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Biomonitoring of Cadmium in Cattle, Pigs and Humans

Ing-Marie Olsson

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

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Abstract

For the general non-smoking population food, and especially cereals, is the main source of exposure to cadmium (Cd), a nefrotoxic element. Cd levels have increased in arable soils during the last century. In the present thesis Cd exposure was studied in livestock and humans, and different indicators were evaluated for biomonitoring of Cd.

Sampling of the outer part of cattle and pig kidney cortex was the optimal sampling technique to detect small differences between groups. Organically raised cows had lower levels of Cd in kidney, liver and mammary tissue than conventionally raised cows. The lower levels in the "organic" cows may be explained by a lower input of Cd to the soil, and lower levels in roughage, different feed composition, or a lower bioavailability of Cd in the feed. Long-term studies in well-defined systems are required to clarify if organic farming can lower the amount of Cd reaching the food chain.

Cd was followed in the chain from soil via crops and feed, to pig blood and kidney and human blood and urine. The levels of Cd in pig kidney were significantly related to the levels in feed, however, there was no relationship to the locally produced cereals, the main ingredient in the feed. Thus, Cd in pig kidneys did not reflect available Cd in the local environment. The Cd content in non-locally produced feed ingredients constitutes an external source of Cd to the local circulation via excretion in feces and application of manure to arable soils.

Food of vegetable origin contributed the major part (83%) of the human Cd exposure. The contribution of locally produced food to the total Cd intake was relatively low and varying. The dietary intake of Cd was higher in males than females living at the same farm, but the women had 1.8 times higher blood Cd (BCd) and 1.4 times higher urinary Cd (UCd) levels than the men. Cd levels in kidneys from pigs, fed locally produced cereals, could not be used to predict BCd and UCd in humans. However, males living in areas with low soil-Cd had lower UCd than the other men, suggesting some local influence. Dietary Cd was not correlated with BCd or UCd. The higher female BCd and UCd are probably explained by higher absorption due to low iron status. BCd and UCd both increased with age and were higher in former-smokers than in never-smokers. Even at the relatively low exposure levels in this study there was an indication of effect on a biochemical marker (β_2-microglobulin-creatinine-clearance) of renal function, an effect that remained also when age was allowed for.

Keywords: bovine, couples, environment, food chain, metallothionein, monitoring, porcine, quality control, sustainable, zinc.

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När du kommer till en trång plats
och allt går dig emot
tills det ser ut som du inte kan hålla ut
en minut längre,
ge aldrig upp då,
för det är den plats och tid
då floden vänder.

Uthållighet är
när händer och fötter
fortsätter arbeta,
fastän huvudet säger
att det är omöjligt.

Birgitta Yavari-Ilan (1944-)

Abstract

Olsson, I.-M. 2002. Biomonitoring of cadmium in cattle, pigs and humans. Doctor's dissertation.

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Keywords: Monitoring, environment, sustainable, food chain, couples, bovine, porcine, zinc, metallothionein, quality control.

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Papers discussed

The thesis is based on the following papers, which will be referred to in the text by their Roman numerals (I-IV):

I Olsson, I.-M. and Oskarsson, A. Sampling of kidneys from cattle and pigs for cadmium analysis. Analyst, 2001, 126:114-120.

II Olsson, I.-M., Jonsson, S. and Oskarsson, A. Cadmium and zinc in kidney, liver, muscle and mammary tissue from dairy cows in conventional and organic farming. Journal of Environmental Monitoring, 2001, 3:531-538.

III Lindén, A., Olsson, I.-M., Bensryd, I., Lundh, T. and Oskarsson, A. Monitoring of cadmium in the chain from soil via crops and feed to pig blood and kidney. Submitted.

IV Olsson, I.-M., Bensryd, I., Lundh, T., Ottosson, H., Skerfving, S. and Oskarsson, A. Cadmium in blood and urine - impact of gender, age, dietary intake, iron status, and former smoking - association with renal effects. Submitted.

Contents

Abbreviations

ANOVA	analysis of variance
Alb-Crea-clearance	albumin-creatinine-clearance
BCd	blood cadmium level/s
β_2- Crea-clearance	urinary-serum-β_2-microglobulin-creatinine-clearance
Cd	cadmium
CdMT	cadmium-metallothionein-complex
DM	dry matter
FAAS	flame atomic absorption spectrophotometry
FFQ	food frequency questionnaire
GFAAS	graphite furnace atomic absorption spectrophotometry
ICP-MS	inductively coupled plasma mass spectrometry
LQS	laboratory quality sample
MRL	maximum residue limit
MT	metallothionein
PCA	principal component analysis
PTWI	provisional tolerable weekly intake
RB	reagent blanks
RBP	retinol binding protein, synonymous with α_2-microglobulin
RSDr	relative repeatability standard deviation
S-Alb	serum albumin
S-β_2	serum-β_2-microglobulin
SD	standard deviation
U-Alb	urinary albumin
U-β_2	urinary-β_2-microglobulin
UCd	urinary cadmium level/s
U-pHC	urinary protein Hc, synonymous with α_1-microglobulin
µg/kgBw/w	µg per kg body weight and week
Zn	zinc

Conversion factors for Cd concentrations

Molecular weight of Cd 112.41 g/mol

1 µg Cd/l ≈ 8.9 nmol Cd/l

1 nmol Cd/l ≈ 0.112 µg Cd/l

Molecular weight of creatinine 113.12 g/mol

1 µg Cd/g creatinine ≈ 1.0 µmol Cd/mol creatinine ≈ 1.0 nmol Cd/mmol creatinine

1 nmol Cd/mmol creatinine ≈ 0.99 µg Cd/g creatinine

Introduction

Cadmium (Cd) is a toxic metal with increasing concentration in arable soils, despite measures taken during the last two decades to reduce the pollution (Hedlund *et al.*, 1997). A balance between input and output of environmental pollutants, such as Cd, in arable soils is one goal for a sustainable production of food (Eriksson, 2000a; MAT21, 2000). Acidification makes Cd more available to plants (Öborn *et al.*, 1995); thus the risk of increasing Cd levels in the food chain is obvious. For the general non-smoking population food is the main source of exposure to Cd (Elinder *et al.*, 1978). Groups of the general population are exposed to Cd levels that put them at risk of developing adverse health effects due to the accumulation and long biological half-life of Cd in the kidney (WHO, 2001a). The uptake of Cd in plants, animals and humans is influenced by a great number of factors. Thus, it is important not only to know the actual concentration of Cd in soil, feed and food, but also to find out the fraction that can be taken up by the organism.

Background

Cd is found at low concentrations in the Earth's crust, often associated with lead, zinc, and phosphates (Schäfer *et al.*, 1999). During the last century the industrial use of Cd has increased and with it the introduction of Cd into the biosphere. The world consumption of Cd ranges between 18,000 and 21,000 ton/year, the main part (about 60%) today being used for batteries (Anonymous, 2000). Cd is also used in alloys and as anticorrosive, pigments, and stabilizer in plastics. Since 1982 this use of Cd is prohibited in Sweden. Cd pollution of the environment originates from metal industries (*e.g.* steel- and iron-industries), combustion of fossil fuels, waste incineration, and production and use of phosphate fertilizers (Hedlund *et al.*, 1997). A disease called itai-itai (ouch-ouch) was diagnosed in 1946 by Dr Noburo Hagino, Japan. In 1961 Dr Hagino and his co-workers presented their hypothesis that the disease was caused by environmental Cd pollution from a lead and zinc mine, resulting in high levels in rice, the staple food in the area (Umemura, 2000). The increasing Cd levels in the agricultural systems (Andersson and Bingefors, 1985; Andersson, 1992; Petersson-Grawé *et al.*, 1997) are worrying as they increase the risk of elevated Cd levels in the food chain.

Cadmium in soils and plants

The level of Cd in the soil depends on the soil parent material, weathering processes and anthropogenic contributions from atmospheric deposition, fertilizers, manure, liming, and sewage sludge. Towards the end of the 1990s a decline of Cd in forest topsoil could be seen in Sweden and also indications of lower wet and dry deposition (Hedlund *et al.*, 1997). In arable soils, however, the situation is not so encouraging. An increase of about 30% of Cd in arable soils

has been seen during the last century (Andersson, 1992). In the early 1990s the yearly increase was 0.16-0.26% depending on geographical location and crop rotation. The increasing rate of Cd in the arable soils has slowed down to approximately 0.1% per year, but the levels are still increasing (Hedlund *et al.*, 1997). The average levels of Cd in Swedish arable soils in remote areas are 0.17 mg/kg dry matter (DM) (Bergbäck and Johansson, 1996), whereas the average for the whole country is 0.26 mg/kg DM (range 0.04-2.93) (Eriksson *et al.*, 1995). Organic farming has rules about restricted use of mineral fertilizers. This makes it interesting to compare the organic and conventional farming for differences in Cd levels. The application of Cd to arable soil is restricted to a total of 1 g per hectare and year from all sources on an average over five years by the Certification Organization for Organic Production (KRAV, 2001).

Of the total amount Cd deposited in Sweden, 90% originates from sources outside the country. Thus, Cd is an environmental pollution problem that requires international agreements on strategies for preventive actions. As Cd levels in mineral fertilizers have been reduced, the major part (approximately 80%) of the contribution to arable soils in southern Sweden today comes from atmospheric deposition, on farms without livestock (Eriksson, 2000). Skåne, the southernmost province of Sweden, is an agricultural area with a long history of intensive farming. Soils in Skåne are above average for Cd concentrations compared to other parts of Sweden. An investigation of winter wheat also shows that arable soils in Skåne have a higher plant availability of Cd than soils from other parts of the country (Eriksson and Söderström, 1996).

Compared to other toxic metals Cd is relatively easily taken up in plants. The uptake is influenced by a number of factors in the soil, *e.g.* clay and organic matter, cation exchange capacity, micro- and macronutrients (Zn, Cu, Mn, Fe, Ca, NH4, PO4, K), moisture content, use of fertilizers, total amount of Cd, and pH (Haghiri, 1974; Hedlund *et al.*, 1997; McLaughlin *et al.*, 1999). Acidification increases the amount of Cd reaching the crop; the plant species and cultivars also influence the degree of Cd uptake (Öborn *et al.*, 1995). Of the cereals grown in Sweden wheat accumulates most Cd, followed by oats, with barley and rye accumulating considerably lower levels (Jorhem *et al.*, 1984; Eriksson *et al.*, 2000; Eriksson, 2001). High concentrations of Cd have been reported in potatoe protein, soybean meal and rapeseed meal used as ingredients in animal feed (Lindén *et al.*, 1999, 2001). The crops grown on arable soils are used for feeding food-producing animals and for direct human consumption.

Dietary exposure in man

Cereals are the main source of dietary Cd exposure in humans. In the western diet more than 75% of the total dietary Cd intake originates from vegetable food, with the highest contribution from cereals (WHO, 2001b). Major contributors to Cd intake are also potatoes and carrots. High concentrations of Cd are found in kidneys and liver from pigs and cattle, some mushrooms and some seeds (*e.g.* sunflower, poppy, and linseeds) and hepatopancreas in shellfish. However, due to a low consumption of these food items, their contribution to the total Cd intake is

low, except in extreme consumers. Thus, the most effective way to reduce the Cd exposure in the non-smoking population would be to reduce Cd concentrations in cereals, roots and tuber vegetables.

Not much is known about the bioavailability of Cd in different foods. Studies have shown that the availability differs *e.g.* depending on the speciation of Cd in respective food item and on interactions with other nutrients (Lind *et al.*, 1995, 1998; Brzóska and Moniuszko-Jakoniuk, 1998; Chan *et al.*, 2001; Reeves *et al.*, 2001). The Cd levels in food have not been regulated in Sweden, but as of April 5, 2002, maximum levels in several food products are regulated within the European Union (EU) (EEC, 2001; Petersson-Grawé, 2001). The maximum levels are 50, 500, 1000, 100 and 100 µg/kg for meat, liver, kidney, cereals (excluding bran, germ, wheat grain and rice, that have a maximum level of 200 µg/kg) and potatoes, respectively. These levels are approximately 50 times above the current average levels found in Swedish meat, 7 to 25 times above those reported in liver, 3 to 9 times those in kidney, 1.5 to 6 times those in cereals and cereal products, and 4.5 to 6 times higher than those in potatoes and roots (Jorhem and Sundström, 1993). Drinking water has a threshold limit of 5 µg Cd/l (SLV, 1989). Reported average weekly intake from mixed diets in Sweden ranges from 63-84 µg/week (Slorach *et al.*, 1983; Becker and Kumpulainen, 1991; Berglund *et al.*, 1994; Vahter *et al.*, 1996; Jorhem *et al.*, 1998). The exposure in Sweden for the average consumer is in the lower range of internationally reported intake levels (WHO, 2001b).

