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Transfer of cadmium, lead, mercury and arsenic from feed into milk and various tissues of dairy cows: chemical and pathological data

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Abstract

Experiments with grazing cows and cows kept indoors were performed to study the transfer of toxic elements from their ration into milk and edible tissues.

The first experiment was carried out with 24 grazing dairy cows. The toxic elements were administered, to 12 of these cows, via wafers of concentrates which contained a mixture of cadmium, lead and mercury acetate and arsenic pentoxide. The dosing period was three months in which the daily intake for each cow was 152, 200, 1.7 and 33 mg for Cd, Pb, Hg and As respectively. The daily intake of these elements for the control cows was 2, 50, 0.2 and 3.4 mg respectively.

The second experiment was carried out with 32 dairy cows which were kept indoors and fed on concentrates and roughage. Four groups of 8 cows each received different treatments: a control group receiving no additional elements, a group receiving cadmium, lead and mercury acetate and arsenic trioxide, a group receiving harbour sludge and a group receiving sewage sludge. The dosing period lasted 28 months or 3 consecutive complete lactations.

At the end of the dosing period 8 cows kept on pasture and 7 cows kept indoors were slaughtered. Samples of feed, water and milk were taken periodically and organs and tissues were sampled at slaughter and stored at –20 °C until analysis.

Histological examination of tissue specimen was also performed. Liver and in particular kidney were the primary sites of element accumulation. Only for cadmium the proposed tolerance levels in liver and kidney were exceeded. Increased dietary concentrations of elements did not result in significantly higher concentrations in milk, blood and muscle tissue. Only soluble arsenic resulted in higher levels of

this element in muscle tissue. Lead showed also a dose-related increase in bone tissue. Regarding the character of the pathological changes, no essential differences were observed between the control and experimental groups.

Introduction

Feed is the major source of intake of heavy metals by dairy cattle (Gründer, 1982). The EEC Directive on Undesirable Substances and Products in Feedingstuffs 74/63 and Amendments includes maximum permitted levels for arsenic (As), lead (Pb) and mercury (Hg). These maximum levels are shown in Table 1. For cadmium (Cd), a maximum permitted level has not yet been established.

To adjust maximum permitted levels of toxic elements in the whole ration to such levels in milk and tissues, data on the transmission of these elements from the ration into milk and tissues are required.

Little information (Baxter et al., 1982; Kreuzer et al., 1981; van de Ven et al., 1977) is available on the transmission of these metals from the whole ration of lactating dairy cows into milk, edible muscle tissues and organs under practical conditions.

The objective of the experiments described in this paper is to obtain supplementary information.

Materials and methods

Experimental design

The first aim was to carry out transfer trials at a level of contamination representative for the practical situation. In the Dutch situation it was impossible to find sufficient quantities of feedingstuffs (concentrates and/or roughages) contaminated at the levels shown in Table 1. However, increased levels of heavy metals in the total ration of dairy cattle can easily be achieved by incorporation of pure compounds of these metals or contaminated sludge (dried sewage or harbour sludge) in concentrates. In practice these sludges are applied to agricultural land as fertilizers and can

Table 1. Prescribed limits for toxic elements in feedstuffs (mg per kg^{-1} product referred to a moisture content of 12 %) according to EEC Directive 74/63.

	Pb	Hg	As
Straight feedstuffs	10	0.1	2
Except meal made from grass, dried lucerne or dried clover	40		4
Phosphates	30		10
Fish meal		0.5	10
Whole feedstuffs	5	0.1	2
Except for dogs and cats	5	0.4	2

Table 2. Summary of experimental data

	Grazing cows		Cows kept indoors			
	control	soluble compounds	control	soluble compounds	harbour sludge	sewage sludge
Number of cows	12	12	8	8	8	8
Age (in years)	4-6	4-6	2-6	2-6	2-6	2-6
Maximum daily milk yield (kg)	21	21	31	33	34	33
Length of trial (months)	3	3	15-28	15-28	15-28	15-28
Daily dry matter intake (kg)						
– roughage	15	15	10	10	10	10
– concentrates	3	3	10	10	11	11
Daily intake of elements (mg)						
Cd	2	152	2	32	10	6
Pb	50	200	50	200	164	168
Hg	0.2	1.7	0.2	1.7	3.1	1.2
As	3.4	33	3.4	33	21	6.8

be taken up by the cows in roughage.

