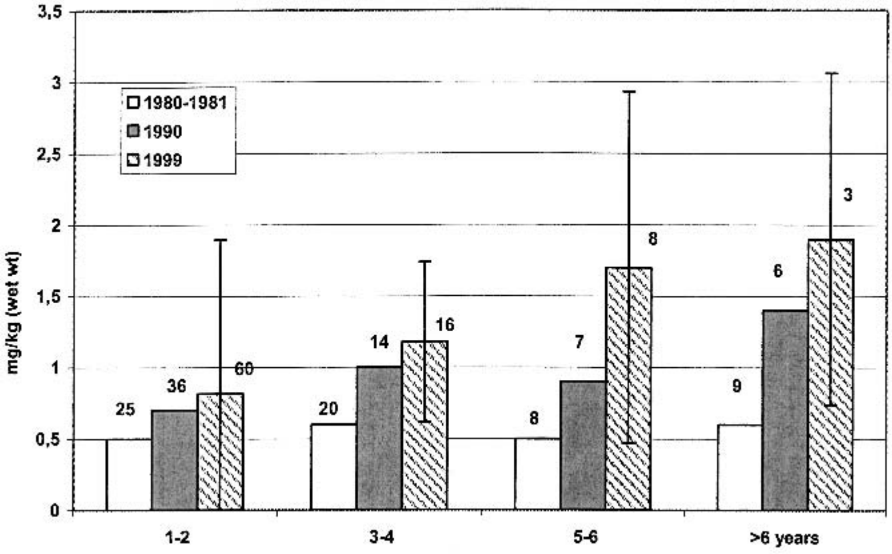

Figure 1. Levels of cadmium in liver of moose in different ages groups. The number at the top of a particular column indicates the number of the samples analyzed. The range can only be provided for the 1999 samples.

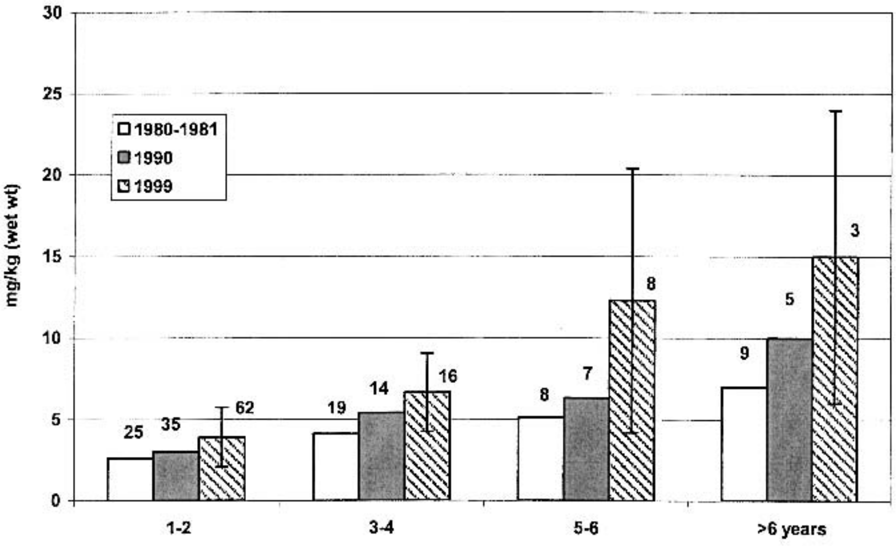

Figure 2. Levels of cadmium in kidneys of moose in different ages groups. The number at the top of a particular column indicates the number of the samples analyzed. The range can only be provided for the 1999 samples.