Kinetics and metabolism

Cd exposure can occur through inhalation and ingestion. The absorption of Cd that reaches the alveoli in the lungs is up to 50% (ICPS, 1992). The absorption in the gastrointestinal tract is much lower, from tenths of a percent up to 20% depending on nutritional status and on chemical and animal species (Neathery *et al.*, 1974; Flanagan *et al.*, 1978, 1980; Sasser and Jarboe, 1980). Absorption of ingested Cd is also affected by the dose, frequency of exposure, age of the animal/individual, pregnancy, lactation, and interactions with various nutrients in the gastrointestinal tract (Groten *et al.*, 1991; ICPS, 1992; Goyer, 1995; Walter *et al.*, 1998). Animal experiments have shown that young individuals have a higher gastrointestinal absorption than older (Sasser and Jarboe, 1980; ICPS, 1992; Lee *et al.*, 1996). A low iron status of the individual enhances Cd absorption in the gastrointestinal tract (Flanagan *et al.*, 1978; Berglund *et al.*, 1994; WHO, 2001b).

Intracellular Cd induces synthesis of metallothionein (MT), and Cd is subsequently bound to MT, forming a cadmium-metallothionein-complex (Cd-MT). Metallothioneins are a group of low-molecular-weight, cystein-rich proteins that can bind up to seven metal ions (Klaassen *et al.*, 1999; Nordberg and Nordberg, 2000). Zinc, cadmium, mercury and copper bind to MT, with the lowest affinity for zinc and the highest for copper (Nordberg and Nordberg, 2000). The function of MT is not fully understood, but there are indications of multiple functions, *e.g.* as a storage protein for zinc (Zn), a free radical scavenger, and moreover it protects against Cd toxicity (Klaassen *et al.*, 1999). After

absorption in the liver Cd induces formation of MT that binds Cd, and Cd-MT is slowly released from the liver into the blood and transported to the kidneys. In the kidneys Cd-MT is filtered through the glomeruli and reabsorbed into the proximal tubular cells. After degradation in lysosomes, the released Cd ions induce synthesis of MT, and Cd is stored bound as Cd-MT (Nordberg and Nordberg, 2000). As long as the proximal tubule cells' capacity to re-synthetize MT is not exceeded Cd will not be deleterious. Studies on MT-null mice have shown that Cd^{2+} is the species causing the actual injury (Liu *et al.*, 1998). The half-life for Cd in human kidney is 10-30 years (ICPS, 1992).

Biological markers of Cd exposure

The most commonly used biomarkers of Cd exposure in humans are Cd in blood and Cd in urine. The blood Cd level (BCd) reflects current exposure, but it is also influenced by the body burden of Cd (Welinder *et al.*, 1977; Järup *et al.*, 1998b). The half-life of Cd in blood is 2-3 months, related to the lifespan of the red blood cell. Non-smoking, non-occupationally exposed persons usually have a BCd between 0.89 and 7.1 nmol/l; smoking usually results in considerably higher levels, 8.9-36 nmol/l (for conversion factors, see page 10).

Urinary Cd (UCd) reflects the body burden of Cd as long as there is no renal dysfunction (Börjesson *et al.*, 1997) and UCd is proportional to the kidney Cd concentrations (Järup *et al.*, 1998b). The UCd excretion increases with age (as the body burden increases) till approximately the age of 50-60 years, when the kidney function starts to decline. At this stage the Cd excretion initially increases, but eventually becomes lower as the Cd content of the kidney decreases. Non-smoking, non-occupationally exposed persons usually have a UCd between 0.02 and 0.7 nmol/mmol creatinine, smokers have concentrations about twice as high (Järup *et al.*, 1998b).

The best measure of the individual Cd load would be Cd in kidney cortex. Kidney Cd can be measured in vivo by non-invasive techniques, x-ray fluorescence and neutron activation (Nilsson *et al.*, 1995; Börjesson *et al.*, 1997). The methods are useful but do have limitations, and BCd and UCd are still by far the most common biomarkers used for Cd exposure assessment. In vivo measurements (x-ray fluorescence) of kidney cortex Cd levels in Sweden have shown that non-smokers have a Cd concentration of approximately 8-18 µg/g (age 25-71 years) and smokers 28 µg/g (age 27-65 years) (Nilsson *et al.*, 1995, 2000). In studies of Swedish autopsy and biopsy material (age span 7-92 years old) levels between 0.5 and 46 µg/g have been found (Friis *et al.*, 1998; Barregård *et al.*, 1999). In the autopsy material (Friis *et al.*, 1998) smokers had levels between 2.8 and 39 µg/g, ex-smokers 2.9-25 and the never-smokers between 0.9 and 22 µg/g.

Kidney anatomy

Kidneys have a gradual change of dominant cell types from cortex to medulla. Due to the anatomy of the kidney and that Cd accumulates mainly in the proximal tubule cells (Friberg and Odeblad, 1957; Berlin *et al.*, 1964; Dorian *et al.*, 1992) a

gradient of Cd is seen through the kidney with the highest levels in cortex and the lowest in the medulla. This gradient has to be considered when sampling kidney tissue for Cd analysis. There are gross- and micro-anatomical differences between kidneys of different species (Henrikson, 1993), *e.g.* horse, cow, pig, and sheep kidney (Figure 1). The bovine kidney has distinctly demarcated lobuli (Figure 1b), a so-called multipyramidal/multilobed kidney, as opposed to the unipyramidal kidney of *e.g.* dogs, horses (Figure 1a) and sheep (Figure 1d). The kidneys of pigs (Figure 1c) and man are of the multipyramidal type but with an externally fused cortex. The cortex contains mainly glomeruli, proximal and distal tubules. The medulla contains mainly the thin tubules and collecting ducts.

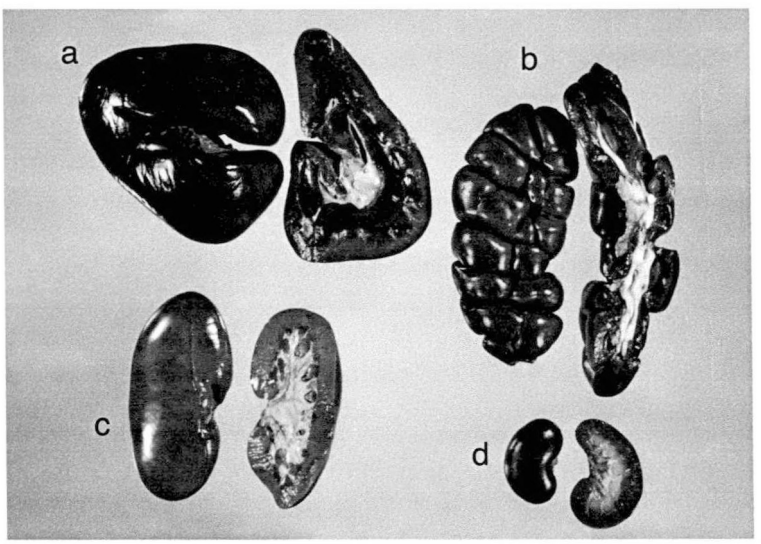

Figure 1. Kidneys from **a**) horse, **b**) cow, **c**) pig, and **d**) sheep; whole kidney to the left and longitudinally sectioned kidney to the right. Foto: Bengt Ekberg © SVA

Descriptions of the sampling procedure of kidney for Cd analyses vary in the literature. Some just state that kidneys have been sampled, others give detailed descriptions of which parts of the kidney have been sampled and the preparation technique used (Kramer *et al.*, 1983; Fitzgerald *et al.*, 1985; Vos *et al.*, 1987; Antoniou *et al.*, 1995; Doganoc, 1996; Lee *et al.*, 1996; Petersson-Grawé *et al.*, 1997; Koh *et al.*, 1998). Scarce information about the distribution of Cd within kidneys of domestic animals and unclear descriptions in the literature about the sampling techniques of kidneys for Cd analysis make it almost impossible to evaluate and compare data.

Adverse health effects at low-level exposure

An early sign of Cd induced kidney dysfunction is the urinary excretion of low-molecular-weight proteins; *e.g.* protein Hc (pHC, synonymous with α_1-microglobulin), retinol binding protein (RBP, synonymous with α_2-microglobulin), β_2-microglobulin, and N-acetyl-β-glucoseaminidase (NAG). In its early stages

tubular proteinuria itself is not accompanied by any specific histological changes, and if exposure is significantly reduced the proteinuria is reversible (Hotz *et al.*, 1999). A reduction of exposure is not easy to achieve for the general non-smoking population, thus, tubular damage of the general population will probably lead to a continued deterioration of kidney function. Early histopathological changes are limited to proximal tubular epithelial cell degeneration. This is, however, followed by cellular atrophy, interstitial fibrosis and glomerular sclerosis (WHO, 2001b). The damage causes decreased reabsorption capacity, and hence a loss from the body of otherwise reabsorbed solutes. Such solutes may include MT with firmly bound Zn and Cu, and a range of low-molecular-weight compounds, such as glucose, phosphate, Ca, amino acids, β_2-microglobulin, and retinol bindning proteins (RBP) (Satarug *et al.*, 2000). The decreased kidney function may induce formation of kidney stones, and reduced glomerular filtration (Järup *et al.*, 1998b). Osteoporosis (Carlsson and Lundholm, 1996; Järup *et al.*, 1998a) and decreased immune response with increasing body burden of Cd (Ritz *et al.*, 1998) are also reported as effects of chronic low-level exposure in humans. Indirect exposure of rat pups through their dam's milk has resulted in reduced serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels in the central nervous system (Andersson *et al.*, 1997). However, the critical effect of Cd is still considered to be the renal tubular dysfunction (WHO, 2001a).

Dose-response for kidney dysfunction – risk assessment

Dose-response analysis of individual critical kidney concentrations showed that after 45 years of exposure to 200 µg/day (70 kg body weight) a 10% prevalence of proteinuria would occur in the general population. Lowering the exposure to 100 µg/day would cause a 2% prevalence of proteinuria in the general population. The World Health Organization (WHO) adopted a provisional tolerable weekly intake (PTWI) based on the assumptions that kidney cortex should not exceed 50 µg/g after 50 years of dietary Cd intake. Assuming an absorption rate of 5% and a daily excretion of 0.005% of body burden, the PTWI was set to 7 µg/kg body weight and week (µg/kgBw/w) (WHO, 1989).

Buchet *et al.* (1990) presented the results of a large epidemiological study of the general population in Belgium (n=1699, age 20-80 years) where they showed a slight renal dysfunction in the form of increased excretion of RBP, NAG, β_2-microglobulin, amino acids, and calcium in about 10% of the individuals having urinary Cd levels of 18-36 nmol/24 hours (approximately 8.9-18 nmol/mmol creatinine for males and 18-36 nmol/mmol creatinine for females). This corresponds to approximately 50 µg/g Cd in the kidney. The kidney Cd levels reported in Sweden (Friis *et al.*, 1998; Barregård *et al.*, 1999) show that there are individuals that are very close to the levels where deleterious effects on the kidney function can be expected.

At the reevaluation of the PTWI by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2001, the previously established PTWI was maintained. It was, however, acknowledged that the safety margins are small and that a proportion of the general population may be at increased risk of Cd-induced

tubular dysfunction. High-risk groups are people with iron deficiency, renal disease and diabetes mellitus (WHO, 2001a). JECFA also pointed out the need of further research of the relationship between dietary intake and urinary Cd excretion in the general population and in high risk groups. Bioavailability of Cd in food and factors that effect bioavailability should also be studied, as should the relationship between biomarkers of exposure and renal tubule dysfunction, and the relationship between renal dysfunction and clinical disease and mortality (WHO, 2001a).

