SZENT ISTVÁN UNIVERSITY
Faculty of Veterinary Science

Study of toxic metals (Cd, Pb, Hg and Ni) in rabbits and broiler chickens

PhD Dissertation

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CONTENTS

CONTENTS (continued)

LIST of ABBREVIATIONS

ALA:	delta-aminolevulinic acid
ALAD:	delta-aminolevulinic acid dehydratase
ALAS:	delta-aminolevulinic acid synthetase
ALP:	alkaline phosphatase
ALT:	alanine aminotransferase
AST:	aspartate aminotransferase
BW:	body weight
Cd-B:	blood cadmium
CF:	crude fibre
CHE:	cholinesterase
CHOL:	cholesterol
CK:	creatine kinase
CoA:	coenzyme-A
CP:	crude protein
CREA:	creatinine
DM:	dry matter
EE:	ether extract
EPP:	erythrocyte protoporphyrin
extd.:	extracted
FCE:	feed conversion efficiency
GGT:	gamma-glutamyltransferase
GSHPx:	glutathione peroxidase
HCT:	haematocrit
HE:	haematoxylin and eosin
HGB:	blood haemoglobin
Hg-B:	blood mercury
LD_{50}:	lethal dose for 50% of the subjects
MCH:	mean cell haemoglobin
MCHC:	mean cell haemoglobin concentration
MCV:	mean cell volume
MTA TAKI:	Soil and Agrochemical Research Institute of the Hungarian Academy of Sciences
NFE:	nitrogen-free extract
OM:	organic matter
Pb-B:	blood lead
PLT:	platelets
PP:	protoporphyrin
PTWI:	provisional total weekly intake
RBC:	red blood cell
solv.:	solvent
TRIG:	triglyceride
TWI:	tolerable weekly intake
UL:	tolerable upper intake level
UREA:	urea
w/w:	wet weight
WBC:	white blood cell
ZPP:	Zinc/Zn protoporphyrin

SUMMARY

Nowadays there is an increasing concern in relation to human consumption of potentially toxic metal-contaminated animal products, namely, that normal cuts of meat, excluding liver and kidney. Therefore a series of trials were designed to evaluate tissue and organ of economical importance animal species, such as rabbit, responses to trace element (Cd, Pb, and Hg) levels that could actually exist in the feedstuffs in a farm. The daily rations included carrot, potato, or beetroot. The effects of toxic metal burden on growth, the digestibility, the changes of haematological and biochemical values as well as of pathophysiological processes were detected. Furthermore, for the investigation of the possible adverse effects of Ni burden, both a broiler chicken and rabbit model experiment were planned.

The carrot samples contained in DM 2.30 mg/kg Cd, 4.01 mg/kg Pb, and 30.00 mg/kg Hg, while the potato samples contained in DM 2.12 mg/kg Cd, 4.1 mg/kg Pb, and 3.44 mg/kg Hg and the beetroot samples contained in DM 4.72 mg/kg Cd, 3.03 mg/kg Pb, and 6.75 mg/kg Hg. Both the grower diet for broilers and the commercial pellet for rabbit were supplemented with 0, 50 or 500 mg/kg Ni.

The results of this study suggest that the adverse effect exerted by high concentrations of Cd, Pb and Hg can hardly be monitored by determining the classical zootechnical parameters (i.e. feed intake, body weight gain). The smaller body weight of rabbits fed carrot, potato or beetroot samples is probably due to the reduced dry matter and, consequently, the lower energy intake from these feedstuffs. Supplemental 500 mg/kg of Ni to the diet significantly ($P<0.05$) reduced weight gain, feed intake and worse FCE were observed in growing broiler cockerels.

After a 4-week ingestion of Cd, Pb or Hg, their concentrations in blood were significantly ($P<0.001$) elevated. RBC, HGB, and HCT are significantly ($P<0.05$) decreased while MCH and MCV are increased by Pb burden; macrocytic hyperchromic anaemia has developed in rabbits. The initial ZPP concentration was insignificantly ($P>0.05$) changed as a consequence of Pb-ingestion.

The increased activity of both AST and ALT and reduced activity of CHE are referring to the damage of the liver parenchyma. Both the reduced activity of GGT and the increased activity of ALP indicate toxicity of trace elements to the kidneys and/or liver. Due to the increased concentration of CREA as well, Cd, Pb and Hg burden of body could cause toxic effect to kidneys. The results of serum biochemistry have been confirmed by pathological focal fatty infiltration found in livers and by slight tubulonephrosis developed in kidneys of rabbits. Even 50 mg/kg of Ni damaged the liver parenchyma induces pathological focal fatty infiltration in broilers and rabbits.

The toxic elements (especially the Pb and Hg) reduced the activity of pancreatic amylase, trypsin and total protease, and lipase.

A large number of syncytial giant cells and degenerated cells indicating abnormal meiosis and reduced rate of spermatogenesis were observed among the spermatogenic cells in rabbit testes by Cd- or Pb-loading. The activity of ovaries could be reduced by supplementation of 500 mg/kg Ni.

The Cd burden of rabbits is highly reflected by kidneys, followed by liver. The highest mean concentrations of Pb, Hg and Ni were observed also in the kidneys. Hairs could also be good indicator of the Hg-burden but not for Cd or Pb. Concentration of toxic metals in kidneys of rabbit could exceed the their maximum permissible limit. Therefore consumption of organs like kidney and liver should be avoided if animal feed has been contaminated with toxic metals.

Motto: Healthy feed, healthy food, healthy human

1. INTRODUCTION

Today most people are to accept as a fact that "to be healthy" a well-balanced diet is required. In other words the ingestion too much or too little of anything, being whatever component of food (proteins, vitamins, or minerals), some disease, at least ill health can be invited.

Scientific data have proved that the disturbance of plant-animal-human food chain is mostly caused by the continuously increasing environmental pollution. In international estimations, human health is affected by lifestyle, genetics, and the environment in 40, 25, and 25%, respectively (KOVÁCS *et al*., 1998).