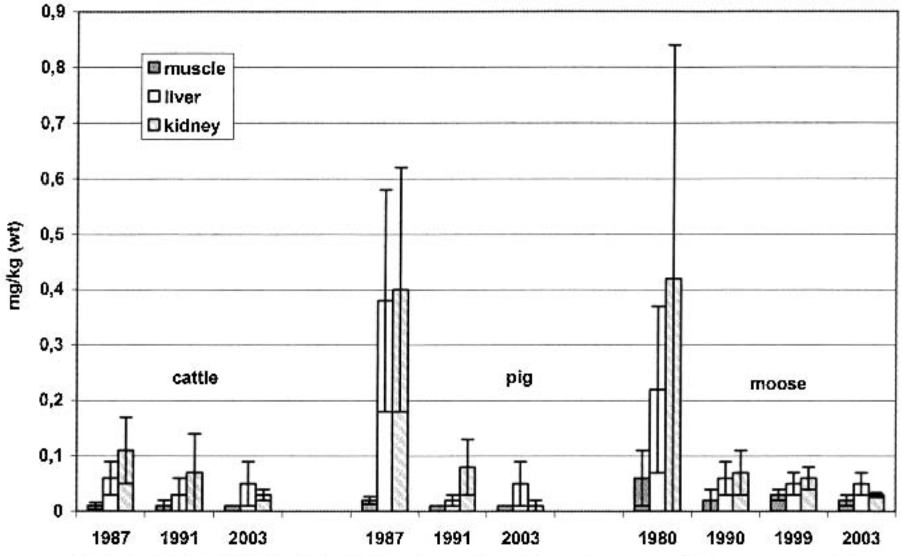

Figure 3. Lead concentrations in cattle, pig and moose. The number at the top of a particular column indicates the number of the samples analyzed. The range is indicated by a bar.

estimated that emissions have declined by as much as 95% even though industrial production of lead has increased over the same period (Melanen et al. 1999). Investments in better purification processes of fuel and gases and advanced combustion technologies together with enhanced process automation have made this possible. However, the most important reason for the decrease of environmental lead level is the use of unleaded gasoline, which has been the case in Finland since 1993 (Melanen et al. 1999). This decreased environmental lead contamination is mirrored in the level of lead in liver and kidney samples which have clearly decreased from those measured in 1980 to 1990 (Niemi et a. 1993).

In contrast, during the time period from 1990 to1999 only minor changes were observed in lead concentrations are in liver and kidney samples. However the lead concentration in muscle has now dramatically decreased from values of 1980 to 1990, and is now close to the determination limit 0.01 mg/kg.

The data from 1980-81 sampling highlighted a geographical difference in the lead concentration in kidney. Then, the samples from southern Finland, where the density of car traffic is most intense, had the highest levels of lead in kidney. By 1990 the geographical locations of the game management area no longer played any role in the lead concentrations in the samples (Niemi et al. 1993). In the present samples the lead content of tissues of moose was at the same level as those detected in bovine tissues (Figure 3, EVI, EELA, MMM 2003).

climate (Louekari et al. 2000). In Central European countries the pH of the cultivated soil is closer to 7 and organic content of the soil is low. The above mentioned factor makes it possible for the soil in Finland to accumulate high amounts of cadmium in a soluble form which then makes cadmium biologically available for plants.
The raw-material for the phosphorous-fertilizers used in Finland contains cadmium only 1 to 5 mg/kg of phosphorous which is by far lower than the average cadmium content in European fertilizers where the Cd content is 138 mg/kg of phosphorous (Louekari et al. 2000). Due to the low cadmium level in Finnish fertilizers and because of a low usage of fertilizers, their share is only 4 % of the total cadmium concentration in cultivated soil of Finland.

Clay soil in southern Finland has a low concentration of zinc which poses a risk by allowing Cd to accumulate in plants (Louekari et al. 2000). Of all the trace elements cadmium is most easily taken up by plants. Uptake of cadmium by plants can be reduced by liming of the soil but this is a costly process and not always successful for

all plant species, for example liming of the soil was not able to reduce the cadmium content of potato tubers (Jackson et. al 1991).