These experiments were performed at the experimental farm of the Institute for Livestock Feeding and Nutrition Research at Lelystad.

Grazing dairy cows

In the first trial 2 × 12 lactating dairy cows were used. Details are summarized in Table 2. The experimental group received a mixture of 4 soluble compounds, containing cadmium, lead and mercury acetate and arsenic pentoxide.

The daily quantity of each element administered was determined by the maximum permitted concentration in the diet multiplied by the daily dry matter intake, which was estimated at 15 kg.

The metal compounds were dissolved in water and an aliquot of this solution was pipetted on wafers of concentrates. Cows in the control group received wafers without additional metal compounds.

Each month samples were taken from the milk on two consecutive days and from feed and drinking water. After 3 months of feeding, 4 cows from the experimental group and 4 from the control group were slaughtered and various samples of tissues were taken (liver, kidney and muscles from 3 sites on the carcass: upper arm, diaphragm and tail head).

Dairy cows kept indoors

The second trial included 32 lactating dairy cows. The cows were divided at random into four groups of eight. The different treatments were: a control group, without additional administration of Cd, PB, Hg and As; a treatment group which received

these elements in the form of water soluble compounds as in the pasture trial, but As as As_2O_3 which is more toxic than As_2O_5; a treatment group which received harbour sludge and a treatment group receiving sewage sludge.

The sludges were dried and included in the concentrates to such an amount that the Pb concentration in the whole ration was about 10 mg kg^{-1} dry matter. The estimated daily intake of these elements by the different groups is shown in Table 2. These quantities were based on the same calculation method as for grazing dairy cows.

The average daily intake of concentrates over the whole lactation was 10-11 kg; this varied from 5 and 7 kg in late lactation to 12 and 13 kg in early lactation, for cows receiving concentrates without and with sludge respectively. Indoors roughage was offered as wilted grass silage of 55 % dry matter at about 18 kg per cow per day.

In general, the dosing period lasted at least 2 whole lactations, with the exception of 10 cows (3 from the control group, 3 from the group which received soluble compounds, 1 from the group which received harbour sludge and 3 from the group which received sewage sludge) for which the dosing period was 28 months or 3 complete lactations. This longer period approached more closely the average lactation period found in practice. During the whole experiment the cows were kept indoors, except for two months at pasture each year to avoid hoof and leg problems. During these months at pasture the ration was mainly grass without addition of water soluble compounds or sludges.

For reasons of costs only 7 cows (2 control, 2 soluble compounds, 1 harbour sludge and 2 sewage sludge) were slaughtered at the end of the dosing period to obtain data on element concentrations in tissues such as muscle, liver, kidney, heart, brain, thymus, spleen and bone. Feed, water, milk and blood were sampled periodically for analyses.

Analytical procedures

Feed samples were dried overnight at 60 °C to about 95 % dry matter and stored in a refrigerator (2-4 °C). Samples of animal products were stored at −20 °C until required for analysis. Samples were carefully prepared for analysis by removing connective and adipose tissue from organs; they were minced and freeze-dried.

The analyses were performed at the State Institute for Quality Control of Agricultural Products at Wageningen.

The applied analytical methods were as follows.

Cd and Pb in feeds and faeces

In a combustion furnace using a programmed increasing temperature scheme up to 450 °C for 16 hours (programming speed 50 °C/h) 5 g of dried material were ashed. Re-ashing occurred with nitric acid/water (1:1) mixture until a white ash was obtained. The ash was dissolved in 15 ml of HCl 3 mol/l and transferred into a 100-ml volumetric flask, followed by addition of 20 ml of citrate buffer (pH 8.5) and 10 ml of saturated NaCl solution were added.

The pH was adjusted to about 7 with NaOH 5 mol/l or HCL 6 mol/l. Subsequently 5 ml of 1 % APDC (ammoniumpyrrolidindithiocarbamate) solution and 10 ml of MIBK (methylisobutylketone) were added. The solution was shaken for 45 seconds and diluted to mark with saturated NaCl solution. After half an hour the absorption of the organic upper layer was measured with flame atomic absorption spectroscopy.

The concentrations were calculated by using calibration curves.