Biological monitoring of Cd

Various organisms can be used to track environmental pollution (Whitfield, 2001). The ideal species to use should retain the contaminant in correlation to exposure levels, be frequently abundant, have a limited homerange, and be large enough to provide enough material for analysis. Depending on the purpose of the monitoring program, the monitoring species used can require different properties. A species' insensitivity to a pollutant can be used to give a measure of accumulation, while a sensitive species can indicate the pollutant by being reduced or even absent. A species with absorption and metabolism/kinetics matching other relevant species can be used to estimate the bioavailability of a pollutant (Frank, 1986; Boening, 1999; Beeby, 2001). Different organisms for biomonitoring of metal pollution have been tested, *e.g.* mussels, eels, fish and river crabs for aquatic environments (Linde *et al.*, 1998; Boening, 1999; Braune *et al.*, 1999; Schuwerack *et al.*, 2001; Whitfield, 2001), eggs from water birds for wetlands (Goutner *et al.*, 2001), squirrels and rats related to soil and vegetation Cd levels (Sharma and Shupe, 1977), and red-fox for comparing rural and urban sites (Dip *et al.*, 2001). Red deer *(Cervus elaphus)* and roe deer *(Capreolus capreolus)* have been used to estimate effects of metal pollution around smelters (Pokorny and Ribaric-Lasnik, 2000; Parker and Hamr, 2001). Moose tissue levels of Cd have been related to spatial differences (Frank *et al.*, 1981; Crête *et al.*, 1987; Glooschenko *et al.*, 1988). Selinus *et al.* (1996) used species from different trophic levels (aquatic roots, mosses and tissues from moose) to monitor Cd in different regions. Even though the perfect species for monitoring purposes does not exist, available species can be used provided there is good knowledge about the chosen species (feeding and other habits, absorption and excretion of the pollutant, etc.) and that the correlations between tissues and source levels and relations to other species are established. This makes it possible to evaluate bioavailability of pollutants for other species exposed to the same sources (Holm, 1993; Beeby, 2001; Cajaraville *et al.*, 2000).

Domestic animals usually spend most of their lives in one region and are fed mainly locally produced feed, giving reason to assume that they would be suitable as indicators of the bioavailable part of pollution within regional agricultural systems (Swarup and Dwivedi, 1998; Lindén *et al.*, 2001). Tissues from pigs and cows that are slaughtered for food production could be used for monitoring of bioavailable Cd. Cattle tissue has been used to evaluate exposure in polluted areas (Milhaud and Mehennaoui, 1988; Farmer and Farmer, 2000) and for regional

comparisons (Lopez-Alonso *et al.*, 2000a, 2000b; Miranda *et al.*, 2001). Temporal changes in pig kidney Cd have also been reported (Petersson-Grawé *et al.*, 1997). Sapunar-Postruznik *et al.* (2001) used the regular official monitoring to follow up cases that exceeded the maximum residue limits (MRL) and take measures to remove sources of contamination.

The movement of Cd within the biosphere is complex, as it occurs at different levels and is influenced by several factors. The flow of Cd from soil to feed and food, animals and man, and the adverse effects caused at different levels within the ecosystem require interdisciplinary cooperation to elucidate and to find ways to deal with Cd contamination in a sustainable way.

Aims of the Thesis

The aims of this thesis were:

- to develop a technique for sampling of kidneys from cattle and pigs for biomonitoring of Cd exposure.
- to compare the levels of Cd in tissues from dairy cows in conventional and organic production.
- to study Cd in the chain from soil, via crops and feed, to pig blood and kidney and investigate the relationship to human Cd exposure in the same environment.
- to evaluate the possibility to use tissues from pigs as indicators of bioavailable Cd in the environment.
- to study low-level Cd exposure and renal effects in men and women living in the same environment.

Materials and Methods

Detailed descriptions of each study are given in the respective paper. Approval of the studies has been given by the Ethical Committees for Animal Experiments in Umeå and Lund, Sweden (Papers II and III) and by the Ethical Research Committee at Lund University (Paper IV).

Cadmium distribution within the kidney

Paper I

Pig and cow kidneys were purchased from the abattoir in Uppsala, Sweden. The kidneys were freed from visible fat, large vessels and connective tissue. One kidney each of 4 cows were sliced and each slice homogenized (Figure S1[1], Paper I) and then frozen and stored at -20°C. The lobules of the other kidney from these individuals and four additional cows were separated and frozen for later analyses of different parts of the lobules. One kidney each of 5 pigs were frozen and stored after homogenization, either of the whole kidney (2 pigs) or of half kidneys (3 pigs). The remaining kidneys from these individuals and from another 3 pigs were frozen whole and stored until analyses. The second kidney from the latter three animals was analyzed fresh.

Study location and collection of material

Paper II

Cows in conventional and organic farming were studied in order to evaluate the possibility to use cows to monitor bioavailable Cd in the environment. At the Öjebyn Research Station, Swedish University of Agricultural Sciences (SLU), Öjebyn (latitude 65°N, longitude 21°22'E), Sweden, a project on milk production in conventional and organic farming has been running since 1990 (Fagerberg *et al.*, 1996). In both systems a six-year crop rotation was practiced. In the organic system no mineral fertilizers were used. Fifty cows in each system were stabled in two cowsheds, the manure and the urine from each system were kept separately and returned to the respective area under cultivation. The feed consisted of roughage, in the form of silage, and concentrate combined to fulfill the recommendations given by Spörndly (1999). Roughage was given *ad libitum* to the cows in the organic system, and they received concentrate according to milk production, but never more than 50% of the daily dry matter (DM) intake. The "organic" cows consumed on average 10.8 kg DM roughage and 7.8 kg DM concentrate per day and the "conventional" cows consumed 8.4 and 10.8 kg DM

of roughage and concentrate, respectively (Simon Jonsson, SLU, personal communication, 2001). Cows sent to slaughter from the two systems from December 1995 till October 1999 were sampled by veterinarians at the abattoirs in Luleå and Piteå. Out of the 113 cows sent to slaughter 79 (70%) were sampled, and totally 79 kidney, 79 liver, 78 muscle and 74 mammary tissue samples were collected. Of these cows 12 were born before 1990, thus entering the conventional or organic system after a period of their lives in another system. Data on age, age at first calving, days as dry cow, number of calves, days since last calving, total milk production, and energy corrected milk (ECM; Spörndly, 1999), were collected for each individual in the study group (Table 1 and S2[1], Paper II).

Papers III and IV

Participants were recruited by a random selection of 800 addresses from a database, at Statistics Sweden (SCB), of approximately 2400 growing/finishing pig producers in the province of Skåne. Skåne is an area with intensive farming and naturally relatively high Cd levels in soil, as well as atmospheric deposition from central Europe. A first questionnaire with inquiries about the farm, the pig production, the resident's smoking and food habits was sent to the farmers in February 1998. Of the 800 questionnaires, 533 (67%) were returned. Of these 465 had complete answers and 224 (48%) volunteered to participate in a more detailed study. From the latter group farms were selected on the basis of the answers given in the questionnaire. The selection criteria were a) production at the farm of more than 50% of the feed used, b) both man and woman on the farm willing to participate and c) both being non-smokers. The farms included in the study totalled 49. Five to ten pigs per farm were blood sampled and tattooed for later identification and sampling of their kidneys at the abattoirs (in Helsingborg, Kävlinge, Ugglarp, Kristianstad, and Kalmar). Two samples of pig feces were collected in the pen where the marked pigs were held at each farm. The freshest droppings were sampled. Samples of water, crops, feed and feed components, and straw were also collected. Water was sampled from the taps in the kitchen and the stable. The Cd concentrations in soils were interpolated from a mapping of arable soils in Skåne (Eriksson *et al.*, 1997) by the geographical location of the farm buildings.

One hundred and nine persons volunteered for sampling. After exclusion of four persons due to smoking or illness, the final study population consisted of 105 persons, 48 females and 57 males. The participants answered a detailed questionnaire about food consumption, current health status and former smoking habits, and blood and urine samples were collected. Based on number of pieces or volume, and frequency of consumption of different food items the weekly Cd intake was calculated using Swedish data, except for coarse-grained whole-meal rye bread and butter where German data were used (Jorhem *et al.*, 1984, 1993;

[1]S in a figure or table reference stands for "supplementary information" which can be found on the internet at the web-addresses given in the respective papers.

Table 1. Food items in food groups with weight transformation factors and cadmium concentration.

FOOD GROUPS Food item	Weight transformation factors	Cadmium concentration (mg/kg wet weight)	Reference
1. BREAD			
White	38.4 g/slice	0.031	Jorhem *et al.* 1984
Sifted rye flour	33 g/slice	0.034	Jorhem *et al.* 1984
Wholemeal	31 g/slice	0.021	Müller *et al.* 1996
Crisp	13 g/slice	0.017[a]	Jorhem & Sundström 1993
2. CEREALS & RICE			
Oat/rice/wheat porridge	13 g flakes/100ml	0.031[b]	Jorhem *et al.* 1984
Rice/rye porridge	13 g flakes/100ml	0.015[c]	Jorhem *et al.* 1984
Cereals	13 g/100ml	0.088	Müller *et al.* 1996
Müsli	38 g/100ml	0.032	Jorhem *et al.* 1984
Rice	15 g/100ml[d]	0.031	Jorhem *et al.* 1984
Brown rice	23 g/100ml[d]	0.025	Jorhem *et al.* 1984
Pasta	17 g/100ml	0.046	Jorhem *et al.* 1984
3. SEEDS & CHOCOLATE			
Sunflower kernels	9 g/tablespoon	0.38	Jorhem & Sundström 1993
Poppy seeds	3.6 g/ teaspoon	0.109[e]	Jorhem & Sundström 1993
Linseeds	10 g/tablespoon	0.42	Jorhem & Sundström 1993
Dark chocolate	-	0.15	Jorhem & Sundström 1993
4. POTATOES & ROOTS			
Potatoes	-	0.017	Jorhem & Sundström 1993
Carrots	-	0.022	Jorhem & Sundström 1993
Other roots	-	0.0305[f]	Jorhem *et al.* 1984
5. VEGETABLES	-	0.016[g]	Jorhem *et al.* 1984 and Jorhem & Sundström 1993
6. MUSHROOMS			
Agaricus hortensis	-	0.011	Jorhem & Sundström 1995
A. augustus	-	14	Jorhem & Sundström 1995
A. campestris	-	0.0275[h]	Jorhem & Sundström 1995
Other mushrooms	-	0.2[i]	Jorhem & Sundström 1995
7. FRUITS & BERRIES			
Fruits	112 g/piece	0.0025[j]	Jorhem et al 1984 and Jorhem & Sundström 1993
Berries	-	0.004[k]	Jorhem & Sundström 1993
8. FATS & OILS			
Margarine	-	0.002	Jorhem *et al.* 1984
Oils	90 g /100ml	0.001	Jorhem *et al.* 1984
9. MILK & MILK PRODUCTS			
Milk, yoghurt	103 g/100ml	0.001	Jorhem *et al.* 1984
Cream	99 g/100ml	0.0027[l]	Jorhem *et al.* 1984 and Müller *et al.* 1996
Cheese	-	0.006	Jorhem *et al.* 1984
Butter	-	0.0044	Müller *et al.* 1996
10. MEAT, FISH & EGGS			
Meat	-	0.001	Jorhem & Sundström 1993
Fish	-	0.003[m]	Jorhem & Sundström 1993
Eggs	-	0.001	Jorhem *et al.* 1984
11. OFFALS			
Liver	-	0.0445[n]	Jorhem & Sundström 1993
Kidney	-	0.23[n]	Jorhem & Sundström 1993
12. SHELLFISH			
Shrimps	6 g/piece	0.028 (canned) 0.079 (meat) 0.27 (all edible parts)[o]	Jorhem *et al.* 1984
Crayfish	5 g/piece	0.009 (meat) 0.083 (all edible parts)[p]	Jorhem *et al.* 1994
Crabs	125g/half	0.081 (meat) 3.4 (all edible parts)[q]	Jorhem *et al.* 1984

Table 1, contd

FOOD GROUPS Food item	Weight transformation factors	Cadmium concentration (mg/kg wet weight)	Reference
13. COFFEE, TEA & JUICE			
Coffee	100 g/100ml	0.001	Jorhem *et al.* 1984
Tea	100 g/100ml	0.0005	Jorhem *et al.* 1984
Juice	104 g/100ml	0.001	Jorhem *et al.* 1984

[a]Rye flour used for all kinds of crisp bread.
[b]Oat flakes.
[c]Average for rye flakes, barley flakes and barley flour.
[d]Grams unboiled per deciliter boiled .
[e]Average of blue and white poppy seeds.
[f]Average of beet roots, parsnips, radishes, Swedish turnips.
[g]Average of Chinese cabbage, green beans, lettuce and green peas (Jorhem & Sundström, 1993) and red cabbage, Savoy cabbage, white cabbage, Brussels sprouts, cauliflower, celery, cucumber, leek, spinach, sweet pepper, and tomatoes (Jorhem *et al.*, 1984).
[h]Average of *A. bisporus* and *A. augustus*.
[i]Average of all analysed species except the *Agaricus* spp. (17 species).
[j]Average of apples, oranges, bananas, peaches, prunes, and pears.
[k]Average of blueberries, blackcurrent berries, lingon berries, raspberries and strawberries.
[l]Average of milk (Jorhem *et al.*, 1984) and butter (Müller *et al.*, 1996).
[m]Average of 11 species, values <0.001, were calculated to be 0.0005 mg/kg.
[n]Average of cattle and pig.
[o]Assumed 6 g meat and 0.3 g hepatopancreas with a Cd concentration of 4.0 mg Cd/kg.
[p]Assumed 77% meat and 23% hepatopancreas with a Cd concentration of 0.33 mg Cd/kg.
[q]Edible parts calculated to 0.85 mg/crab.