In this study, the high cadmium content was found in the moose samples originating from south western and central Finland. A part of the total Finnish emissions of cadmium and also copper originate from southwestern Finland in the vicinity of non-ferrous metal smelting plants located in Harjavalta and Kokkola. In south-eastern Finland, the soil cadmium content is higher (0.38 mg/kg) than in other parts of Finland, which possibly could be one of the reasons for the high cadmium concentrations in moose living there (Louekari et al. 2000). The lowest cadmium concentrations were found in southern Finland. The level of cadmium in liver and kidney samples has increased during the sampling years. The cadmium level is also higher in older animals than in young animals (figures 1 and 2). However, the cadmium concentration in muscle tissue has decreased during the monitoring years, being now close to the determination limit (0.001mg/kg). In liver samples, the cadmium level is about 1/5 of that found in kidney (Table 1). The maximum cadmium level in cattle meat is 0.05 mg/kg, in liver 0.5mg/kg and in kidneys 1.0mg/kg (European Commission 2001).

The detection limit of our assay for lead was 0.01 mg/kg. The mean value of lead in the kidney samples grouped according to the hunting area varied from 0.05 to 0.07 mg/kg wet weight with the highest levels of lead being detected in samples derived from south and south western Finland. However, the difference in lead levels between different geographical areas was not statistically significant (p=0.1529). The data also indicates that the level of lead was the highest in kidney (0.07 mg/kg), followed by liver (0.05 mg/kg) and muscle tissues (0.02 mg/kg). The difference between kidney and liver was statistically significant (p=0.0009) and if the levels are compared to the muscle level, then a statistically significant difference was observed between kidney and muscle sample (p=0.0000) and between liver and muscle samples (p=0.0000).The data of the lead levels in muscle, liver and kidney in samples from four hunting areas are summarized in table 1.

The atmospheric emissions of lead decreased substantially in the 1990s; it has been

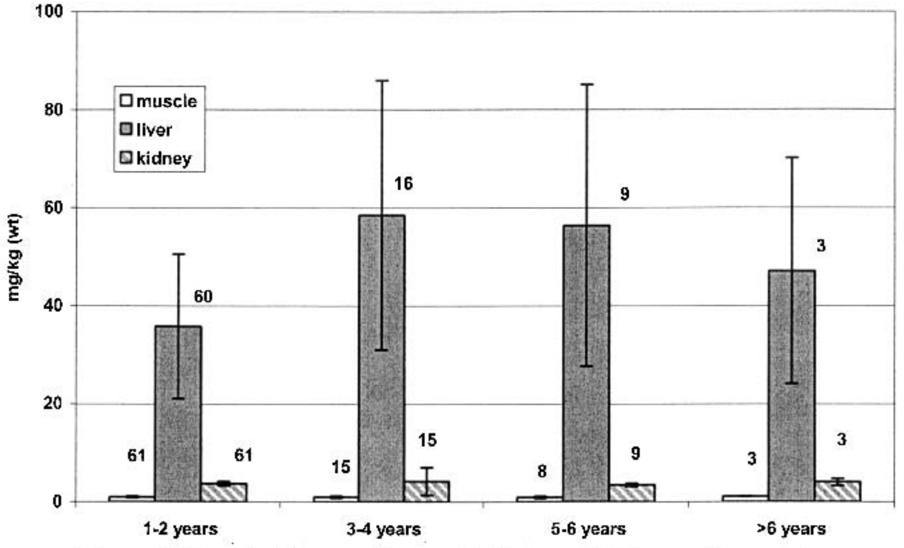

Figure 4. Levels of copper in muscle, liver and kidneys of moose in different age groups. The number at the top of a particular column indicates the number of the samples analyzed. The range is indicated by a bar.