Cd and Pb in water, milk, blood and tissues

1 g of freeze-dried tissue or the freeze-dried residue of 25 g of milk or 1 g of blood or the dry residue of 25 g of water were ashed similar to that for feeds and faeces (see above).

Re-ashing of the sample was performed with a nitric acid/water solution (1:1) and/or $Mg(NO_3)_2$ 0.1 mol/l until a white ash was obtained. The white ash was dissolved in 0.5 ml of hydrochloric acid 12 mol/l, adding 5 ml water and 5 ml acetic acid/sodium acetate buffer (pH 3.5).

The measurement was based on differential pulse anodic stripping voltammetry (DPASV, from −0.850 to −0.300 V versus Ag/AgCl in KCl 3 mol/l. The halfwave potentials for Cd and Pb were −0.625 and −0.425 V, respectively). The concentrations were calculated with standard addition.

Cd and Pb in bone tissue

– *Sample preparation.* Discs of bone were prepared (bone marrow was removed) and dried for 10 minutes at 105 °C. A known quantity of bone was dissolved under heating in concentrated nitric acid and transferred into a 250-ml volumetric flask, diluted to volume and homogenized.

– *Measurement by differential pulse anodic stripping voltammetry.* Of the prepared solution 5 ml was evaporated. The dry material was ashed and analysed by DPASV as indicated above (standard addition).

Hg in feeds, faeces, tissues, milk, blood and water

200 mg of dried feeds or freeze-dried material or 2 g of fresh milk, 1 g of blood or water was digested for 2 hours at 140 °C in a Uni-seal decomposition vessel with 3 ml of concentrated nitric acid. To the mineralized solution $SnCl_2$ was added. The generated mercury vapour was blown into a 30-cm measuring vessel by using a stream of nitrogen. The absorption of mercury was measured by cold vapour atomic absorption spectrometry. The concentrations were calculated by using calibration curves.

Hg in bone tissue

2 ml of the mineralized solution, obtained under 'sample preparation' for Cd and Pb in bone tissue, was reduced with $SnCl_2$ and measured as indicated above.

As in feeds, faeces, tissues, milk, blood and water

1 g of dried feeds or freeze-dried material (1 ml in the case of blood and water or the

freeze-dried residue of 25 g of milk) was mixed with 5 ml of nitric acid and 4 g of $Mg(NO_3)_2$ and digested on a hot plate. Dry ashing was done at 450 °C by temperature programming. Arsenic was determined by hydride atomic absorption spectrometry. The hydride was evolved by reduction of the arsenic compound with sodium borohydride solution and swept by a flow of nitrogen into a measuring vessel at 1000 °C. The concentrations were calculated with standard addition.

As in bone tissue

5 ml of the mineralized solution, obtained as described previously for Cd and Pb in bone tissue, was evaporated and treated as indicated above.

Methods of pathological examination

A post-mortem examination was performed on the 7 slaughtered cows and for gross inspection the following organs were collected: liver, kidney, spleen, heart, rumen, abomasum, small intestine, colon, uterus and skeletal muscle. Based on macroscopic examination tissue specimen were selected and fixed in 10 % neutral buffered formalin for histological examination. After fixation the tissues were dehydrated and embedded in paraffin by standard methods. Sections (5 μg) were stained with haematoxylin-eosin and van Gieson.

Results

Concentrations in milk and blood at the end of the dosing period

Concentrations of the elements in milk are listed in Table 3. Increased intake of administered elements during 28 months did not result in higher concentrations of the elements in milk. Because of the low level the differences found were unimportant. Mean concentrations of Cd, Pb, Hg and As in whole blood did not increase for the treatment groups and amounted 0.5, 8, 0.2 and 0.3 μg per litre of blood respectively.

Table 3. Concentrations (mean ± s.e.m. in $\mu g\ kg^{-1}$) of the elements in milk.

	n	Cd	Pb	Hg	As
Cows on pasture					
control	12	0.26 ± 0.03	7 ± 1	2.3 ± 0.6	<1.0
soluble compounds	12	0.39 ± 0.10	15 ± 2	0.9 ± 0.2	<1.0
Cows kept indoors					
control	4	0.15 ± 0.03	2 ± 0	<0.5	<1.0
soluble compounds	4	0.20 ± 0.04	6 ± 1	0.6 ± 0.1	2.0 ± 0.7
harbour sludge	2	0.10 ± 0.10	5 ± 2	2.4 ± 0	<1.0
sewage sludge	4	0.20 ± 0.06	4 ± 1	1.3 ± 0.5	<1.0

n = number of samples analysed.