Considerable amounts of trace elements get into the environment by the human activity. Nowadays, industries (e.g. mining, steel, paint, or accumulator production, fossil fuels), traffic (e.g. exhaust fumes), and agricultural technologies (e.g. phosphate fertilizers, sewage sludge, or town-refuse composts applications) are accounted for the largest discharge of trace elements (i.e. Cd, Cr, Hg, Ni, Pb, As, Co, Se, V) to soil (KÁDÁR, 1993), water, and air.

The vegetation of Soroksár Botanical Garden (suburb of Budapest) had the lowest trace element content, including Cd and Ni, whereas that of Mechwart Park (area of Budapest full of traffic) had the highest concentrations and the flora in both Gellért-hill and Kamara-woods (areas of Budapest with less traffic) had moderate levels of toxic metals (SCHMIDT, 1999). The findings confirm the results of KERÉNYI *et al*. (1986) and REGIUSNÉ *et al*. (1990b), who noticed Cd and Pb loading of the vegetation and consequently of the livestock in the emission-areas exceed the national average values.

The composting of town refuse, in order to produce a material which would be of value as a soil conditioner or manure, becomes a new recycling process for organic waste. The nutritive value of such composts is low, but they contain considerable amounts of trace elements as shown in Table 1 (VAN BRUWAENE *et al*., 1984).

Table 1 Total trace elements contents of composted town refuse (mg/kg in DM)

Sampling place	Pb	Cu	Cd	Ni	Zn	Fe	Mn	Cr
Rome	57	130	0.8	6	365	2430	29	19
Switzerland	396	483	7.1	41	1875	3500	100	65
Belgium	595	251	5.2	87	1363	24548	856	69

Some of these elements are generally or partially essential for both plants and animals/humans, but each of them may have a mild to severe toxic effect (PAIS, 1998).

Trace metals as the Cd, Pb, and Hg are known to be environmental pollutants. Of particular importance to man is the fact that these elements affect all the ecosystem components, both in the aquatic and terrestrial system. Since little or no recycling occurs, the amount entering the environment is continually accumulated. Consequently, the contaminated environmental air and water enter the biological cycle by incorporation into plants and animals used for food and thereby eventually becomes available for absorption by man. One of the main risks of these trace metals to health is due to their effects on enzyme activities. Moreover, after accumulation these agents can be chronically toxic, mutagenic, carcinogenic,

teratogenic; they reduce fertility, injure the cardiovascular and nervous system, and other systems.

Apart from the occupational exposure, for populations thus the main source of heavy metal body burden derives from food (>70%). Drinking water and ambient air (except Pb) contribute relatively little to the daily intake.

Classical examples for bio-accumulation and toxic effects in the food chain are the *itai-itai* disease for cadmium and the *Minamata* disease for mercury, both in Japan. And now, lead poisoning in population is becoming a social and health problem in most of the industrialized countries.

In 2000, after bursting of the reservoir dam belonged to company "Aurul", which mines precious metals in Transylvania (Romania), the living world of both the rivers *Tisza* and *Szamos* in Hungary were badly damaged and thereby fish died of order of several hundred tons. Besides the cyanide, the concentration of Cu was dominant in the rivers, while the Pb was negligible at 0.02 mg/kg. The other metals (e.g. Cd) in measured concentration did not have any toxic effects (SÁLYI *et al.*, 2000). Examination of livers, however, indicates that both Pb and Cd accumulated in the different fish species with a range from 0.05 to 0.26 mg/kg (w/w) and from 0.035 to 0.35 mg/kg (w/w), respectively.

Since these health hazards, mentioned above, were recognized, significant steps have been taken nowadays to reduce Pb exposure, such as the switch from leaded to unleaded gasoline usage in motor vehicles. The production of leaded interior paint has been diminished to 0.5% by weight in Canada. Other changes to reduce Pb exposure include replacement of old lead plumbing with copper or plastic fixtures, use of silver in place of Pb in solder for pipes and cans used for food, and the reduction of Pb emission by industries into the environment (LEUNG *et al*., 1993).

In case of cadmium has also been done a lot to reduce emissions to the air and to reduce the dispersal of Cd-contaminated industrial waste to the environment. However, Cd-containing effluents can still be discharged into the sewers, resulting in the high concentration of Cd in sewage sludge. Much of the sewage sludge produced is disposed of on land where it provides plant nutrients. Many countries have recommended maximum limits to regulate the addition of Cd in sewage sludge to agricultural land: in the United Kingdom the level is <5 kg/ha (SHERLOCK, 1984), in Hungary the maximum levels are for Cd, Pb, Hg, and Ni 0.15, 10.0, 0.15, and 2.0 kg/ha/year, respectively (ÁGAZATI MŰSZAKI IRÁNYELVEK, 1990). It is only by controlling the discharge of Cd into the sewers that limitation of future increases in the Cd content in the soil and, therefore, in food, can be achieved.

Meanwhile, recently there has still been a growing concern at the accumulation of heavy metals (i.e. Cd and Pb) in pasture soils from New Zealand via Greece to Romania (BRAMLEY, 1990; ANTONIOU and ZANTOPOULOS, 1992; LĂCĂTUSU *et al*., 1996). The increased accumulation of these trace metals in the soil and their uptake by pasture species is likely to result in increased Cd and Pb accumulation in grazing ruminants.

The liver and kidneys are the most hazardous raw materials for the consumers of animal products which have been exposed previously to excessive quantities of toxic metals. To ensure the acceptability of animal products in markets and the food safety, it is desirable that the concentrations should not exceed the limit values (e.g. maximum Cd level of 1 mg/kg fresh weight). Mean concentrations in the kidneys, liver, and muscle of farm stock are, generally, lower. According to an estimation of the National Food Investigation Institute (Budapest), the Cd content in the muscle of beef, swine, and poultry is reduced from 42-46 µg/kg to 10-16 µg/kg between 1989-90 and 1993 due to the decreased utilization of phosphate fertilizers (KÁDÁR and NÉMETH, 2002). But in some areas, the accumulation of Cd in sheep, particularly offal, exceeds this permissible level. A limited increase in dietary Cd could be tolerated without causing concern to animal health.