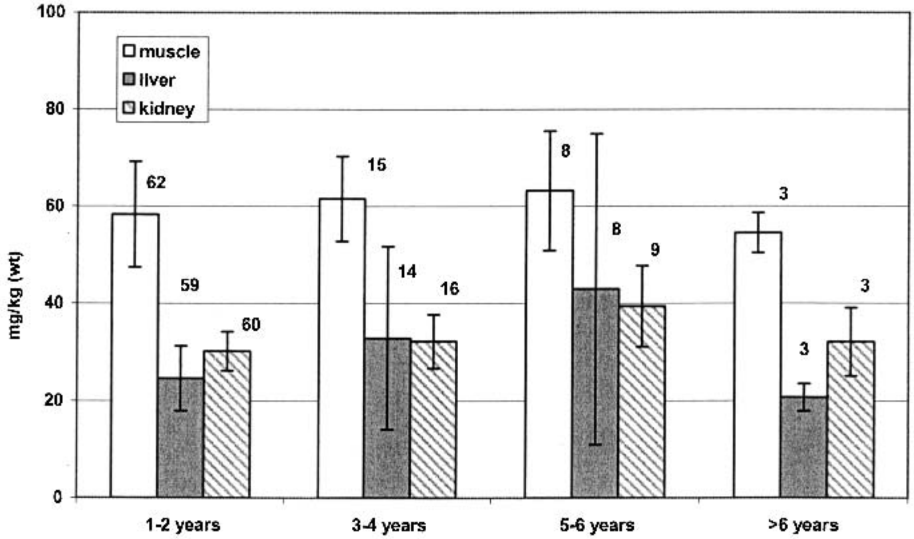

Figure 5. Levels of zinc in muscle, liver and kidneys of moose in different age groups. The number at the top of a particular column indicates the number of the samples analyzed. The range is indicated by a bar.

The detection limit of zinc and copper was 0.2 mg/kg. The level of zinc was highest in muscle tissues (59.6-62.1 mg/kg) followed by kidney (30.0- 32.4 mg/kg) and liver

(23.7- 32.1). The level of zinc in muscle tissues was statistically higher than the levels in kidney (p=0.000), but the sampling area did not influence the zinc level.

Copper accumulates in the liver and this organ has clearly the highest level of this particular metal of the tissues studied. Samples (n= 28) originating from south eastern Finland had the highest level of copper in liver tissues and a statistically significant difference was seen comparing south eastern Finland and south western Finland (p=0.0191). If compared to the other sampling areas, then the difference was not statistically significant. The level of zinc and copper was not dependent on age (Figure 4 and 5). The data on zinc and copper are summarized in table 1.

Copper concentrations have decreased slightly in southern and central Finland but considerably more in south western Finland. The highest copper concentrations are found in south eastern Finland and the lowest concentrations in south western Finland (Niemi et al. 1993). This is interesting because the only processing plant for copper is located in south western Finland and copper production has actually increased from the mid-90s. The copper processors now pay more attention to installing effective

purification systems for fuel gases and have improved combustion techniques. The high copper concentrations in the other parts of Finland, and also in south western Finland may be caused by long-distance deposition. In general liver, seems to accumulate copper (29.9- 50.5 mg/kg) more than kidney (3.6-3.8 mg/kg) and muscle tissues (0.97-1.07 mg/kg) (table 1). The level of copper seems to be constant in muscle and kidney but some variation occurs in liver samples.

The zinc concentrations have increased slightly during the last ten years but there are no significant differences between different game management areas.
The samples collected in 1980 or 1990 were rather similar to the zinc levels determined in this study (Niemi et a. 1993). However, in all areas where samples were collected in 1999 muscle had the highest level of zinc.

We studied the consumption of moose meat, liver and kidneys as foodstuffs in hunters and estimated their cadmium intake (Vahteristo et al. 2003). We conclude that the consumption of moose meat will not cause any significant increase in cadmium intake if compared to the average daily intake of cadmium (about 10 μg) in Finland. However, consumption of liver and kidney will have a major impact on cadmium intake. A person spotted to the highest deciles of consumption of liver and kidney would be estimated to have a cadmium intake of 13.9 % of PTWI and 25.5 % of PTWI from liver and kidney respectively (WHO 2001, Vahteristo et al. 2003).
Cd does not pose a health risk as far as moose meat is concerned and the high concentrations of cadmium in liver and kidneys may be relatively unimportant in terms of dietary intake of the Finnish population, but may have a significant impact on daily cadmium intake of individual moose hunters. (Vahteristo et al. 2003).

Acknowledgments. The authors wish to express special thanks to the hunters and the laboratory assistants Irja Jokinen and Henna Punkkinen for analysing the samples and laboratory assistants Sulo Mahosenaho and Jarmo Korpenfelt for preparing the teeth for age determination.