Table 4. Concentrations (mean ± s.e.m. in μg per kg fresh weight) of Cd, Pb, Hg, and As in muscle tissue.

	n	Cd	Pb	Hg	As
Cows on pasture					
control	12[1]	5 ± 1	51 ± 6	3 ± 1	5 ± 1
soluble compounds	12	4 ± 1	47 ± 6	4 ± 1	24 ± 3
Cows kept indoors					
control	2	2 ± 0	10 ± 0	2 ± 0	4 ± 1
soluble compounds	2	7 ± 1	20 ± 0	2 ± 1	30 ± 0
harbour sludge	1	4	20	1	13
sewage sludge	2	3 ± 1	20 ± 1	2 ± 1	6 ± 1

n = number of samples.
[1] From 4 cows the upper arm, diaphragm and tailhead were analysed, resulting in 12 samples.

Concentrations in muscle tissue

Mean concentrations and the standard error of mean in muscle tissue are given in Table 4. The mean levels of Cd ranged from 2 to 5 μg kg^{-1} for the control groups and from 3 to 7 μg kg^{-1} for the treatment groups.

The mean concentrations of Pb were about 50 μg kg^{-1} tissue for both groups of cows on pasture and ranged from 10 to 20 μg kg^{-1} tissue for the groups of cows kept indoors.

Administration of arsenic trioxide resulted in a significant effect on arsenic concentration in muscle tissue. The mean levels for the control groups were 4 and 5 and for the groups fed on wafers with arsenic 24 and 30 μg kg^{-1} fresh tissue. Intermediate levels were found for the other groups fed on concentrates with sludge material.

Table 5. Concentrations (mean ± s.e.m. in μg per kg fresh weight) of elements in liver.

	n	Cd	Pb	Hg	As
Cows on pasture					
control	4	65 ± 14	210 ± 30	7 ± 1	8 ± 1
soluble compounds	4	1240 ± 200	470 ± 60	10 ± 2	30 ± 10
Cows kept indoors					
control	2	60 ± 20	110 ± 5	3 ± 1	12 ± 1
soluble compounds	2	1500 ± 160	560 ± 35	26 ± 7	100 ± 20
harbour sludge	1	160	260	14	48
sewage sludge	2	100 ± 30	280 ± 5	9 ± 3	20 ± 2

n = number of cows slaughtered.

Concentrations in liver

Table 5 presents the concentrations of Cd, Pb, Hg and As in liver of slaughtered cows. Increased concentrations of elements in the ration resulted in most cases in significantly higher concentrations in liver, particularly for the groups which received soluble compounds. For example, a daily intake of 32 mg of soluble Cd per cow, which was equal to about 2 mg kg^{-1} of the whole ration, based on 88 % dry matter, resulted after 28 months in a mean concentration of 1500 μg kg^{-1} fresh liver, whereas a daily intake of 152 mg of soluble Cd per cow (= 10.1 μg kg^{-1} of the whole ration) after 3 months resulted in a mean Cd concentration of 1240 μg kg^{-1} fresh liver.

Concentrations in kidney

The mean concentrations of the elements are presented in Table 6. A dietary concentration for Cd of about 2 mg kg^{-1}, based on 88 % dry matter, led to a mean concentration of about 6 mg kg^{-1} fresh weight after 28 months. Grazing cows receiving 10 mg additional Cd per kg feed based on 88 % dry matter for 3 months had a mean Cd concentration in the kidney of about 2.2 mg kg^{-1} fresh weight.

In all but one case the concentrations of elements in kidney were significantly higher than those in liver.

Other tissues

From the cows kept indoors only one sample of heart, thymus, spleen, brain and bone tissue was analysed.

Accumulation of Cd was only found in the spleen and to a lesser degree in thymus. The concentration for the control group was 6 μg kg^{-1} fresh weight whereas the highest concentration of 55 μg kg^{-1} was found for the group treated with soluble compounds.