It is important to ensure that these organs and bone from such animals should not be included in products intended for animal (e.g. domestic pets) or human consumption.

The tolerable weekly intake (TWI) of Cd in the human diet is 7 µg/kg body weight or 1 µg/kg per day. For a 5 kg infant, the permissible intake is thus only 5 µg/day. This level may be easily exceeded because of liver and kidney are preferred by children. The WHO (WORLD HEALTH ORGANISATION, 1996) report states that 2-year-old Australian children sometimes have received almost 3 times the TWI.

Nevertheless, many of the results raise an important point in relation to human consumption of potentially Cd-contaminated animal products, namely, that normal cuts of meat, excluding liver and kidney, will probably be quite safe for human consumption and will contain Cd at concentrations below the acceptable limit (BRAMLEY, 1990).

The incorporation of Ni into the food chain of soil, plant, animal, and man can lead to chronic diseases in human beings and animals.

Hungary can join the European Union (EU) mainly with agricultural products, which have been produced according to the strict quality standards and are harmless to human health.

Rabbits were involved into our studies because of their economic importance for export in Hungary. After several years of decline, in 2001 the quantity of live rabbits purchased (12,761 tons) and of the export as slaughtered and processed carcass (5,615 tons) increased as well as export income (20,643 USD). Hungary is one of the most important exporting countries in the World. Even if the importance of Italy as a buyer has drastically decreased, it remains the first customer for Hungarian rabbits (46%), followed by Switzerland (42%), Germany (8%), Belgium (2%), and Russia (1%). In the opinion of EU experts, the Hungarian rabbit meat has a stable market for many years. These appear to be confirmed by the fact that the Hungarian rabbit meat products can be exported to EU without volume restriction and are free of duty (BLEYER, 2002).

Aim of study. A series of trials were designed to evaluate the animal tissue and organ, especially the edible parts of the body, responses to trace element (Cd, Pb and Hg) levels that could actually exist in the feedstuffs in a farm. The daily rations included carrot, potato, or beetroot as home-grown vegetables. Due to the increasing interest in bio-monitoring the heavy metal exposure of humans, not only in case of occupational burden, but also in normal populations (DRASCH *et al*., 1997), the effects of toxic metal load depending on the ingestion way (dietary or oral administration) on growth and digestibility, the changes of haematological and biochemical values as well as of pathophysiological processes were detected.

Furthermore, for the investigation of the possible adverse effects of Ni burden, both a broiler chicken and rabbit model experiment were planned. Accordingly, the differences between the species and the interactions between Ni and other elements were also studied.

2. LITERATURE

2.1. CADMIUM (Cd)

Cadmium was identified as an element in 1817. Due to its favourable chemical properties, the large scale use of Cd (e.g. galvanization and alloy of other metals as well as for making paints, batteries and catalysers) dates from the 1940s (SHERLOCK, 1984; MÜLLER *et al*., 1994; LEHOCZKY *et al*., 1996).

Although the attention focused on Cd as a food contaminant only around 1970 when it was recognized that a severely painful and crippling condition in humans, known as *Itai-Itai* disease, was caused primarily by an elevated intake of Cd in rice (*Oryza sativa* L.) and drinking water. The syndrome was characterized by damage of the proximal renal tubules, bone mineral loss with multiple fractures, enteropathy, and anaemia. The affected population was multiparous, postmenopausal Japanese women, who probably had low intakes of Ca, Fe, protein, fat, and vitamin D, particularly during the period of exposure to Cd (FOX, 1988). Some studies conclude that bone disease occurred independent of kidney changes, while others suggest that skeletal deterioration resulted as a secondary response to Cd-induced renal dysfunction (WHELTON *et al*., 1997a, b).

2.1.1. Cadmium in nature

Cadmium occurs widely in nature, it is present in air, in all soils and aquatic systems. The concentration of Cd in the air of non-industrialized areas rarely exceeds 0.0025 µg/m^3. Toxic heavy metals in high concentrations are infrequently found in soils. The concentration of Cd in most soils is in the range of 0.5-1.0 mg/kg air-dry soil (VAN BRUWAENE *et al*., 1984; MÜLLER *et al*., 1994). Average Cd content in the 0-30 cm upper layer of the main soil types in Hungary varies between 0.09 and 0.54 mg/kg (LEHOCZKY *et al*., 1996); the experimental fields of Soil and Agrochemical Research Institute of the Hungarian Academy of Sciences (MTA TAKI) in Nagyhörcsök being calcareous chernozem soils naturally contain 0.19 mg/kg of Cd (KÁDÁR, 1991). The total concentration of Cd in unpolluted seawater is generally <1 µg/kg (SHERLOCK, 1984).

Considerable amounts of Cd get continuously into the environment as a consequence of human activities. The steel industry, waste disposal, volcanic action, zinc refinery, fossil fuels, and traffic are accounted for the largest part of emissions of atmospheric Cd. Raised concentrations of Cd in soil may be found as a result of industrial activities (e.g. mining) or agricultural activities (e.g. sewage sludge, phosphate fertilizers, and pesticides containing high concentrations of Cd). Consequently, concentrations of Cd above 20 mg/kg occur naturally in some mining areas; in agricultural soils excess of 2.4 mg/kg are abnormally high (KOSTIAL, 1986).

Since little or no recycling occurs, the amount entering the environment is accumulated; the soil must be regarded as a "cadmium reservoir", because Cd remains in the soil for a long time. The Cd levels in the air leads to an average inhaled 0.02-0.03 µg/day for adults. The amount inhaled from the air is insignificant compared with that ingested with food, with the exception of heavy smokers who have a Cd intake of 3 to 5 µg/day or more from this source alone. Moreover, such inhaled Cd is absorbed much more efficiently than ingested Cd (see below). Drinking water also contributes relatively little to the average daily intake, not more than 3-4 µg/day, and food is the major source of Cd for animals and non-smoking human beings.