Müller *et al.*, 1996). Food items reported to be consumed at least once a month was included. Different food items were grouped into 13 food groups (Table 1).

Sample preparation

The gradients of Cd in the kidneys of cattle and pigs were studied as described in detail in Paper I. In short, Cd in homogenates, slices of pig kidneys and cattle kidney lobules, as well as cortex, intermediate and medulla zones (Figures 1 and 2 in Paper I) were analyzed to evaluate the effect of the kidney anatomy on the Cd concentration.

For the studies in Papers II and III the outer 50% of the kidney cortex was sampled in order to optimize detection of small differences between groups in Cd concentrations in kidney (Figures 1c and 2c in Paper I). Liver and muscle samples (Paper II) were cut into five slices. The outer layer of the udder quarter was discarded and the remaining central part was cut into five slices (Figure 1 in Paper II). Duplicate samples from the third slice, for all three tissues, were analyzed. Evaluation of the sampling technique was not performed for liver, muscle and mammary tissue as it was for kidney (Paper I). However, pilot studies for all three tissues were performed to evaluate the degree of variation in multiple samples from the same individual. Ten pieces (0.48-0.68 g) from one liver slice, and 8 pieces (3.9-4.1 g) from one muscle slice, 10 pieces (3.7-4.5 g) of mammary tissue

from the second slice from the dorsal end of the udder (slice 2, Figure 1 in Paper II) were sampled and analyzed (for results see "Results and Discussion" below).

Feed, feed components, water, blood and urine samples for element analysis were frozen (-20°C) until analyses (Papers III and IV).

Analytical procedure, quality control and limit of detection

The concentrations of Cd in biological samples are generally very low, thus, contamination of samples at sampling, preparation and analysis is a problem of high concern (Vahter, 1982). To prevent and control this strict routines were used. All chemicals used were of *pro analysi* quality or a higher degree of purity, materials used were checked for leakage of Cd, and all utensils were acid-washed (Papers I, II, III and IV).

Analyses

Animal tissues, feces, crop, feed and feed components (Papers I, II and III): Samples were mineralized by either dry-ashing in a Lenton programmable furnace (Market, Harborough, Leicestershire, UK) or microwave digested in closed Teflon vessels in a microwave labstation (MLS 1200 H MEGA, Milestone, Sorisole, Italy) under control of temperature and pressure. Analysis was done using either flame atomic absorption spectrophotometry (FAAS) with deuterium lamp background correction (Perkin Elmer FAAS 4100, Bodenseewerk Perkin Elmer GmbH, Überlingen, Germany) (Cd and Zn) or graphite furnace atomic absorption spectro-photometry (GFAAS) with Zeeman background correction (Perkin Elmer GFAAS 4100ZL) (Cd) (Papers I, II and III). Linear calibration was chosen as the most suitable method for calibration, based on the analyses of reference samples. With every round of samples duplicate samples of reagent blanks (RB) and reference material (Table 2) were prepared (40 crucibles and 36 vessels per round for dry-ashing and microwave digestion, respectively). The limit of detection was set to 3 standard deviations [SD] of at least 20 RB. Limits of detection for Cd were 6.0 µg/l for dry-ashing-FAAS, 0.32 µg/l for dry-ashing-GFAAS (Paper I) and for microwave-GFAAS 0.24 µg/l (Paper I), 0.17 µg/l (Paper II), and 0.087, 0.096 and 0.070 µg/l for kidney, feed and feces, respectively (Paper III). For Zn the limit of detection was 8.3 µg/l (Paper II).

Our laboratory has regularly participated in the proficiency-testing program of trace elements in food, organized by the Swedish National Food Administration, with a mean Z-score of -0.7 for Cd (n=5) and -0.1 for Zn (n=1) (Jorhem and Merino, 1997; Jorhem and Engman, 1999; Sundström and Jorhem, 1999; Åstrand and Jorhem, 2000, 2001).

In Paper I a laboratory quality sample (LQS) of homogenized bovine kidney was also used for internal quality control. The average Cd concentration for the LQS analyzed by dry-ashing-FAAS was 495±17 µg/kg (n=48) and for microwave-GFAAS 444±14 µg/kg (n=20), with relative repeatability standard deviation (RSDr) (NMKL, 1997) of 2.2 and 2.6% respectively.

Table 2. Reference material used in Papers I – IV.

Material	Certified value	Analyzed	Analytical technique[a]	Paper
BCR[b] 184 lyophilized bovine muscle	13±2 µg/kg	13.6±1.9	MW-GFAAS	I
BCR[b] 185 lyophilized bovine liver	298±25 µg/kg	279±43	MW-GFAAS	II
		299±25	MW-GFAAS	III
BCR[b] 186 lyophilized pig kidney	2.71±0.15 mg/kg	3.1±0.17	DA-FAAS	I
		2.5±0.18	DA-GFAAS	I
		2.7±0.16	MW-GFAAS	I
		2.63±0.13	MW-GFAAS	II
		2.76±0.41	MW-GFAAS	III
Wheat flower GBW8503[c]	31±4 µg/kg	28.2±2.3	MW-GFAAS	III
Water SLRS-2[d]	0.028 µg/l [f]	0.035±0.003	ICP-MS	III, IV
Seronorm[e] 404107	0.7 µg/l [f] (0.67-0.76)	0.65±0.06	ICP-MS	III
		0.80±0.07	ICP-MS	IV
Seronorm[e] 404108	6.4 µg/l [f] (6.3-7.9)	5.98±0.27	ICP-MS	III
		6.17±0.07	ICP-MS	IV

[a]MW-GFAAS = microwave – graphite furnace atomic absorption spectrophotometry, DA-FAAS = dry-ashing - flame atomic absorption spectrophotometry, ICP-MS = inductively coupled plasma mass spectrometry.
[b]Community Bureau of Reference, Brussels, Belgium.
[c]Cereal and Oil Chemistry Institute, Ministry of Commerce, Beijing, China.
[d]Riverine Water Reference Material for Trace Elements SLRS-2, National Research Council, Ottawa, Canada.
[e]Nycomed AS, Oslo, Norway.
[f]Recommended values.

Each round of samples was evaluated on the basis of the RB, the reference samples and the results from the duplicates of each sample. When the coefficient of variation (CV) was >10% or more than 0.003 absorbance seconds (As) between duplicate injections on the spectrophotometers the samples were reanalyzed. The duplicate sample results after calculation of Cd concentration per kilogram tissue were not allowed to differ more than a certain value from each other, as this might indicate contamination of single crucibles or Teflon digest vessels and/or poly-propylene sample tubes. The difference between duplicate samples was not allowed to be more than 8%, 10%, 30%, and 95% for kidney, liver, muscle and mammary tissue, respectively (based on results from the pilot study, see "Liver, muscle and mammary tissue" under "Sampling of tissues from cattle and pigs for Cd analysis" in Results and Discussion). At larger differences new duplicate samples were extracted, prepared and analyzed.

Blood, urine and water (Papers III and IV): Blood, urine and water samples were analyzed for Cd by inductively coupled plasma mass spectrometry (ICP-MS) at the Department of Occupational and Environmental Medicine, University Hospital Lund, Sweden. Samples of human urine and human and pig blood were diluted in duplicates with a dilution reagent. Internal standards, indium (In), bismuth (Bi), and gallium (Ga) were used. The ICP-MS instrument was a PQ2+ from Thermo Elemental (Winsford, Cheshire, UK) with a Gilson 222 autosampler (Gilson, Villiers, France). Sample introduction was in a segmented-flow mode. The samples were analyzed in the peak-jumping mode (3 points per peak, 15 ms dwell time for internal standards, and 75 ms for Cd). Interference corrections were made for ^{114}Cd (corrected for spectral overlap from tin, Sn, measured at m/z 118). For calibration, the procedure in the PQ2+ software was used. One-point calibration curves were obtained using outdated blood or urine from donors. For all sample results, a RB was subtracted. Detection limits for Cd were in water 0.007 µg/l, in pig blood 0.046 µg/l, in human blood 0.059 µg/l and in urine 0.044 µg/l. The accuracy of the analyses was checked against reference materials (Table 2).

Analyses of blood and urinary parameters (Paper IV) were performed at accredited laboratories (Lund University Hospital, Sweden). All urinary parameters were adjusted for creatinine. From serum-albumin (S-Alb), S-creatinine, urinary-albumin (U-Alb) and U-creatinine the albumin-creatinine-clearance (Alb-Crea-clearance) was calculated, as was the β_2-microglobulin-creatinine-clearance (β_2-Crea-clearance), from serum-β_2-microglobulin (S-β_2), S-creatinine, urinary-β_2-microglobulin (U-β_2) and U-creatinine.

Metallothionein (MT) was analyzed by the Cd-hemoglobin affinity (Eaton and Cherian, 1991) and total protein (Hartree, 1972) assays in sub-samples of kidneys from 20 cows, selected to represent a wide range of Cd levels (Paper II).

Statistical methods

Results were tested for normality by Kolomogorov-Smirnoff and the homogeneity of variances tested by Bartlett's test. Depending on the outcome of these tests parameters were log-transformed and re-evaluated. Further analysis was performed by parametric or non-parametric tests as required. Data were evaluated with *t*-tests (unpaired or paired), Mann-Whitney *U* test, Kruskal-Wallis, analysis of variance (ANOVA) (simple and multiple), correlations (Pearson or Spearman) and regression analyses (simple, stepwise and multiple). *Post-hoc* testing was done with Scheffe's test or Games-Howell. The level of significance was set to $p \leq 0.05$. For samples with levels below the formal detection limit the measured values were used in the statistical evaluation in order not to distort means and distributions. The Statview(r) 5.0 software (1998, SAS® Institute Inc., Cary, NC, USA) was used. Data was also evaluated by principal component analysis (PCA) using the multivariate program UNSCRAMBLER (The Unscrambler™, CAMO ASA, Sales & Marketing, Oslo, Norway).

Results and Discussion

Sampling of tissues from cattle and pigs for Cd analysis

Kidney

The purpose of the study in Paper I was to obtain an optimal sampling technique to detect small differences between different groups in Cd concentrations in kidney and to increase the knowledge of Cd distribution within bovine and porcine kidneys. The study showed that there was a gradient of Cd in both cattle and pig kidney, with the highest concentrations in cortex and the lowest in medulla. For cattle kidney two slightly different methods of sampling were used for division into zones, one where a whole lobule was divided into zones (cortex, intermediate, and medulla) (n=4 lobules x 4 animals, individuals 2-5, Table 1 in Paper I) and one where a central slice of the lobule was divided into zones (n=4 x 4, individuals 6-9, Table 1 in Paper I). The latter method was considered the best for sampling specific parts of a cattle kidney. This could be seen as a lower SD for the cortex concentrations (individuals 2-5, *vs.* 6-9, Table 1 in Paper I) and a larger difference between cortex and medulla concentrations (25 times) for the slices (Table 3) than for the lobules (8 times).