The highest increase for lead was found in bone tissue. The control group showed

Table 6. Concentrations of elements (mean ± s.e.m. in μg per kg fresh weight) in the kidney.

	n	Cd	Pb	Hg	As
Cows on pasture					
control	4	270 ± 46	510 ± 70	9 ± 1	17 ± 2
soluble compounds	4	2250 ± 660	910 ± 150	24 ± 3	45 ± 9
Cows kept indoors					
control	2	300 ± 130	420 ± 5	5 ± 1	53 ± 3
soluble compounds	2	6080 ± 700	1190 ± 135	79 ± 7	160 ± 20
harbour sludge	1	1670	1000	50	90
sewage sludge	2	430 ± 70	660 ± 0	27 ± 5	87 ± 33

n = number of cows slaughtered.

2.6 mg kg^{-1} and the group with soluble compounds 7.3 mg kg^{-1} fresh weight.

Spleen showed the highest mercury concentrations, which were 3 μg kg^{-1} for the control group and 7 μg kg^{-1} for the group treated with soluble compound.

Arsenic showed accumulation in heart, thymus, spleen and brains. The highest concentrations were found in spleen tissue and were 22, 120, 160 and 88 μg kg^{-1} for respectively the control group, the group treated with soluble compounds and the groups treated with harbour and sewage sludge.

Pathomorphological examination (Table 7)

Gross lesions

Liver. In 4 cows (1 control and 3 experimental) chronic inflammatory processes (2-3 cm in diameter) were observed, often with local chronic peritonitis with adhesion of the omentum minus. In one cow, on a diet including soluble compounds, chronic abscesses were found.

Kidney. In 3 experimental cows a focal nephritis was observed, in one case with a large number of foci scattered throughout the cortex. In the other 2 cases the foci were less numerous.

Spleen. 2 cows (one from the control group and one fed soluble compounds) revealed a chronic perisplenitis.

Table 7. Survey of pathological lesions[1] in the screened organs.

Organ	Type of lesions	group[2] →	I		II		III	IV	
		cow No →	166	242	298	334	117	214	362
Liver	abscesses/chronic inflammatory processes		+	–	–	++	++	+	+
	granulomas/portal cell infiltrates		++	+	–	++	++	+	+
Kidney	interstitial focal nephritis		+	+	++	+++	+	++	++
	calcium deposits in the medulla		+	–	+	+	–	–	–
Spleen	perisplenitis		–	+	+	–	–	–	–
	germinal centre formation in Malpighian corpuscles		+	+	+	+	+	+	+
	haemosiderosis		+	+	+	+	+	+	+
Heart	sarcosporidiosis		+	+	+	+	+	+	+
	mononuclear cell infiltration in the myocardium		++	–	+	–	–	+	+
Abomasum	mononuclear cell infiltration		+	++	+	+	++	+	+
Small intestine	mononuclear cell infiltration		++	++	++	+	++	+++	++
Colon	mononuclear cell infiltration		+	++	+	+	+	+	++
Uterus	endometritis		–	–	–	+	–	–	–
Skeletal muscles	sarcosporidiosis		–	–	–	–	–	+	–

[1] + slight; ++ moderate; +++ serious.

[2] I control; II soluble compounds; III harbour sludge; IV sewage sludge.

Genital tract. One cow from the control group showed a unilateral hydrosalpynx. No macroscopical abnormalities were found in the other organs screened.

Histopathological lesions
Liver. In 6 cows (2 control and 4 experimental) portal mononuclear infiltrates and tiny granulomas were found. In general, the lesions had a mild character. In two cows the lesions were more serious and consisted of portal fibrosis (No 166) with necrotic foci and micro abscesses (No 117).
Kidney. Besides the cows with a macroscopically observed nephritis the other cows also showed lesions characteristic of interstitial focal nephritis. In the latter, the lesions were of lesser degree. In one case (cow 334) the nephritis had a chronic character. Calcium deposits were present in the medulla of 3 cows (1 control and 2 experimental).
Spleen. In all cows a varying degree of haemosiderosis was found. The Malpighian corpuscles showed more or less pronounced germinal centre reaction. However, there was no essential difference between control and experimental cows.
Heart. Sarcosporidiosis occurred in all cows. In the myocardium of 3 cows (1 control and 2 experimental) mononuclear cell infiltrates were seen, mostly perivascularly localized.
Rumen. No lesions were observed.
Abomasum. In 6 of the 7 cows a mild cellular infiltration of the propria mucosae was observed. The infiltration was predominantly lympho- and plasmacellular with a small number of globular leucocytes and eosinophils.
Small intestine. All cows displayed a mononuclear cell infiltration of the propria mucosae of varying intensity. In addition many globular leucocytes were present. The picture by mild infiltration was mostly diffuse and by severe infiltration follicles were also observed. In 2 cows (1 control and 1 experimental) haemosiderin was found in the propria mucosae.
Colon. In all cows the picture was characterized by a mild infiltration in the propria mucosae. The infiltration was mainly lympho- and plasmacellular with a different number of globular leucocytes.
Uterus. In one cow (No 334) a slight endometritis was seen with small haemorrhages and haemosiderin in the propria mucosae.
Skeletal muscles. In 1 cow sarcosporidiosis was observed.