2.1.2. Cadmium in human foods and animal feeds

Plant based foods. Cadmium is a non-essential element for plants, so there are no lower critical concentrations below which deficiency of the element would occur. All plants contain detectable concentrations of Cd (VAN BRUWAENE *et al*., 1984; BRAMLEY, 1990; MÜLLER *et al*., 1994). Individual samples of plant-based foods grown in uncontaminated environments rarely contain more than 0.2 mg/kg (on a w/w basis) and average values for Cd in specific foods are unlikely to exceed 0.1 mg/kg. Some root crops, such as carrot and parsnip (0.05 mg/kg), and some leafy crops, such as lettuce (0.06 mg/kg) and spinach (0.08 mg/kg), tend to contain more Cd than the other plant foods, such as cabbage, potato, corn grain and plums (0.01, 0.03, 0.03, and <0.02 mg/kg, respectively).

In Hungary, the maximum limit for Cd in human foods ranges 0.03-0.5 mg/kg (w/w); the lowest value is related to fresh vegetables and fruits, while the highest one is for dried vegetables and fruits (ÁGAZATI MŰSZAKI IRÁNYELVEK, 1990).

Most forages and plant materials fed to animals contain levels of Cd well below 0.5 mg/kg (on a DM basis). It is established that increasing Cd content of soil increases the Cd content of plants grown in those soils. In general, roots, including tubers, stems, leaves, fruits, and seeds, in that order, accumulate the largest amount of Cd (VAN BRUWAENE *et al*, 1984; KOSTIAL, 1986).

Aquatic food species (fish, shellfish, crab etc). Most species contain so little Cd that it is difficult to determine the concentration accurately. The average concentration of Cd is certainly <0.2 mg/kg and the Cd concentrations in fish are often <0.005 mg/kg. Shellfish contain higher concentrations of Cd than the most other species. Pollution of marine ecosystem, for example by discharge of Cd-containing sludge to rivers or seawaters, appears to have resulted in increased concentrations of Cd in shellfish but not fish (SHERLOCK, 1984).

Meat and offal. The concentration of Cd in meat is low, average concentrations being <0.05 mg/kg. Animal offal, especially liver and kidney, generally contains an average Cd concentration in excess of 0.05 mg/kg; individual samples of kidney often contain more than 0.5 mg/kg of Cd. This is not surprising because the kidneys and, to a lesser extent, the liver of animals accumulate about 65% of the Cd absorbed by the body (SHERLOCK, 1984).

Animals grazing on land contaminated by Cd or consuming fodder grown on contaminated land yield meat which contains normal or slightly elevated concentrations of Cd. However, the liver and kidneys from animals consuming elevated amounts of Cd contain significantly more Cd than is usual and this offal, therefore, should not be consumed.

Dairy products and other foods. Milk, cheese, butter, margarine, lard, and eggs contain uniformly low concentrations of Cd (0.005, 0.05, and 0.01 mg/kg, respectively). Milk from cows of high Cd intake does not appear to contain elevated levels of the element.

Rats fed a commercial lab diet retained markedly smaller doses of Cd than rats receiving milk or meat, and bread. The phenomenon may be explained by the higher fibre content of the laboratory feed. Similar effects were observed in adult male mice (SHERLOCK, 1984; KOSTIAL, 1986).

In Hungary, the maximum limit for Cd in feeds ranges 0.5-2.0 mg/kg DM; the lowest value is related to plant based feeds, while the highest one is for concentrates, except for dog and cat foods involving maximum 0.5 mg/kg of Cd (CODEX PABULARIS HUNGARICUS, 1990).

2.1.3. Cadmium in human and animals (metabolism)

The salient features of Cd metabolism are the lack of an effective homeostatic control mechanism, retention in the body with an unusually long half-time, accumulation in soft tissues, mainly in kidney and liver, and powerful interactions with other divalent metals (KOSTIAL, 1986).

2.1.3.1. Uptake and absorption. The FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (1972) has recommended a TWI for Cd, which is 0.4-0.5 mg for adults, based on a tolerable intake of 1 µg/kg body weight/day for a 60-70 kg adult. For children the tolerable intake is less. Human dietary Cd intake shows regional differences (Table 2).

Table 2 Typical dietary intakes of cadmium (mg/week) in some countries

Country	Intake
Australia	0.15
Belgium	0.35
Denmark	0.21
Germany	0.40
Great Britain	<0.15
Italy	0.38
Japan	0.27
New Zealand	0.11
Poland	0.13
USA	0.23

Maximum tolerable levels of dietary Cd for bovine, ovine, porcine, and avian species were set at 0.5 mg/kg diet (NRC, 1980a; FOX, 1988).

It may be concluded from the data in the table above that the "average" consumer in the countries for which data are presented is not endangered by Cd in the diet. Although analysis of rice indicates that the daily intake of Cd might be high in several areas in Asia (SHERLOCK, 1984).

Farm and wild animals can be exposed to Cd pollution by two main route: inhalation of polluted air and ingestion of polluted food (VAN BRUWAENE *et al*., 1984; KOSTIAL, 1986, BOKORI, 1994). The respiratory absorption is between 10 and 40% of the inhaled Cd (approximately 50% in human), depending upon the cadmium compounds. The absorption of ingested Cd differs by animal species and by type of compounds. No evidence exists for a homeostatic mechanism to limit Cd absorption and retention below a non-toxic threshold when toxic levels are consumed. In general, the intestinal absorption is low: 0.3% in goat; 0.035-0.2% in lactating dairy cow, and 5% in swine, while 2-6% of dietary Cd is absorbed in human.

Gastrointestinal absorption is influenced by a number of physiological and dietary factors. The influence of age on Cd absorption is well established in animals. Experiments on mice and rats show a 10 times higher absorption of Cd in suckling and young animals than in adults. Some human and animal data indicate that Cd absorption might be higher in females than in males (REGIUSNÉ *et al*., 1984a).