Table 3. Cadmium distribution in different parts of cattle and pig kidney relative to the Cd concentration in the medulla and relative to the calculated Cd concentration in homogenate.

Zone	Cattle		Pigs	
	Conc. relative to medulla[a]	Conc. relative to calculated homogenate	Conc. relative to medulla[b]	Conc. relative to calculated homogenate
Cortex	25	1.37	4.4	1.14
Intermediate	14	0.79	3.1	0.78
Medulla	1	0.10	1	0.23

[a]For zones from slices of cattle kidney lobules.
[b]For slices of growing finishing pig kidneys.

What sampling technique one should choose depends on the purpose of the investigation. To detect small differences in renal Cd levels, as is the case in biological monitoring of Cd exposure, a standardized sampling of outer cortex seems to be the optimal method. Analysis of whole kidney tissue homogenate might, on the other hand, be preferable for assessment of Cd intake from consumption of kidney. Homogenization eliminates uncertainty due to uneven distribution of the element, but introduces the risk of sample contamination.

Cattle have a proportionally larger renal medulla than pigs, and the difference in Cd concentrations between kidney cortex and medulla is larger in cattle (25

times) than in pigs (4.4 times) (Table 3). Thus, the sampling technique of cattle kidney is of greater importance for the results of the Cd analysis than the sampling technique for pig kidney. In our study, pig kidney cortex contained about 1.14 times the levels of kidney homogenate (cortices ranging from 49.9-884 µg/kg). In cattle with Cd concentrations in kidney cortices ranging from 252-980 µg/kg, the cortex contained on average 1.37 times the levels of kidney homogenate (Table 3). The variation in Cd distribution in the zones was small among the studied animals. Nevertheless, conversion factors should be used with caution. The dose as well as exposure regimen and length of exposure may also influence the distribution of Cd within the kidney.

Using the data from Paper II as an example can show the importance of the sampling technique. The kidney cortex Cd concentration in the "conventional" cow group was 410 µg/kg and in the "organic" 330 µg/kg. Converting these concentrations on an individual level into homogenates (conversion factor 1.37) gives an average for the groups of 310 and 273 µg/kg, respectively, thus reducing the difference between the groups from 80 to 37 µg/kg. According to the Games-Howell analysis the calculated difference between these homogenates is below the critical difference (48 µg/kg) needed to give a significant difference between the two groups. If homogenates would have been analyzed the difference between the systems probably would not have been found.

The results in Paper I are in line with the findings in other studies on the distribution of Cd within the kidney. Livingston (1972) found the highest levels of Cd in the outer cortex and a gradual decrease towards the renal pelvis in human kidneys. Svartengren *et al.* (1986) reported approximately 1.25 times higher Cd concentration in human kidney cortex than in whole kidney. Scott *et al.* (1987) demonstrated a small intra-kidney variation and a ratio 2:1 for cortex-medulla concentrations also in human kidneys. Schenkel *et al.* (1979) studied pigs and found 1.84 times higher Cd levels in the cortex than in the medulla. This is considerably lower than what we found (Table 3). However, Schenkel *et al.* divided their kidney samples along the cortico-medullary line and, thus, the medulla would have higher, and the cortex lower levels due to the "dilution" of tissue from the "intermediate zone". No difference between the Cd concentrations in the two kidneys from one cow was reported by Lücker *et al.* (1987) using solid micro-sampling. In concordance with our results, they found approximately 1.3 times higher concentrations in the cortex than in the medulla.

Liver, muscle and mammary tissue

The anatomical structures of liver and muscle are more homogenous than the kidney's. The distribution of Cd within the liver has been studied in several species. For porcine, bovine and equine liver, solid micro sampling is reported to give similar levels of Cd as homogenization (Klüßendorf *et al.*, 1985; Lücker, 1992; Lücker *et al.*, 1993b). However, in 3 of 6 livers from mallards *(Anas platyrhynchos)* heterogeneity was reported showing the need for knowledge about different species in order to use a suitable sampling procedure (Lücker *et al.*, 1993a).

The variation in Cd levels between different samples from the same slice of liver (n=10), muscle (n=8), and mammary tissue (n=10) was tested in a pilot study. The mean±SD (range) concentrations were 88±3.7 (83-97) µg/kg 0.66±0.10 (0.56-0.84) µg/kg, and 1.9±0.12 (1.7-2.1) µg/kg for the respective tissues. This variation can be due to both variation in tissue concentrations and variation between replicate analyses (precision of the method). Expecting a normal distribution of multiple analytical results from the same tissue, concentrations of ±2SD from the average for the multiple samples were accepted. This would include 95% of the normally distributed samples. Using this criterion a variation of 8, 30 and 12% from the average was calculated for liver, muscle and mammary tissue, respectively. This resulted in a 5.0% and 33% RSDr for the liver and muscle samples analyzed in Paper II. The distribution of Cd in mammary tissue would be expected to be more homogenous than in kidney but less than in both muscle and liver, as the mammary tissue gradually changes character from the alveoli, where milk is produced, to the duct system. There are large individual variations in the proportions of gland parenchyma, connective tissue and fat in the cow's udder, and the lactational state also affects the proportion of different cell types in the udder. The anatomy of the cow udder will also make the presence of lactiferous epithelium less abundant closer to the teats (Dyce *et al.*, 1987). To reduce the effects of the anatomical differences the sampling of mammary tissue was standardized (Paper II). The Cd concentration in the mammary tissue used in the pilot study was relatively high compared to samples in Paper II, where 23 of the samples of mammary tissue were below the limit of detection and the variation between duplicate samples was large. Thus, a practical limit of 95% difference of the duplicate samples from the average had to be accepted. Despite this generous criterion for accepting analytical data, a final RSDr of 31% for mammary tissue was achieved.

Cadmium in animal tissues

Cadmium in dairy cows in conventional and organic farming

Statistically significantly higher tissue Cd concentrations were found in kidney, liver and mammary tissue from "organic" than from "conventional" cows (Figure 2 "within") by ANOVA. When animals that had been reared in another system before entering the Öjebyn project 1990 were included, the statistically significant difference between the systems disappeared for all tissues except the mammary tissue (Figure 2 "all"). Cadmium in mammary tissue was correlated to system, age and production related parameters (Table 3 in Paper II), both with and without inclusion of the older animals. This indicates that Cd in mammary tissue might be a more sensitive indicator of Cd status in cows than Cd in kidney. However, the results from analysis of Cd in mammary tissue should be interpreted with caution due to the low Cd levels, with 37% of the samples below detection limit. This limits the possibilities to use mammary tissue as a bioindicator of Cd in cattle and

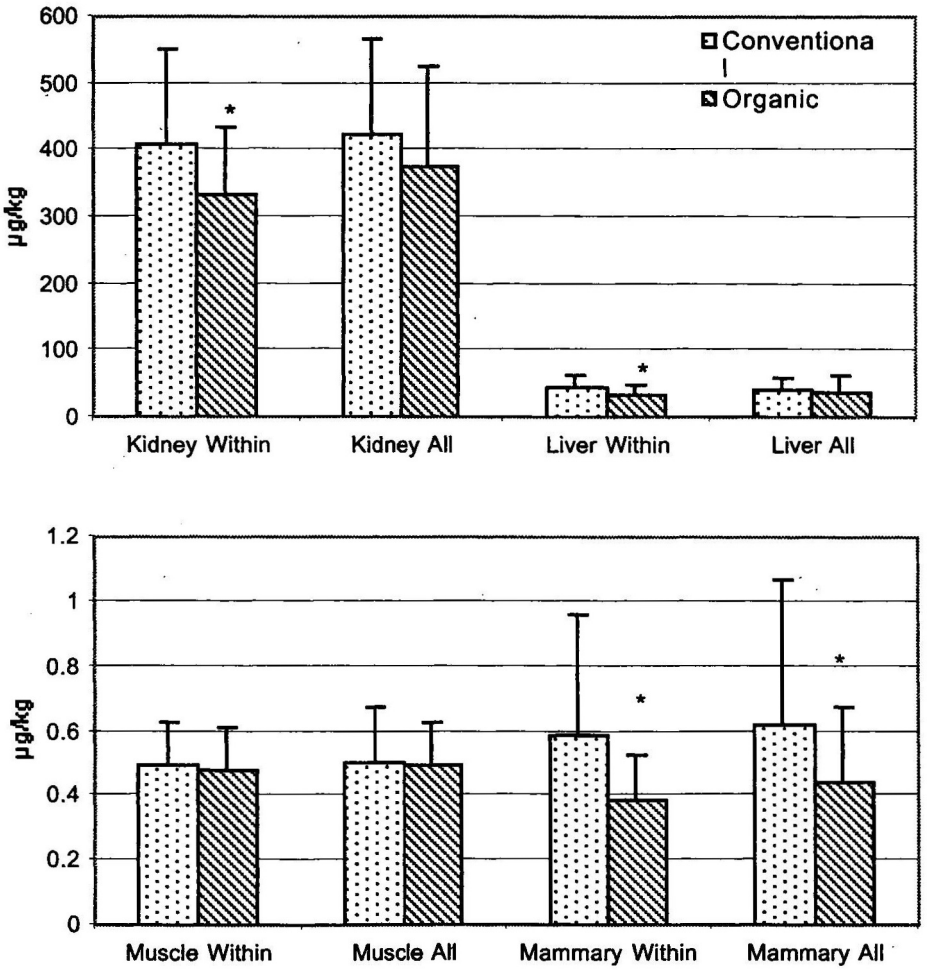

Figure 2. Group average of tissue cadmium (Cd) concentrations for conventional and organic cows born **within** the Öjebyn project (1990 and later) and for **all** cows sampled at the Research Station in Öjebyn (including cows that were already born when the project started). Statistically significant differences between Cd concentrations in tissues for conventinal and organic cows are marked with an asterix (* denotes $p \leq 0.05$).

makes the analytical results extremely sensitive to contamination, sampling technique, and dependent on a high sensitivity and accuracy of the analytical method.

Including the older animals in the material showed that age and several production related factors (number of calves, total milk production and months in production) influenced the levels of Cd in the kidney. A high feed consumption in response to elevated energy need, due to high production, is probably associated with a higher Cd exposure that may be reflected in increased tissue levels of Cd in the dairy cow. The production index was statistically significantly correlated with Cd in liver and mammary tissue (Table 3 in Paper II). This may reflect the high metabolic activity and blood flow through these tissues associated with milk production (Hanwell and Linzell, 1973), which may lead to a higher uptake of Cd from the blood into the liver and the mammary gland. Transfer of Cd to milk is very low. In rats Cd is retained in the mammary tissue during lactation (Lucis *et al.*, 1972; Bhattacharyya, 1983; Bhattacharyya *et al.*, 1986). Stevens (1991) reported a biotransfer factor of 1.3 x 10-6 of the daily intake transferred per liter produced milk. Petersson-Grawé and Oskarsson (2000) showed a high retention and a non-uniform distribution pattern of Cd in mammary tissue after administration of 109Cd to lactating mice, with a high uptake of Cd in the lactiferous epithelium, and a low excretion in the milk.

To detect subtle effects on Cd levels due to different management strategies, samples from well-defined, comparable systems are needed. The possibility to collect material from the research station instead of from different farms, one for each system (conventional and organic), reduced the number of confounders in the study, *e.g.* influence of climate, differences in location, agronomic management techniques, genetics of animals.

The lower tissue Cd concentrations in animals reared in the organic system may be explained by a lower input of Cd to the soil. The ceased use of phosphate fertilizer in the organic system probably leads to decreased levels of Cd in the roughage, which is the main feed component in the organic system. In addition, there are differences in feed composition between the systems. The "conventional" cows received approximately 38% higher amount of concentrates and 22% less roughage compared to the "organic" cows. Lindén *et al.* (1999) showed that certain protein and mineral components in concentrates to pigs can contain high levels of Cd, and even though constituting a small proportion of the feed they contribute a major part of the total Cd content in the feed. The concentration of Cd in crops depends on a number of factors, *e.g.* soil Cd concentration, soil pH, type of soil, crop species (Bruwaene *et al.*, 1986; Öborn *et al.*, 1995; Eriksson *et al.*, 2000). It is also known that different varieties of the same species have different abilities to accumulate Cd. Soya beans and sugar beets, constituents of concentrates, are both known to have a high uptake of Cd via the root system (Haghiri, 1973; Sillanpää and Jansson, 1991). Thus, the "organic" cows had a higher intake of roughage, with lower Cd levels (Sillanpää and Jansson, 1991), compared to "conventional" cows that had a higher intake of concentrates, containing higher levels of Cd. Another factor to take into account is the difference in bioavailability of Cd from different feed ingredients (Lind *et*

al., 1998; Lindén *et al.*, 1999, 2001) (*e.g.* phytic acid reduces and phytase increases the availability of Cd in the gastrointestinal tract). Without feed or manure analyses it is not possible to determine which system has the highest exposure of Cd through the feed. However, the kidney, liver and mammary Cd concentrations indicate that the "conventional" cows were exposed to higher levels of Cd or to Cd with a higher bioavailability than the cows in the organic system.