Discussion

Milk and blood

The low concentrations of Cd, Pb, Hg, and As in milk, listed in Table 3, can be compared with the results of monitoring studies. De Ruig (1976 and 1977) analysed 162 samples of cow milk in the Netherlands and found mean values of 0.7 μg Cd, 30 μg Pb, and 1.2 μg Hg per kg. Heeschen (1982) found in consumption milk from West Germany 1.5 μg Cd, 20 μg Pb, 5 μg Hg and 4 μg As per kg. In carry-over studies Sharma et al. (1982) found in milk from dairy cows which were not supplemented,

approximately 15 μg kg^{-1} for both cadmium and lead. This level for Cd is extremely high. Dietary supplementation of $CdCl_2$ up to 11.3 mg Cd per kg dry matter did not provoke increased levels of cadmium in milk. In contrast, dietary Pb at 30 mg per kg dry matter increased Pb in milk to about 60 μg kg^{-1}.

At similar levels of supplementation, as in Table 2, Sharma et al. (1982) found no increased concentrations in the blood.

Muscle tissues (meat)

The concentrations of the elements found in meat are in general comparable with those of monitoring studies (van der Veen, 1983; Kramer et al., 1983; Sahli, 1982) and of carry-over studies (Kreuzer et al., 1981; van de Ven et al., 1977; Sharma et al., 1982). The measured concentrations are considerably lower than the proposed tolerance values, which are for beef 0.05 mg Cd, 0.4 mg Pb and 0.05 mg Hg per kg (Klitsie, 1983). In the Netherlands, a so-called action level has been chosen for arsenic in meat of 0.03 mg per kg fresh weight. If during monitoring work such a level is reached or exceeded it is considered to be essential to trace the sources of contamination. The group of cows fed on soluble arsenic (dietary concentration of 1.6 mg As per kg, based on 12 % moisture) just reached this level.

Liver and kidneys

Cadmium

The review of published carry-over and monitoring studies (Tables 8 and 9) shows a significantly lower concentration in liver than in kidney. However, this does not al-

Table 8. Results of transfer experiments for cadmium with dairy cows reported in recent literature.

Ration	Concentration in ration (mg per kg DM)	Experimental period	Chemical form	Mean concentration (mg/kg) in			Remarks	Reference
				kidney	liver	meat		
Lucerne, silage and concentrates	0.1	9 months	–	7.4± 2.5	1.2± 0.3	0.12± 0.08	concentrations in tissue on DM basis	Baxter et al. (1982)
	11.5	9 months	unknown	54.0± 6.0	19.4± 6.0	0.27± 0.04		
Common	0.47-0.68	2.5-7 years	unknown	0.65-3.18	0.09-0.88	0.005	fresh weight	Kreuzer et al. (1981)
Lucerne, barley and soya beans	0.18	3 months	–	1.35± 0.89	0.64± 0.39	0.054	fresh weight	Sharma et al. (1982)
	2.40	3 months	$CdCl_2$	3.58± 1.66	0.73± 0.24	0.064		
	11.29	3 months	$CdCl_2$	8.83± 4.34	3.21± 0.18	0.018		
Grass	0.2-0.4	5±1.5 years	unknown	0.8 ± 0.4	0.23± 0.06	0.02± 0.02	fresh weight	van de Ven et al. (1977)

Table 9. Results of monitoring studies on cadmium in edible products of animal origin reported in (recent) literature.