Cadmium absorption is influenced by different dietary factors (KOSTIAL, 1986; FOX, 1988). Intakes of the interacting nutrients include Zn, Cu, Fe, Se, Ca, vitamin D, ascorbic acid, protein, and fibre, in amounts greater than the requirement, decrease, and deficiencies increase the absorption and effects of Cd. Competition for binding sites provide a reasonable explanation repeatedly for the antagonism between Cd and essential elements such as Zn and Fe. The elevated dietary Zn (200-600 mg/kg) would be a practical means of reducing Cd in liver, kidney, and muscle of domestic animals. Increased sensitivity to Cd in mice fed low Cu diets has been detected.

Women with low body Fe stores had on average twice higher gastrointestinal absorption rate than in the control group of women. These findings have been confirmed in animal species including Japanese quail, chicken, and mouse. The effect of ascorbic acid is believed to increase Fe absorption. Diets low in Ca are associated with significantly higher levels of absorption and deposition of Cd in the tissues of mice. Diets deficient in vitamin D also lead to increased Cd absorption. On the other hand, vitamin D_3 supplementation is a suitable method to reduce the risk of Cd burden in poultry (KORÉNEKOVÁ *et al*., 1997).

Compared with rats fed optimum protein (21%), the Cd concentrations in the body were lowest with the low-protein diet (5.5%) and highest with the high-protein diet (67.5%). The high-protein diet caused more severe renal tubular necrosis than the other diets. The adverse effects of the high-protein appear to be due to its cystine content.

In vitro experiments resulted in the following sequence of fibres binding Cd (in increasing order): cellulose, glucomannan, pectin, sodium alginate, sodium carboxymethyl cellulose, and lignin. Incorporation of 5% levels of either lignin or carboxymethyl cellulose into a purified casein rat diet caused decreased concentrations of Cd in the liver and kidneys. Cellulose had no such effect.

Researchers have long postulated that the chemical form in which Cd occurs in foods may affect its bio-availability. Tissue Cd uptake from the food was less than that from the inorganic reference sources (FOX, 1988).

2.1.3.2. Cadmium in tissues. Cadmium is virtually absent both from the human and the animal body at birth and its concentrations increase with age up to approximately 50 years. At this age the total body burden of a "standard" non-exposed middle-aged person varies from about 5 to 20 mg (5-7 mg for non-smokers and 8-13 mg for smokers).

Cadmium is taken up from the blood into the liver, where incorporation into metallothionein occurs (KOSTIAL, 1986; NORDBERG and NORDBERG, 1987; MASSÁNYI and UHRÍN, 1996). Metallothioneins are a class of low molecular weight (6000-7000), cysteine-rich (30%) metal-binding (5 to 10% w/w) proteins found in highest concentrations in liver and kidney tissues. The protein is known to bind various metal ions such as Cd, Zn, Cu, and Hg, and its biosynthesis is closely regulated by the levels of exposure of an organism to salt of these metals. SZILÁGYI *et al*. (1996) reported the concentration of metallothionein may be induced by Cd in the liver and kidney cortex of rabbits and chickens. There are great differences in the metallothionein concentrations of the different animal species, which may explain the great differences among species in sensitivity to metal burden.

Cadmium is then slowly released from the liver into the blood for transport to other organs. Normal human blood is low in Cd. In non-occupational exposed persons the mean blood level is usually <1µg/L. In rats receiving Cd in drinking water, the blood concentration increased to plateau values after 3 months and was proportional to the concentration in drinking water. Blood is therefore considered to reflect recent exposure.

About half the body burden of Cd is localized in the human kidney cortex and liver. The Cd concentration in the kidneys of "normal" people is about 10 to 15 times higher than in the

liver, values from 10 to 14 mg/kg. Women often have higher renal concentrations than men. Beyond 50 years of age the levels of renal Cd remain essentially constant or decrease.

It is generally recognized that ruminant and especially cattle are more exposed to local pollution situation than pigs or other intensively bred domestic animals for human consumption. Total retention observed in goats was about 0.3-0.4% of the administered dose. In cows total retention was estimated to be about 0.75% of the dose, 14 days after an oral dosing; whereas 131 days after dosing, 0.13% of dose was retained. In both goats and cows, the highest cadmium concentrations were found in kidneys followed by liver, pancreas, and small intestinal wall. The Cd content in kidneys and in livers are closely correlated. Nevertheless, the Cd concentration observed in kidneys being 2-5 times more important than the Cd concentration in liver. In goats a high concentration of cadmium was also found in the wall of abomasum.

After application a mixture of $Cd(Cl)_2$ in 0-3 mg/kg and $Pb(NO_3)_2$ in 0-20 mg/kg, only minuscule quantities ($1x10^{-5}$) of the Cd and Pb in the soil pool were transferred and retained in hen tissues via ingestion of the wheat grain (BRAMS and ANTHONY, 1983).

In conclusion, both in human and animals the largest stores of cadmium are in the liver and kidney cortex (Table 3).

Table 3 The "normal" cadmium content (in mg/kg DM) of kidneys and liver of several monogastric species (MASAOKA *et al*., 1986), (mean and ±SD)

Species	Kidneys	Liver
Duck	2.0 ±0.9	0.4 ±0.1
Goose (3 yr.)	7.0 ±5	0.8 ±0.2
Goose (6 mo.)	0.8 ±0.2	0.4 ±0.1
Hen	2.3 ±1.1	0.4 ±0.2
Horse	113.0 ±56	13.0 ±0.10
Mink	1.4 ±0.9	0.7 ±0.5
Pig	0.9 ±0.6	0.2 ±0.1
Rat	0.5 ±0.3	0.13 ±0.1
Sparrow	7.0 ±4.0	0.9 ±0.6

When the Cd exposure level and/or time of exposure increased, the concentrations of Cd in these tissues increased. There is considerable evidence that when the cadmium concentration in the renal cortex reaches 200 mg/kg (w/w), proximal tubular damage occurs with marked urinary losses of Cd, Ca, glucose, amino acids, and small protein molecules (KOSTIAL, 1986; FOX, 1988).