The differences between the systems were not seen in kidneys and liver after inclusion of older animals that had a prehistory in a conventional system before entering the organic system. A higher gastrointestinal uptake of Cd at lower ages (Kostial *et al.*, 1983; Lee *et al.*, 1996) and different levels of exposure during the years before entering the project may partly explain this phenomenon. The mammary tissue develops after the first calving; thus differences in uptake during the first two years of life are probably not as strongly expressed in the mammary tissue as in kidney and liver. This may explain why we could see the difference between the systems in mammary tissue even when the older animals were included.

Metallothionein (MT) was analyzed in a sub-sample of the kidneys; the MT-factor was 0.14±0.041 µg MT per mg protein (min 0.101- max 0.291), corresponding to 9.6 mg MT per kilogram kidney tissue. The kidney Cd concentration for this group of cows was 454±225 µg/kg (140-809). Cd accumulates in the kidney mainly bound to MT. We found a significant correlation between kidney levels of Cd and MT. MT is believed to protect the kidney from Cd-induced toxicity by binding Cd (Nordberg *et al.*, 1975; Klaassen *et al.*, 1999; Nordberg and Nordberg, 2000). When the MT synthetizing capacity of the kidney is exceeded, unbound Cd can exert its toxic effects. In this study we found a molar Cd/MT ratio in the kidney of approximately 3, indicating a sufficient Cd-binding capacity of MT and protection from renal dysfunction from Cd (Klaassen *et al.*, 1999). The Cd concentrations found in the kidneys in this study are far below the levels reported to cause histopathological changes as a sign of deleterious renal effects (ICPS, 1992). A positive relationship was found between Cd and MT ($r=0.49$, $p=0.028$), but not for Zn and MT in the kidney. Zn and Cd usually bind simultaneously to MT. We did not, however, analyze the metal content of MT but of the kidney cortex as a whole, and about 53 times higher levels of Zn than Cd were found. Zn is an essential element under homeostatic control and required for the function of several enzymes within cells. Zn levels in kidney were negatively related to months in production, and Zn levels in muscle were negatively related to the production index. In mammary tissue the Zn levels were positively correlated to age, days as dry cow, number of calves, total milk production, ECM, and the months in production. The negative relations between production related factors and Zn levels in kidney and muscle, and the positive correlations for the mammary tissue, might be related to the increased demand for Zn for milk production and a concomitant mobilization of Zn from muscles.

In this study with comparable and controlled conditions such as climate, agricultural management factors, animal age and genetics, we could show that organically raised cows had lower levels of Cd in kidneys, liver and mammary tissue

than conventionally raised. The circulation of Cd in the biosphere is complex, and further long-term studies are required to clarify if organic farming decreases the amount of Cd reaching the food chain.

Cadmium in the chain from soil via crops and feed to pig blood and kidney

Cd concentration in collected samples from 49 farms in southern Sweden (Papers III and IV) and the interpolated Cd levels for soils are presented in Table 4. The flow of Cd was followed from soil to pigs in the agricultural system of growing/finishing pig production. Correlations were found between Cd levels in soil *vs* wheat ($r=0.46$, $p=0.02$), Cd in wheat *vs* barley ($r=0.67$, $p=0.0002$), concentrate *vs* pig feed Cd levels ($r=0.44$, $p=0.009$), pig feed *vs* pig kidney Cd concentrations ($r=0.34$, $p<0.0001$), Cd in feed *vs* feces ($r=0.79$, $p<0.0001$) and Cd in kidney *vs* feces ($r=0.35$, $p<0.0001$).

Two correlations, the ones between soil and wheat and between Cd levels in feed and kidney, are links in the chain from soil to kidney. However, there was no statistically significant correlation between the cereals and the feed. Barley is a crop that has a relatively low and varying uptake of Cd from soil, which may decrease the possibility to detect any correlation between Cd in barley and soil. However, there was an association between Cd in barley and Cd in wheat, and the Cd levels in wheat were correlated to Cd levels in soil.

Table 4. Cadmium levels in soil and sample material from 49 farms in Skåne, Sweden.

Sample	n	Median	Min	Max
Soil (µg/kg) [a]	49	260	120	840
Wheat (µg/kg)	26	43.8	18.1	69.3
Barley (µg/kg)	44	12.8	3.8	35.3
Concentrate (µg/kg)	34	152	42.3	631
Feed (µg/kg)	49	48.6	12.6	84.2
Stable water (µg/l)	47	0.010	0.0003	1.04
Kitchen water (µg/l)	49	0.007	-0.0001	0.206
Pig kidney (µg/kg)	49	143	68.6	451
Pig blood (µg/l)	330	0.080	0.000	1.19
Pig feces (µg/kg)	98	270	121	553
Male blood[b] (nmol/l)	41	1.41	0.38	17.7
Female blood[b] (nmol/l)	38	2.33	0.66	5.66
Male urine[b] (nmol/mmol creatinine)	39	0.17	0.065	0.408
Female urine[b] (nmol/ mmol creatinine)	38	0.26	0.097	0.993

[a]Interpolated from Eriksson *et al.* (1997).
[b]Never-smokers

Pigs are fed a mixture of locally produced crops, usually barley, approximately 80-85% of the feed, and a non-locally produced vitamin-mineral-protein mixture (Lindén *et al.*, 1999, 2001). The proportion of concentrate in the feeds is relatively small (15%), but it contributes a significant part of the Cd content, so that the Cd levels in feed were more dependent on the Cd levels in concentrate than in barley. The relatively low and varying contribution of Cd in feed from

cereals can explain the lack of correlation between Cd in pig kidney and the cereal ingredients of the feed. These results show that pig kidney did not reflect Cd levels in the local environment. However, due to the relatively low gastrointestinal absorption, most of the Cd content of the feed will be excreted in the feces, which will be applied to arable soils as manure. Thus, Cd from concentrates constitutes an external source to the arable soils when farmyard manure is applied. The positive correlations for Cd between feed and kidney, feed and feces, and between kidney and feces indicate that the results from the Cd analysis of pig kidneys, which are routinely done in official control programs, could be used as indicators of inputs of Cd to arable soils. Within the official control program in Croatia levels of up to 12 mg Cd/kg in pig kidneys were found. The source of Cd in these kidneys was tracked to mineral premix with levels of 900 mg Cd/kg, and actions could be taken to prevent this in the future (Sapunar-Postruznik *et al.*, 2001).

The individual Cd concentration in kidneys differed between pigs at the same farm, and the quotient of the max and min Cd levels of kidneys was on average 2.1, ranging from 1.2 to 3.6. A high intra-farm variation of Cd levels in kidney was also seen in a study of eight Swedish farms with a mean quotient between the highest and lowest Cd levels of 2.1 and a range of 1.3-4.1 (Petersson-Grawé *et al.*, 1997). In two studies performed at research farms quotients of 2.8 (Lindén *et al.*, 1999) and 2.4 (Lindén *et al.*, 2001) can be extracted. There was a great variation also in pig blood Cd levels within farms. The mean max/min quotient was 3.6, ranging from 1.1 to 24 (levels below limits of detection were excluded when calculating the max/min quotient). The intra-farm variation shows that there must be other factors than Cd in feed and environment that is of importance for the Cd level in kidneys and blood, as these pigs were of the same breed, raised indoors in the same environment and given the same feed fulfilling the nutritional requirements of the pigs. Age at slaughter has previously been shown to have a significant but relatively small impact on Cd levels in kidneys of growing/finishing pigs (Lindén *et al.*, 1999). However, the small variation in age at slaughter can probably not explain the large inter-individual differences. Non-identified individual factors, genetic and environmental, evidently have a high impact on Cd levels in pig kidney.

There were differences between geographical areas (the northeastern [NE], northwestern [NW], southeastern [SE], and southwestern [SW] part of Skåne) for Cd in kidney, barley, wheat and soil. Significantly higher Cd levels in kidneys were found in the NE area. On the contrary, significantly higher Cd levels in soil, wheat and barley were found in the SE and the lowest levels in the NE.

Animals from the same farm, raised in the same environment, given the same feed, and slaughtered at the same age had Cd levels in kidney that could differ up to four times. As long as the reason for this variation is not known and the Cd contribution from the purchased concentrates in the feeds is so dominating, Cd in pig kidney is unsuitable as an indicator of the available Cd in the local environment. Cd in pig kidney does, however, have the potential to be used as an indicator of changes in temporal and spatial Cd input to the agricultural system.

Cadmium in men and women (Paper IV)

Males and females, with presumably similar Cd exposure, were compared and the effect of different factors on Cd levels in blood and urine, and renal effects were studied. Furthermore, Cd levels in couples living at the same farm were evaluated.

Cadmium intake

The calculated total weekly Cd intake in the study population was on average 121±43 µg/week (1.63±0.64 µg/kgBw/w). Men (n=57) had a statistically significantly higher weekly Cd intake (136±49 µg/week) than women (104±28 µg/week) (pANOVA<0.0001). The intake per kg body weight and week did not differ statistically significantly between sexes (women 1.53±0.51 µg/kgBw/w *vs* men 1.73±0.73) for the whole study population. However, a paired comparison of the intake per kg body weight between man and woman for the 24 never-smoking couples in the study population showed a lower Cd intake for women (1.50 µg/kgBw/w) than for men (1.68 µg/kgBw/w) (p=0.05). In the group of 24 female/male never-smoking couples a strong correlation was seen for Cd intake within the couples (Rho=0.753, p=0.0002), with a higher Cd intake per kg body weight for men than for women.

The Cd contribution from each food group for respective sex is shown in Figure 3. The first eight food groups, all derived from plants, constituted only 29 weight percentage (w%) of the consumed food items (Table 5 in Paper IV), although they contributed 81% and 84% of the total Cd intake in men and women, respectively. Bread was the largest contributor of Cd followed by potatoes and roots, and vegetables. Drinking water (Table 4) contributed 0.2% of the total Cd intake. The individual intake from water varied from 0 to 1.7% of the total Cd intake.

The weekly intake of Cd found in this study was 1.4-2.0 times higher than what has previously been reported for mixed diets in Sweden (Slorach *et al.*, 1983, 1991; Vahter *et al.*, 1990; Becker and Kumpulainen, 1991). Overestimation of Cd from the food items calculated from data in Jorhem *et al.* (1984) (22 out of 45) (Table 1) might have occurred, as Cd concentrations in different food items from Sweden in 1984 generally are higher when compared with data available from 1993 (Jorhem *et al.*, 1984; Jorhem and Sundström, 1993). On the other hand, the food questionnaire was designed to detect food items known to have high Cd concentrations (seeds and chocolate, mushrooms, offal, and shellfish) (Jorhem *et al.*, 1994; Jorhem and Sundström 1993, 1995). The analytical data on Cd levels for seeds, chocolate and mushrooms are limited. However, the four groups constituted 0.6 w% of the totally consumed amount of food, but the contribution to the total Cd intake was 14 µg/week, which is approximately 10% of the total Cd intake for the individuals consuming these food items (n=93). This shows that for individual consumers, Cd from specific food items might be a considerable source for Cd intake. Including these food items probably gives a more accurate estimate of the Cd intake and can, at least partly, explain why our data are somewhat higher than those previously reported.