Mean concentration (mg per kg fresh weight) in				Remarks	Reference
kidney	liver	meat	milk		
0.43 ± 1.07	0.08 ± 0.10	–	–		Flanjak & Lee (1979)
			0.0015	range: 0.0005-0.07	Heeschen (1982)
0.25	0.10	0.05			Solley et al. (1981)
0.37 ± 0.52	0.06 ± 0.09	0.001 ± 0.003			Kramer et al. (1983)
0.34	0.09	0.003			Van der Veen (1983)

ways imply a higher quantity of Cd retained by the kidneys than by the liver (see Table 13), because the weight of the liver is 5-6 times higher than the weight of both kidneys of a cow. Calculations show that the percentage of Cd consumed which was recovered in liver and kidneys was lower for cows fed on sludge material than for cows fed on cadmium acetate. This may be due to a lower availability of Cd in sludge.

The proposed Cd tolerance levels of 1.0 mg kg^{-1} in liver and of 3.0 mg kg^{-1} in kidney (Klitsie, 1983), were exceeded only for cows fed on cadmium acetate (see Tables 5 and 6). Sharma et al. (1982) found kidney cadmium concentrations higher than 3.0 mg per kg fresh weight after a dosing period of only 3 months at mean dietary levels of 2.4 mg Cd per kg dry matter (Tables 8 and 9).

Dutch monitoring studies showed a mean level of 0.09 and 0.47 mg Cd per kg fresh weight for liver and kidney, respectively (van der Veen, 1983).

Lead

Although the lead concentration in kidney was higher than that in liver, the total quantity of lead in kidney is lower than that in liver (see Table 13). Lead acetate treatment showed a higher quantity retained in these organs than harbour and sewage sludge treatment. The proposed tolerance levels in liver and kidney are 1.0 and 1.5 mg Pb per kg fresh weight respectively (Klitsie, 1983). Such high levels were not observed (Tables 5 and 6). Baxter et al. (1982) used an addition of 50 mg Pb per kg dietary dry matter in the experimental group for 9 months (see Table 10) resulting in mean concentrations in liver and kidney of about 5 and 4 mg kg^{-1}, respectively.

Sharma et al. (1982) used lead acetate and found with heifers after 3 months exposure similar levels in liver and kidney as we did in our study with grazing dairy cows.

Recent Dutch monitoring studies (van der Veen, 1983) revealed median concentrations of 0.14 and 0.38 mg Pb per kg fresh weight for liver and kidney, respectively. Also Flanjak & Lee (1979) and Kramer et al. (1983) observed similar levels of lead in these organs as a result of monitoring research.

Kreuzer et al. (1981) carried out a monitoring study based on practical situations and found up to about 1.4 mg Pb per kg in kidney and up to about 1.6 mg Pb per kg in liver, based on fresh weight, whilst the dietary concentrations in general ranged from 36 up to 64 mg Pb per kg dry matter.

Table 10. Results of lead reported in recent literature: transfer experiments and monitoring studies.

Ration	Concentration in ration (mg per kg DM)	Experimental period	Chemical form	Mean concentration (mg/kg) in				Remarks	Reference
				kidney	liver	meat	milk		
Lucerne, silage and concentrates	1.8	9 months	unknown	1.4 ± 0.6	0.6 ± 0.6	0.4		tissue on dry matter basis	Baxter et al. (1982)
	50.0	9 months	unknown	4.3 ± 1.1	4.9 ± 1.0	0.2			
Common	36-64 (1 farm: 163)	6 years	unknown	1.91-6.75	1.12-6.20	0.05-0.17		idem	Kreuzer et al. (1981)
Lucerne, barley and soya beans	3.67	3 months	–	0.63± 0.32	0.17	0.02		tissue concentrations on fresh weight	Sharma et al. (1982)
	9.23	3 months	lead acetate	1.24± 0.19	0.42	0.07			
	31.45	3 months	lead acetate	4.04± 0.09	1.73± 0.91	0.03			
Grass	210	6 months	as in automobile exhaust	mean concentration in blood: 0.286 μg/ml (control level: 0.109 μg/ml)				toxic level in feed 250 mg per kg DM	Verhoeff et al. (1981)
				0.22± 0.29	0.14± 0.15			fresh weight	Flanjak & Lee (1979)
							0.02	0.0001-0.09	Heeschen (1982)
				0.04± 0.08	0.05± 0.07	0.02		fresh weight	Kramer et al. (1983)
				0.43	0.17	0.02		monitoring	van der Veen (1983)

Verhoeff et al. (1981) indicated that the minimum toxic dose for adult cattle is set at 250 mg of lead per kg feed.