REFERENCES

Artic Monitoring and Assessment Programme, AMAP (2002) The Artic Pollution 2002: 37-59

Davister A (1996) Studies and research on processes for the elimination of cadmium from phosphoric acid. OECD Proceedings- Fertilizers as a Source Of Cadmium. IOMC. Paris. p. 21-30

European Commission, Food science and techniques (1996) Reports on tasks for scientific cooperation. Dietary exposure to cadmium: Report of experts participating in Task 3.2.4. European Commission, Directorate-General III, Brussels

European Commission (2001) Commission Regulation (EC) No. 466/2001 of March 2001. Setting Maximum Levels for Certain Contaminants in Foodstuffs

EVI, EELA (2003) Results of residue examinations of products of animal origin in Finland in 2002, EVI-EELA-MMM Publications 1/2003, Helsinki

Ermala A (2001), Annual Game Bag 2000, Finnish Game and Fisheries Research Institute, Helsinki

Jackson AP, Alloways BJ (1991) The transfer of cadmium from sewage-sludge amended soils into edible components of food crops. Water, Air Soil Pollut 57-58:873-881

Jarup L, Berglund M, Elinder CG, Nordberg G, Vahter M (1998) Health effects of cadmium exposure – a review of the literature and risk estimate. Scandinavian J Work Environ Health 24 (Suppl 1): 1-51

Jorhem L (1993) Determination of metals in foodstuffs by using atomic absorption spectrophotometer after dry ashing: NMKL1 interlaboratory study of lead, cadmium, zinc, copper, iron, chromium and nickel. JAOAC Int 76: 798-813

Louekari K, Mäkelä-Kurtto R, Pasanen J, Virtanen V, Sippola J, Malm J (2000) Cadmium In Fertizers, Risk to human health and environment. Publications 4/2000. Ministry of Agriculture and Forestry in Finland, Helsinki

Matson G (2000) Cementum age report. Matson's laboratory, LLC, Milltown

Melanen M, Ekqvist M, Arun B, Munkherjee, Arun B, Aunela-Tapola L, Verta M, Salmikangas T (1999) Atmospheric emissions of heavy metals in Finland in the 1990s. The Finnish Environment 329, Ministry of the Environment, Helsinki

Ministry of Agriculture and Forestry (1997) Decision MMMp 20/EEO/1997, Ministry of Agriculture and Forestry, Helsinki (in Finnish)

Nielsen U, Kamp JJ, Grandjean P, White RF (2002) Environmental lead exposure and neurodevelopmental outcome in Danish preschool children. Neurotoxicology 21: 896-897

Niemi A, Venäläinen E-R, Hirvi T, Karppanen E (1991) The lead, cadmium and mercury concentrations in muscle, liver and kidney from Finnish pigs and cattle during 1987-1988. Z Lebensm Unters Forsch192: 427-429

Niemi A, Venäläinen E-R, Hirvi T, Valtonen M (1993) Heavy metals in muscle, liver and kidney from Finnish elk in 1980-81 and 1990. Bull Environmental Contam Toxicol 50: 834-841

Nordic Council of Ministers (1992) Atmospheric heavy metal deposition in Northern Europe 1990. Nord 12, 41

Puls R (1989) Mineral levels in animal health: Diagnostic data. Sherpa International p 240

Tjel JC, Hovmand MF (1978) Metal concentrations in Danish arable soils. Acta Agric.Scandinavia. 28: 81-89

Treble RG, Thompson TS (1998) Trace metals in moose (alces alces). Bull Environ Contam Toxicol 60: 531-537

Vahteristo L, Lyytikäinen T, Venäläinen, E-R, Eskola M, Lindfors E, Pohjanvirta, R, Maijala R (2003) Cadmium intake of moose hunters in Finland from consumption of moose meat, liver and kidney. Food Add Contam 20: 453-463

Venäläinen E-R, Kilpi M, Hirvi T, Hallikainen A (1999) The Effects of Chemical Contamination Sources on Food Control in Finland; A Regional Comparison of Cadmium and Lead Concentrations in Cattle and Swine. Research Notes 1/1999 National Food Administration, Helsinki

WHO (2001) Evaluation of Certain Food Additives and Contaminants. Fifty-fifth Report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series 901, Geneva