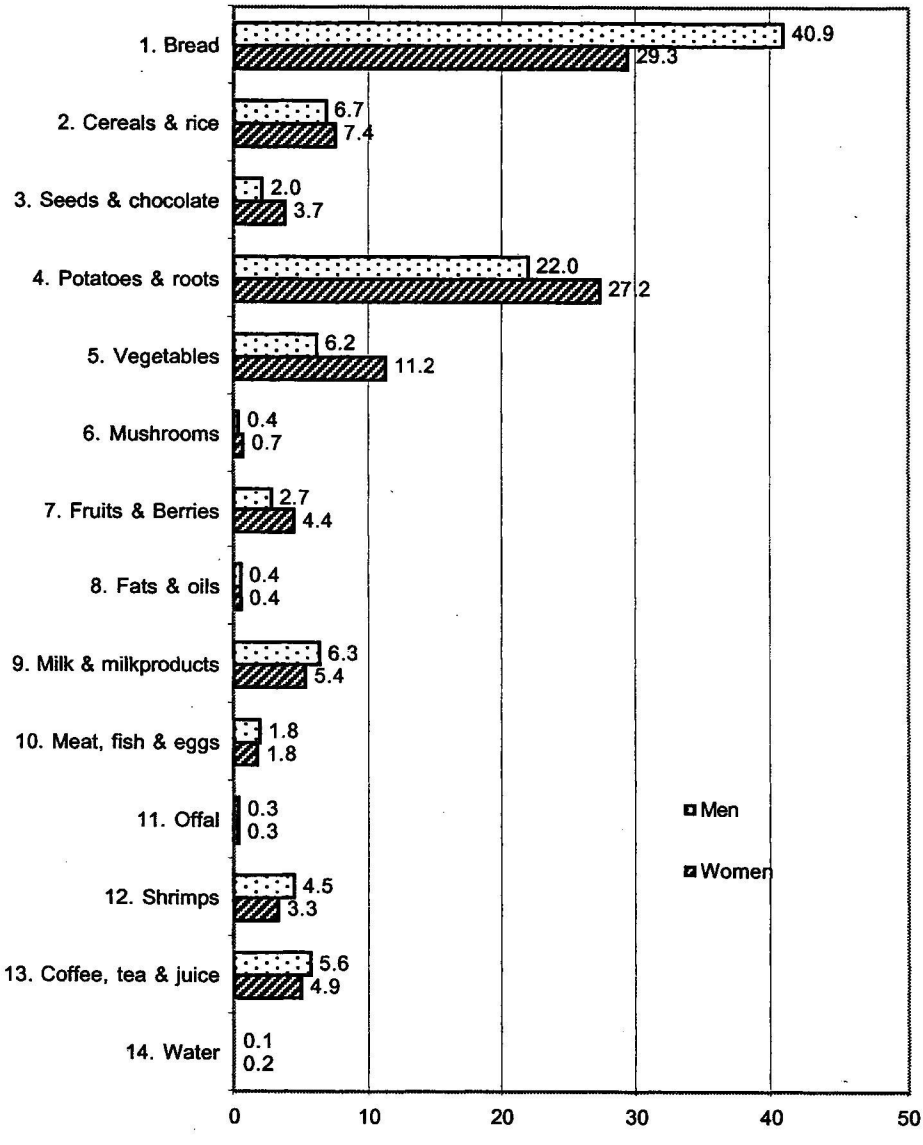

Figure 3. Percentage (%) of total weekly Cd intake from different food groups for men (n=57) and women (n=48).

Comparing the consumption of different food groups shows that the study population consumed larger quantities than the average Swedish population. The weight consumption of most of the food groups was from slightly below the 75th

to over the 90th percentile of the average population (SLV, 1994). This is expected, as the study population is physically active in their occupation, thus, requiring more energy. This may also partly explain why higher Cd intake levels were found in this study than in other studies.

Cd has a slow turnover in the body. Thus, a food frequency questionnaire (FFQ) which reflects long-term food consumption should be relevant. The final estimated Cd intake is, however, also dependent on sampling and analytical quality of Cd concentrations used for each food item, and on the accuracy of the consumption reported by the participants (Louekari, 1992). Duplicate portion studies give a more accurate figure of recent intake, but have the disadvantage of only covering a short period of time and usually comprising only a low number of participants.

The consumption of locally produced food items varied considerably within the study population. However, all persons but two reported eating locally produced food. The Cd consumption from locally produced food was on average 21 (0.6-50) µg/week, which corresponds to 17% of the total Cd intake per week, the individual contribution varying from 0.5 to as high as 45%. No correlations were found between BCd or UCd and the Cd contribution from the locally produced food items in the present study.

In studies by Reeves and co-workers (Reeves and Vanderpool, 1997; Reeves *et al.*, 2001) sunflower kernel consumption was reported to give a significant increase to the intake of Cd. However, no increase in BCd or UCd was seen, indicating a low bioavailability of Cd in sunflower kernels. The availability of Cd differs in different foods, probably depending on the speciation of Cd in the respective food item. In mice the uptake of Cd from boiled crab hepatopancreas was slightly lower than for Cd from mushrooms (Lind *et al.*, 1995), and Cd in carrots was more efficiently taken up than Cd in wheat bran (Lind *et al.*, 1998). Higher accumulation in liver has been found in rats fed caribou kidney with naturally high Cd concentrations compared to rats fed veal kidney spiked with CdCl2 (Chan *et al.*, 2001). BCd and UCd were not statistically significantly correlated to the calculated Cd intake (µg/kgBw/w), neither in the whole group nor among the never-smokers. In a duplicate portion study by Berglund *et al.* (1994) no positive relations were found between BCd or UCd and the Cd intake. Among Japanese women, with a weekly intake of 170 µg/week and BCd of 16 nmol/l and UCd of 3.9 nmol/mmol creatinine, positive correlations between dietary Cd intake and blood and urinary levels were found (Shimbo *et al.*, 2000). The Cd intake in Sweden is in the lower range of internationally reported levels (WHO, 2001b).

Cadmium in human blood and urine

For the whole study population BCd was on average 2.3 (range 0.38-18) nmol/l and UCd 0.26 (0.065-0.99) nmol/mmol creatinine (Table 5). The BCd and UCd found in this study correspond to earlier reported levels for non-smokers in the general population of Sweden (Järup *et al.*, 1998b), and blood levels are in the same order as for non-smokers in the Baltic region (Skerfving *et al.*, 1999) and

Swedish adolescents (Barany *et al.*, 2002). The average UCd found in this study was approximately 10-50% of the levels previously reported to give proteinuria (Buchet *et al.*, 1990; Nortier *et al.*, 1997; Järup *et al.*, 2000).

Table 5. Blood and urinary cadmium (Cd) concentrations in the study population from 49 farms in Skåne, Sweden.

Group	Number of persons	Blood Cd (nmol/l)	Urinary Cd (nmol/mmol creatinine)
All	105	2.3±2.0	0.26±0.15
Males	57	2.0±2.2	0.20±0.09
Females	48	2.8±1.5	0.32±0.18
Never smokers	79	2.2±2.1	0.24±0.14
Ex-smokers	46	2.7±1.3	0.31±0.16

Women had approximately 1.4 times higher BCd and 1.6 times higher UCd than men (Table 5). This confirms that women have higher levels of Cd both in blood and urine as can be extracted from other studies (Jawaid *et al.*, 1983; Bäcklund *et al.*, 1999; Björkman *et al.*, 2000). Blood and urinary Cd increased with age in both sexes. A higher correlation between blood Cd and age was seen for men (Rho=0.5, p=0.0002) than for women (Rho=0.33, p<0.0001).

In the group of 24 female/male never-smoking couples a very close correlation within the couples was seen with age (Rho=0.967, p=<0.0001). Age-adjusted BCd and UCd were not correlated for male and female from the same farm. However, an intra-couple female/male-ratio showed that the women had 1.8 times higher BCd and 1.4 times higher UCd than the men, despite the lower Cd intake in women. Using a biokinetic model Choudhury *et al.* (2001) also showed that women have higher UCd levels than men in spite of a lower dietary intake.

S-Ferritin was shown to be of importance for BCd levels in the whole study population, but when divided into separate sexes S-Ferritin was related only to the BCd concentrations for women (Rho=-0.410, p<0.0001). No man was defined as having iron deficiency using S-Ferritin <10 µg/l as the cut-off level for low iron stores (Fernlund *et al.*, 1991). Eight (17%) women were defined as iron deficient; of these all but one had at least one more of the iron-parameters below normal ranges. When the cut-off point for low iron stores was set at 30 µg/l, 35% of the women and 2% of the men had low iron stores. Flanagan *et al.* (1978) showed similar retention curves of 109Cd for one male and two females with low S-Ferritin. The lack of relationship for men in the present study is probably due to the fact that the men had normal to high S-Ferritin levels. Berglund *et al.* (1994) have earlier shown that S-Ferritin in women is related to BCd levels.

Ex-smokers (n=27) had higher Cd levels in blood (p=0.03) and urine (p=0.01) than never-smokers (n=78) (Table 5). Years of smoking and total amount smoked (packyears = packs smoked per day x years as smoker) did not differ between the

sexes. The ex-smokers had smoked on average 16±11 (1-40) years, and 18±12 (1-46) years had elapsed since they stopped smoking. Two women had stopped one year ago and the rest more than 5 years ago. Exclusion of the two women in the analysis did not change the outcome of the analysis. BCd reflects current Cd exposure; the half-life of Cd in blood is approximately 2-3 months. BCd levels are, however, also influenced by the body burden of Cd, which is elevated for long periods of time after exposure due to the long-term retention of Cd in kidney and liver (Welinder *et al.*, 1977; Berglund *et al.*, 1994). Thus, former smoking, even more than 5 years since discontinued smoking, causes increased BCd as well as UCd levels, which should be considered in biomonitoring of Cd exposure (Hoffmann *et al.*, 2001).

Stepwise multiple linear regression showed that the BCd were best predicted by age (years), sex (men=0, women=1), S-Ferritin (µg/l) and former smoking (never-smokers=0, ex-smokers=1) for the whole study population ($LogBCd_{n=105}$ = - 0.043 + 0.007 Age + 0.106 Sex - 0.001 S-Ferritin + 0.099 Former smoking [r=0.57, R2=0.30, p<0.0001]). For men (n=57) age was the only statistically significant predictor of BCd. For women (n=48) age and S-Ferritin were statistically significant.

Even when taking several predictors into consideration a large part of the variation remains unexplained. Björkman *et al.* (2000) showed that inter-individual variations in BCd concentrations are not entirely attributable to environmental exposure but there are also genetic factors.

Stepwise multiple linear regression showed that the UCd were best predicted by age, sex, and former smoking for the whole study population ($LogUCd_{n=105}$= - 1.20 + 0.009 Age + 0.20 Sex + 0.088 Former smoking [r=0.72, R2=0.53, p<0.0001]). For men (n=57) age was the only statistically significant predictor of UCd. For women (n=48) age, former smoking and S-Ferritin were statistically significant.

Kidney function

Despite the low UCd levels the kidney function parameters β_2-Crea-clearance, U-pHC, U-NAG and Alb-Crea-clearance were positively correlated to the Cd levels in urine for the whole study group (n=105) (β_2-Crea-clearance Rho=0.208, p=0.04; U-pHC Rho=0.225, p=0.024; U-NAG Rho=0.278, p=0.0055; Alb-Crea-clearance Rho =0.216, p=0.030). However, when the respective kidney function parameter was tested *vs* UCd and age in a multiple regression analysis, only the β_2-Crea-clearance was statistically significantly related to UCd (p=0.01). This may indicate that the Cd exposure in this study population, with an average intake of 1.64 µg/kgBw/w (approximately a fifth of the PTWI), is at the limit of detection for renal effects. The urinary Cd concentrations found in this study were below 1nmol/mmol creatinine for most of the study group, a level at which the first elevated levels of U-NAG excretion have been reported (Nortier *et al.*, 1997). 33% of the women and 14% of the men had β_2-Crea-clearance above 0.1%, indicating a slightly decreased reabsorption of β_2-microglobulin in the proximal renal tubules (Järup *et al.*, 2000). Tubular proteinuria in itself does not give rise to

symptoms or clinical disease. However, Cd-induced tubular damage is in most cases irreversible, and unless Cd exposure decreases the damage may become worse (Hotz *et al.*, 1999; Järup *et al.*, 2000). The results registered in a population from an area with environmental Cd pollution with only slightly higher levels of U-pHC and moderately higher UCd than in this study indicate that the renal effects may be of clinical significance (Hellström *et al.*, 2001).