Mercury (Table 11)

Table 13 shows that the total quantity of Hg in liver in most cases exceeds that in the kidneys. Concentrations in both organs of all groups are below the provisional tolerance level of 0.05 mg Hg per kg fresh liver and 0.1 mg Hg per kg fresh kidney (Klitsie, 1983). Van de Ven et al. (1977), Flanjak & Lee (1979) and van der Veen (1983) also found low concentrations.

Arsenic

Retained As is higher in the liver than in the kidneys (Table 13). Van der Veen (1983) found low concentrations in livers and kidneys, namely 0.010 and 0.050 mg As per kg fresh weight, respectively. Flanjak & Lee (1979) found similar concentrations in these organs. Sahli (1982) analysed arsenic in liver and kidney of healthy cattle and found values up to 0.40 mg kg^{-1}. Values for cattle associated with arsenic poisoning were higher than 5 mg per kg fresh weight (see Table 12) in both organs.

Table 11. Results on mercury reported in (recent) literature.

Ration	Concentration in ration (mg per kg DM)	Experimental period	Chemical form	Mean concentration (mg/kg) in				Remarks	Reference
				kidney	liver	meat	milk		
Grass, outer marches	0.03-0.05	5±1.5 years	unknown	0.01 ± 0.01	0.006± 0.002	0.004± 0.001	–	fresh weight	van de Ven et al. (1977)
–	–	–	–	0.006± 0.016	0.005± 0.005	–	–	fresh weight	Flanjak & Lee (1979)
–	–	–	–	0.01-0.15	0.01-0.03	0.01-0.02	–	fresh weight	Kramer et al. (1983)
–	–	–	–	–	–	–	0.005	range: 0.0-0.025	Heeschen (1982)
				0.006	0.002	0.001		monitoring	van der Veen (1983)

Table 12. Results on arsenic reported in literature.

Ration	Concentration in ration (mg per kg DM)	Experimental period	Chemical form	Mean concentration (mg/kg) in				Remarks	Reference
				kidney	liver	meat	milk		
Grass, outer marches	0.3-0.7	5±1.5 year	unknown	0.10	0.04	0.02	–	fresh weight	van de Ven et al. (1977)
–	–	–	–	0.018± 0.021	0.013± 0.015	–	–	fresh weight	Flanjak & Lee (1979)
–	–	–	–	–	–	–	0.004 (0.0-0.018)		Heeschen (1982)
Grass, contaminated	normal	–	–	0.15-0.40	0.03-0.40	–	0.0005-0.07	fresh weight	Sahli (1982)
	increased	–	–	5.0-53	7.0-70	–	0.07-1.5	fresh weight	
–	–	–	–	0.03 ± 0.04	0.02-0.09	0.02-0.20		fresh weight	Kramer et al. (1983)
				0.051	0.011	0.004		monitoring	van der Veen (1983)

Evaluation of pathological data

In the experimental as well as control cows various lesions were found. There are no indications that these lesions can be attributed to the oral application of metals. Most of these lesions can be regarded as frequently observed in slaughtered cows.

Table 13. Present results regarding mean total quantity of elements in liver and kidneys compared with the mean daily quantity in the total ration (mg).

	Ration	Liver	Kidneys
a. cadmium			
control	2	0.6	0.5
soluble compounds	32	14.1	11.6
harbour sledge	10	1.4	2.8
sewage sludge	6	1.1	0.8
b. lead			
control	50	1.1	0.7
soluble compounds	200	5.3	2.3
harbour sludge	164	2.3	1.7
sewage sludge	168	3.1	1.2
c. mercury			
control	0.2	0.03	0.01
soluble compounds	1.7	0.25	0.15
harbour sludge	3.1	0.12	0.14
sewage sludge	1.2	0.10	0.05
d. arsenic			
control	3.4	0.12	0.09
soluble compounds	33	0.94	0.30
harbour sludge	21	0.42	0.15
sewage sludge	6.8	0.22	0.16

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