In spite of the much lower concentrations of Cd in muscles (both cardiac and skeleton), bone, and skin, these tissues might represent a significant contribution to the body burden due to their mass. Organs that accumulate Cd include testes, lungs, pancreas, spleen, and various endocrine organs. In contrast, the concentration in bone, brain, fat, and muscle tissues is very low. The placenta and mammary gland effectively limit Cd transport into foetus and milk, thus, the concentration in organs of embryo, foetus, or newborn baby is lower by three orders of magnitude than in adult woman. The Cd concentration of cow's milk is also low (approximately 5 µg/L). The Cd concentration in hair ranges from 0.5 to 3.5 mg/kg. The Cd concentration of 0.55 to 1.2 mg/kg in wool was not significantly increased by dietary supplementation.

Since only unimportant Cd transferred to muscle (meat) or to milk it is apparent that the main animal products (other than liver and kidneys) from even exposed areas may also be used as food (MASAOKA *et al.*, 1986).

2.1.3.3. Biological half-life. The binding of Cd by metallothionein and deposition in the kidney and other soft tissues apparently accounts for its very long half-life in the body. For humans values of 10 to 38 years have been reported (KOSTIAL, 1986; FOX, 1988); in case of cattle liver and kidney this value is <2 years and >12 years, respectively (SCHENKEL, 1988).

Experimental studies, whatever the conditions may be, produce much shorter estimates of half-life of Cd in animals than in humans, ranging from several weeks in mice to 2 years in monkeys. Variations in exposure time, basal metabolic rate and lifespan of animal species, and interactions between Cd and other exposure factors may explain the wide differences.

2.1.3.4. Excretion. The continuous synthesis of Cd-binding metalloprotein in the liver and kidneys causes very slow elimination of Cd. It has been estimated that 0.01% of the body load is excreted daily, to a large extent via urine (0.5-2.0 µg/L), but also via bile, the gastrointestinal tract, saliva, and sweat. Cadmium is also eliminated through hair fall, skin scurf, but these routes are of limited importance. Cadmium exposure from whatever source tends to increase the daily urinary output of the element. Although total urinary excretion observed in goats and cows was low: 0.03-0.05% of the administered dose after oral ingestion.

Animal studies show that the faecal excretion is considerably higher than the urinary excretion: in goats and cows, about 80-90% of the total ingested Cd is excreted via the faeces within 5-14 days after the end of the application. Faecal excretion therefore appears to reflect the dietary intake closely. The excretion in the urine and bile was negligible, no more than 0.05% of the dose (KOSTIAL, 1986).

2.1.4. Health effects

2.1.4.1. General. Cadmium is a toxic trace element for the population, whether ingested, injected, or inhaled. Its toxicity has been demonstrated experimentally in numerous animal species including cattle, sheep, goat, swine, chicken, Japanese quail, dog, rat, and mice. Many of the data are derived from these studies using relatively high parenteral doses. Of much greater practical importance are studies that investigate adverse effects with chronic exposure at lower levels as they may be found in the environment. There are large differences between the effects of acute and chronic exposures of Cd.

The signs after single injections of high Cd doses into animals relate to reproductive organs (e.g. testicular and placental necrosis) and the nervous system, but a number of lesions in other organs may also occur. Nevetheless, testicular necrosis can be induced by relatively low doses that do not damage other organs. However, in acute toxicity experiments (i.p. or s.c. 0.5-1.0 mg/kg Cd) where only mortality is recorded, the effects of Cd on the liver may be the most important.

In animals, lung damage is predominant after short-term inhalation, and at high inhalation exposures (5 mg/m^3) lethal oedema might occur.

After short-term oral exposure the type of damage is to some extent dependent on animal species. Fortunately, acute Cd toxicity caused by food consumption is rare. In contrast to human, rats may tolerate large concentrations without gastrointestinal reactions. Therefore, both liver necrosis and other lesions may be observed after oral exposure.

With higher concentrations of Cd in the diet (chronic exposure) a wide range of adverse effects can occur in animals, including reduced food intake, depressed growth (loss of

weight), enteropathy, anaemia, poor bone mineralization, severe kidney damage, cardiac enlargement, hypertension, and foetal malformation (abortion). The exposure to Cd causes a special type of proteinuria (probably metallothionein) as well. A dose of 50 mg/kg Cd caused only a slightly reduced feed intake and weight loss in both adult cows and mature ewes but above a dose of 200 mg/kg Cd, anaemia was observed. Cadmium decreased RBC (red blood cell) and HGB (blood haemoglobin) values.

More or less severe growth retardation and tubulonephrosis in the kidneys in almost all chickens exposed to Cd load were observed (BOKORI *et al*., 1995).

In conditions of oral Cd exposure it is claimed that at least 5 μg/kg are usually required to produce physiological effects. However, minimum toxic levels or maximum safe dietary Cd levels can not be given with any precision, because Cd metabolism is so strongly influenced by dietary interactions (see above).

2.1.4.2. Renal Effects. The kidneys are the organs that exhibit the first adverse effect following long-term moderate to excessive exposure by both inhalation and ingestion. Cadmium causes primarily renal tubular lesions, but there may also be glomerular lesions. The main feature of renal dysfunction caused by Cd is an increased excretion of low molecular weight proteins. In addition, a reduction in glomerular filtration rate has been observed. These effects are generally seen at average renal cortex concentrations of 200-300 mg/kg (w/w). Such results were obtained in different animal species such as rabbits, rats, swine, and monkeys. A dietary concentration greater than 200 mg/kg increased the blood urea nitrogen levels in both cow and sheep during the exposure to Cd.

The renal toxicity of Cd is due to its rapid uptake and degradation by renal proximal tubular cells. Effects on lysosomal enzyme activities and possibly membrane lesion processes appear to be due to the rapid release of toxic cadmium ions (Cd^{2+}). Tubular dysfunctions are irreversible.