Human BCd and UCd in relation to Cd in pig kidney and wheat (Papers III and IV)

In the evaluation of human BCd and UCd in relation to pig kidney Cd concentrations found at the farm only those persons that never had smoked were included in the analysis (Table 4). Statistically significant simple regressions were found for male (n=30) UCd ($p<0.0001$) and for female (n=34) UCd ($p=0.0003$) and BCd ($p=0.0059$) *vs* pig kidney Cd concentrations (n=421). However, the relationships were negative and may be explained by the geographical differences shown below. Even though cereals are a substantial part of both the human and the pig diet, Cd in pig kidneys could not be used to predict Cd concentrations in human blood and urine in the present study. This is probably because in humans, the cereals and other foods consumed are mainly from non-local sources, and in pig feed other ingredients than locally produced cereals were shown to contribute to a large part of the Cd intake (Lindén *et al.*, 1999, 2001; Paper III). Thus, the association between pig Cd exposure and human Cd exposure is too weak to show any significant relationships. An interesting positive relationship of borderline significance (n=15, r=0.50, $p=0.059$) was found for urinary Cd in men and Cd in wheat.

Geographical differences

Significantly higher Cd levels were found in pig kidney in the NE (198 µg/kg, the other areas ranging from 134-145 µg/kg), whereas the NE had the lowest levels of Cd in soil, barley, and wheat. The age adjusted LogBCd and LogUCd for never-smoking males and females were analyzed by ANOVA for geographical area (NE, NW, SE, and SW). Men living in the NE part of Skåne had lower UCd levels (0.14 nmol/mmol creatinine) than men living in other parts of Skåne (0.21 nmol/mmol creatinine) ($p=0.0019$). Sartor *et al.* (1992) showed that higher 24 hours UCd excretion was found in areas with Cd polluted soils. Higher UCd has also been shown for persons living closer to industries causing environmental Cd pollution (Roels *et al.*, 1981; Staessen *et al.*, 1994; Järup *et al.*, 1995).

Monitoring

Possible routes for the flow of Cd are shown in Figure 4. In the Skåne study correlations for Cd were not found for all links in the agricultural system. The influences from certain links are obscured, most likely by a greater influence of Cd from other sources, as shown for pig feed where Cd in cereals could not be correlated to Cd in the feed due to the high contribution from non-local feed components. Differences in Cd levels between cows in different agricultural systems were shown in the Öjebyn study. However, the difference between "conventional" and "organic" cows could not be seen when older animals with a prehistory in another system were included. There are indications of a lower input of Cd to soils in the organic system than to soils in the conventional system (Helena Bengtsson, SLU, personal communication, 2001). The data from Paper II will be further evaluated in cooperation with scientists who have studied Cd and Zn in soils and crops at Öjebyn.

In spite of the obvious source of Cd exposure from food in non-smoking persons, so far the relationship between intake and BCd and UCd has only been shown where levels in food are high, and mainly from one food source, *e.g.* rice. At low Cd level exposure this relationship is obscured by other factors, *e.g.* iron status as well as low and various bioavailability of Cd in food. Using Cd in cattle and pig kidney as indicators has the advantage that it reflects long-term exposure and is routinely analyzed in programs for food control. However, there are several factors influencing the level of Cd in each link of the chain from soil to animal and man (Figure 4), and substantial knowledge is needed about each of these steps in order to evaluate and interpret the data (Cajaraville *et al.*, 2000; Beeby, 2001).

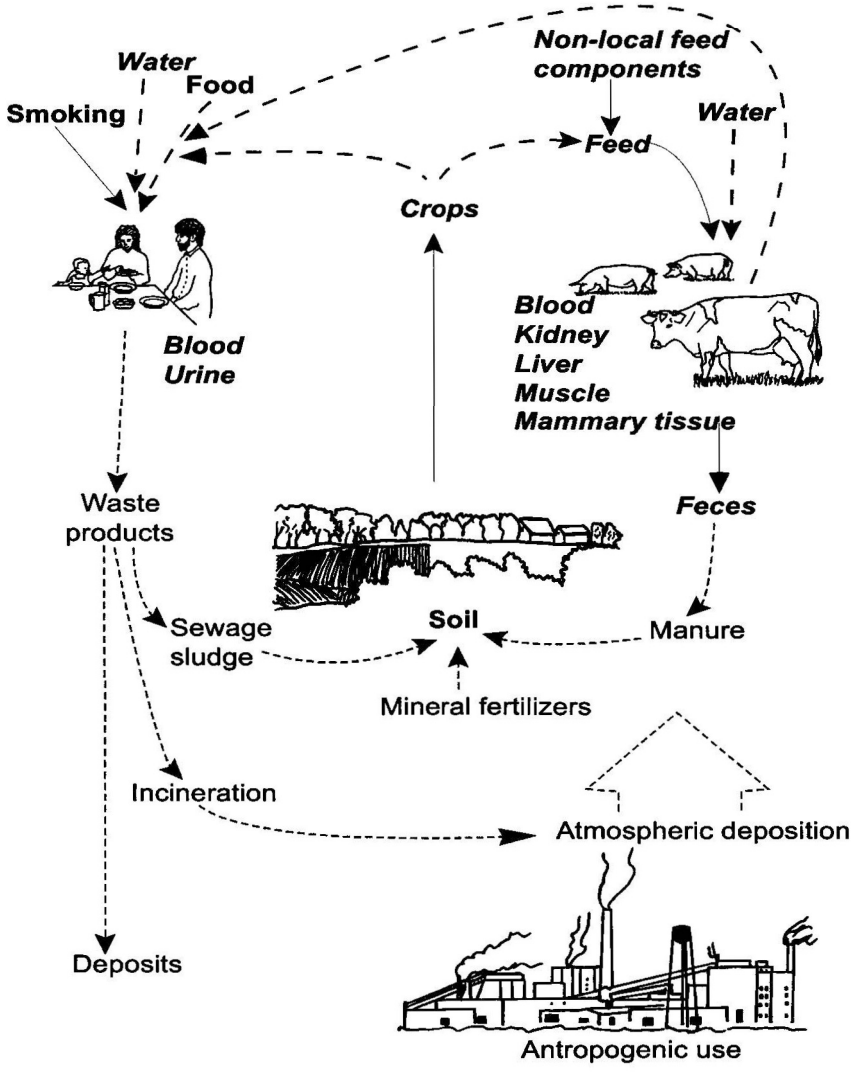

Figure 4. Flow of cadmium (Cd) in the environment, exposure routes for livestock and humans and the specimens for biological monitoring of Cd that was sampled in this thesis. Bold letters show parameters measured or calculated. Black full arrows indicate correlations found, black dotted arrows show examined linkes that did not show statistically significant correlations. Grey arrows show links in the circulation that was not studied.

Concluding remarks

The heterogeneous distribution of Cd in the kidney and the species variation in kidney anatomy should be considered when sampling kidney for Cd analysis. A detailed description of the used sampling technique is needed for comparison of results from different studies.

The optimal sampling technique of bovine and porcine kidney with the purpose of biomonitoring is to sample the outer part of the kidney cortex. In cattle the difference between Cd concentrations in kidney cortex and medulla is larger than in pigs, and in cattle the medulla is proportionally larger than in pigs. Thus, the sampling technique is of greater importance for the analytical results for cattle kidney than for pig kidney.

Organically raised cows had lower levels of Cd in kidney, liver, and mammary tissue compared to conventionally raised cows. Cd levels in mammary tissue were positively correlated to age and milk production. Cd in kidney was positively correlated to metallothionein in the kidney. Long-term studies in well-defined systems are required to clarify if organic farming can lower the amount of Cd reaching the food chain.

Cd in pig kidneys could not be used to predict human BCd or UCd even though cereals are a substantial part of both the human and the pig diet. Cd levels in pig kidney were significantly related to Cd levels in feed. However, there was no relationship between the locally produced cereals, constituting the main part of the feed, and Cd in pig kidneys. In pig feed other ingredients than locally produced cereals contributed to a large part of the Cd in feed. Hence, Cd in pig kidney did not reflect Cd in the local environment. The Cd in non-locally produced feed ingredients constitutes an external source of Cd to the local circulation via excretion in feces and application of manure to arable soils. Men living in the area with the lowest soil Cd levels (NE Skåne) had lower UCd than men from the other Skåne areas, thus indicating some local influence on the Cd body burden.

When using livestock for biomonitoring of Cd, knowledge on sources of exposure and other environmental as well as individual factors that influence Cd levels within the production systems is required.

Women have higher BCd and UCd than men. The higher female BCd and UCd may be explained by higher absorption due to low iron status (S-Ferritin). In addition age and former smoking were important determinants for BCd and UCd. BCd and UCd were not correlated to the calculated weekly Cd intake.

Even at the low dietary exposure levels in this study there was an indication of effect on a biochemical marker (β_2-microglobuline-creatinine-clearance) of renal function. The effect remained even when age was allowed for.

For certain individuals the Cd intake from locally produced food contributes significantly to their Cd intake. For the population in general it is important that further introduction of Cd to arable soils is prevented and that measures are taken to reduce the uptake of Cd in vegetable food in order to avoid increasing levels of Cd in staple foods.

Sammanfattning

Livsmedel, framförallt spannmål, är den huvudsakliga källan till kadmium-exponering (Cd) för icke-rökare. Njuren är målorgan för Cd-toxicitet. Cd-halterna i åkermark har ökat under det senaste århundradet. I föreliggande avhandling redogörs för studier om Cd-exponering hos kor, svin och människor. Dessutom diskuteras olika indikatorer som kan användas vid biomonitoring av Cd.

Genom provtagning av de yttre delarna av njurbarken hos ko och gris optimerades möjligheterna att hitta skillnader i Cd-halter mellan olika grupper. Ekologiskt hållna kor hade lägre Cd-halter i njurar, lever och juvervävnad än kor hållna i ett parallellt konventionellt system. De lägre halterna i de "ekologiska" korna kan bl.a. bero på en lägre Cd-tillförsel i åkermarken och därmed lägre Cd-nivåer i grovfodret samt skillnader i fodersammansättning och i biotillgänglighet av Cd i fodret. För att avgöra om ekologisk odling på sikt kan minska mängden Cd som når våra livsmedel behövs långtidsstudier i väl definierade system.

Cd har studerats i kedjan från jord via gröda och foder till grisblod och -njure och till humanblod och -urin. Cd-halterna i grisnjure var signifikant relaterade till Cd-halterna i foder. Däremot fanns inget samband mellan Cd i lokalproducerat spannmål, vilket var huvudingrediensen i grisfodret, och halten i helfoder eller i njure. Cd-halterna i grisnjure avspeglade alltså inte Cd-halterna i den lokala miljön. Cd-innehållet i icke lokalproducerade foderkomponenter innebär en extern tillförsel av Cd till det lokala kretsloppet. På grund av låg absorption i mag-tarmkanalen utsöndras Cd till största delen i träck, som används till gödsel och därmed hamnar i åkermarken.

Vegetabila livsmedel utgjorde den största delen av Cd-intaget (83%) hos en grupp män och kvinnor boende på svingårdar i Skåne. Andelen Cd från lokal-producerade livsmedel av det totala Cd-intaget var relativt lågt och varierande. Män hade ett högre Cd-intag via maten än kvinnor, trots detta hade kvinnor 1,8 gånger högre blod-Cd (BCd) och 1,4 gånger högre urin-Cd (UCd) än män. Det fanns inget signifikant samband mellan Cd-halten i njurar, från grisar ur besättningar med lokalproducerad spannmål i foderstaten, och BCd eller UCd hos människor från samma gårdar. Män, boende i områden med låg Cd-halt i åkermarken, hade lägre UCd än män från andra områden, vilket tyder på en lokal påverkan. Varken BCd eller UCd kunde relateras till det dietära intaget. Kvinnors högre BCd och UCd beror troligen på högre absorption av Cd p.g.a. låg järnstatus. Både BCd och UCd ökade med stigande ålder och var högre hos före detta rökare än hos personer som aldrig rökt. Trots den relativt låga exponeringen av Cd fanns en lindrig påverkan på njurfunktion (β_2-mikroglubolin-kreatinin-clearance) relaterat till UCd. Denna effekt kvarstod även sedan hänsyn tagits till ålder.

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