2.1.4.3. Effect on Reproduction (and Foetal Development). Cadmium is taken up in reproductive tissues such as gonads and uterus. The animal and human placenta accumulates cadmium, but Cd transport into the conceptus is low (KOSTIAL, 1986).

Damage to reproductive tissues is considered to be the critical effect of Cd after acute parenteral doses. Acute effects include acute hemorrhagic necrosis in testes, haemorrhages and necrosis in non-ovulating ovaries, and destruction of the placenta during the last third of pregnancy. Acute Cd dosing also blocks embryonic implantation in sexually mature rats.

There are some indications that low-level Cd exposure can affect the placental blood vessels in animals. In pregnant cows and sheep for all doses (50-500 mg/kg Cd) aborted foetuses, neonatal death, and newborns with birth defects, as infertility were observed. After stopping the Cd treatment, the declined milk production has increased within 10 days to the previous production in lactating cows.

Signs of Cd toxicity were exhibited in 8-week-old swine receiving 450 and 1350 mg/kg Cd for 6 weeks. The skin covering the inner part of the hind legs and ears were red and scaly. Growth rate decreased only in the 1350 mg/kg group. While HCT values were the most sensitive criteria of toxicity, they decreased in all Cd fed (i.e. 50-1350 mg/kg) pigs.

Syrian hamster are the most susceptible species to $CdCl_2$-induced ovarian hemorrhagic necrosis at all ages. (MASSÁNYI and UHRÍN, 1996)

Toxicity of Cd in chickens has been already observed at Cd concentrations of 60-90 mg/kg feed. Body weight and feed conversion efficiency were reduced as a function of Cd intake. In laying hens fed 7-10 days a ration containing 50 mg/kg, a total stop of egg laying has been observed. BOKORI *et al*. (1994) noticed that the egg production of Japanese quails exposed to Cd load rapidly decreased.

2.1.4.4. Hypertension. Much evidence exists linking Cd exposure to hypertension in man and animals, but the mechanism of chronic Cd hypertension is still poorly understood. A number of mechanisms have been postulated to explain the effects of Cd on the cardiovascular system, including interference with catecholamine metabolism, direct action on vascular walls, modification of cardiac performance, and involvement of the renin-angiotensin-aldosterone system, possibly triggered by changes in sodium reabsorption (KOPP *et al*., 1983; KOSTIAL, 1986).

2.1.4.5. Carcinogenicity. Lung carcinomas in rats exposed to Cd chloride aerosols for 18 months by inhalation (12-50 $\mu g/m^3$) provide sufficient evidence for carcinogenicity of Cd. Testicular tumours in mice and rats given Cd metal or salts also indicate carcinogenicity of certain Cd compounds. However, no carcinogenic response has been observed with ingested Cd, and its potency via the oral route is at least 200 times less than that via inhalation in experimental animals. Epidemiological studies do not provide sufficient evidence of a risk of prostate cancer from exposure to Cd. However, evidence from the same studies seems to provide an excess risk of lung cancer in Cd smelter workers.

2.1.4.6. Effect on Skeletal System. Renal dysfunction can cause mineral disturbances that eventually may cause uroliths or osteomalacia. In humans, there are large differences between men and women with regard to secondary effects caused by long-term exposure to Cd. Renal stones have been common among the male workers, whereas osteomalacia has been found in women with Cd-induced renal damage. Most results indicate that Cd can induce osteomalacia or osteoporosis in animals, but negative results have been observed in rabbits, mice, and monkeys. One of the reasons for decreased calcium absorption could be the inhibition by Cd of vitamin D_3 hydroxylation in the renal cortex. The formation of the metabolite 1,25-dihydroxycholecalciferol can be almost totally suppressed by high dietary Cd exposure in rats. Consequently, the concentration of the calcium-binding protein in intestinal mucosa is also decreased by Cd exposure.

2.1.4.7. Other Effects. Slight anaemia has been found in exposed workers but is not common. Animal studies have shown that it is an iron deficient anaemia, and a decreased gastrointestinal absorption of Fe due to Cd might be the same mechanisms. Animal experiments have indicated that morphological and enzymatic changes may occur in the liver. Cadmium has the potency to interfere with the immune system, increasing the susceptibility of rabbits, rats, mice, and carps to infections (VAN BRUWAENE *et al*., 1984; KOSTIAL, 1986; SÖVÉNYI and SZAKOLCZAI, 1993), however, a sub-toxic dose of Cd (25 mg/kg p.o.) apparently had no effect on the *in vivo* immune response in rats (GRO *et al*., 1983).

2.2. LEAD (Pb)

The Pb content of the uppermost layer of the earth's crust (16 km thick) amounts to 0.0016%. Lead occurs principally as the ore known as galena, or lead sulphide (PbS), which was already known to the Egyptians 5000 years ago. The properties of Pb stimulated a diversified application of this metals, especially in alloys with other metals. Egyptians used Pb salts in ancient times to kill. Due to its widespread use, Pb intoxication is common and has been already recognised in ancient Greece. Hippocrates (370 B.C.) connected Pb exposure for the first time with subsequent clinical signs. It has even been suggested that the decline and fall of the Roman Empire partly may have attributed to Pb intoxication of the upper classes, leading to low fertility. (REICHLMAYR-LAIS and KIRCHGESSNER, 1984; PEEREBOOM-STEGEMAN, 1987; PAIS, 1998). Over the last half century, human exposure to Pb have changed in origin, but have probably not changed significantly in amount. At the levels to which human beings are exposed in the workplace as well as the overall environment, Pb has been shown to be a toxic element in most of its chemical forms.

Of particular importance to man is the fact that Pb contaminating environmental air and water enter the biological cycle by incorporation into plants and animals used for feed and food and thereby eventually becomes available for absorption by animals and man (DEMICHELE, 1984).

2.2.1. Lead in nature

In nature, Pb is primarily in inorganic form, ubiquitous and varies widely in concentration. Under normal conditions the Pb content in air ranges from 0.04 to 0.27 $\mu g/m^3$, in drinking water from 10 to 30 µg/L. The WHO recommended limit for Pb in drinking water is 100 µg/L, the EU limit of 50 µg/L. The concentrations in reality are often higher because of increasing environmental pollution. This is especially the case in the areas surrounding the emission sources, such as automobile and industrial pollution. Fifty to 70% of the Pb compounds are emitted with the exhaust fumes. Consequently, the Pb content of the vegetation near busy streets may rise above 300 mg/kg in extreme cases. The Pb concentration of the air may reach levels between 2 and 20 $\mu g/m^3$, depending on traffic density and climatic conditions. Lead-containing equipments (e.g. plumbers, linoleum, ammunitions, accumulators and so on) and paints also contribute to increasing levels of Pb in the environment.

The daily Pb intake of human has been estimated at 0.1-2 mg. The provisional TWI of 3 mg per person established by the joint FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES in 1972. Intake is predominantly oral, and a minor amount is taken through the respiratory tract. The lipid soluble compound (e.g. tetraethyl Pb) may also be absorbed through the skin (REICHLMAYR-LAIS and KIRCHGESSNER, 1984; QUARTERMAN, 1987; HUMPHREYS, 1991).

2.2.2. Lead in human foods and animal feeds

Lead present in food and feed may be the accidental result of technological operations, or of environmental origin. The four main sources of contamination of food and feed are soil, industrial pollution of air, agricultural technology (e.g. Pb-containing pesticide, phosphate fertilizers) and food processing. Since all soil contains Pb, practically there is no Pb-free food, a natural level of Pb exists in food according to natural levels in the soil. The calcareous chernozem soils of experimental fields of MTA TAKI in Nagyhörcsök naturally contain 4.24 mg/kg of Pb (KÁDÁR, 1991).

Human foods. Lead is widely distributed in foods, but the Pb content of staple foods such as bread, vegetables, fruit, and meat is generally small (0.02-3 mg/kg, w/w). A principal sources of Pb can be the solder in cans containing food. The mean Pb contents of fruit juices was higher for such cans (1.16 mg/kg) than for plain cans (0.09 mg/kg). However, cans made without solder should decrease human Pb intake.

In general shellfish contains more Pb than other foods, but the widest variation has been found in root and green vegetables. The deposited Pb is not all (approximately 50%) removed from plant by washing (QUARTERMAN, 1987). It also seems probable that cooking may alter the trace element content of vegetables. Lead content of potatoes was determined in the United States and in the United Kingdom. The results suggested that the amounts of Pb in potatoes vary more than is generally realised, ranging from 0.2 to 31.7 mg/kg DM. There is a general consensus that possibly only 5-10% of the Pb ingested from potatoes and other vegetables is absorbed (WARREN, 1967). High concentrations of Pb have also been recorded in carrot (0.2-10 mg/kg DM), and lettuce (0.1-20 mg/kg DM). However, in root crops most of the Pb is concentrated on the surface (PETERSON, 1978).

Meat products are little affected by the Pb content of the animals' diet. When the dietary Pb of sheep, pigs, or chickens was increased to 500 or 616 mg/kg, there was only a slight increase in muscle Pb content, that of other tissue rose to 4 mg/kg. Only kidneys and bone showed larger increases, to 20 and 90 mg/kg, respectively.

Concentration of Pb in milk is normally approximately 0.02 mg/kg. When the supply from one locality is contaminated with Pb, the final product may be unacceptable. For example, in an area of high soil Pb originating from previous mining activities, silage containing up to 300 mg/kg DM Pb was produced and fed to dairy cow. Some samples of milk were found to contain up to 0.14 mg/kg Pb (QUARTERMAN, 1987).

In Hungary, the maximum limit for Pb in human foods ranges 0.5-2.0 mg/kg (w/w); the lowest value is related to fresh vegetables and fruits, while the highest one is for dried vegetables and fruits (ÁGAZATI MŰSZAKI IRÁNYELVEK, 1990).

Animal feeds. In a survey of 588 individual animal foodstuffs, including grains, milling by-products, oilseed meals, and protein concentrates, most were found to contain less than 1 mg/kg Pb, although the mean Pb contents of some cottonseed and coconut cake meals and meat and bone meals exceeded 4 mg/kg (QUARTERMAN, 1987).

In herbage, generally, the Pb concentrations were less than 3 mg/kg DM. The average concentration of Pb in plants depends not only on the plant species but also on the soil type. Since the capacity of soils to bind Pb depends on both pH and soil composition. Plants growing on ultra basic soils contained the highest concentration of Pb (49 mg/kg) whilst those on calcareous soils contained the lowest concentration (26 mg/kg). Plants collected from silicic rocks contained intermediate Pb contents (34 mg/kg) (PETERSON, 1978).

The application of sewage sludge to agricultural land leads to a large increase in the soil content of a number of toxic metals, including Pb. The fresh sludge in soil will not induce a significant increase in Pb concentration of the plant, especially those parts above ground. No significant response by wheat grain to soil Pb was observed where soil Pb levels ranged from 5 to 19 mg/kg, and Pb concentrations in grains averaged 3±1.5 mg/kg. This lack of accumulation of Pb by grain may, in part, be due to the retention of Pb in root tissues restricting movement into the grain. By contrast, sludge used long-lasting or permanent, and weathered in soil will increase in plant metal concentration (BRAMS and ANTHONY, 1983).

Moreover, changes in aerial Pb rapidly produce corresponding changes in Pb concentrations in the leaves. This is particularly apparent in herbage and other crops growing near roads. Near busy roads (20-100 m) an increase in herbage Pb is detectable and may be twice or three times the concentration in distant herbage. Animals grazing land with a high soil Pb content are at risk, because they necessarily ingest large amounts of plants

(QUARTERMAN, 1987). These are in agreement with RÓZSA *et al*. (2002), who noticed that Pb content of plants from the agricultural areas had been under the critical level (5 mg/kg) in Hungary. Lead contamination of plants from industrial areas and near busy roads was always higher than that of plants from agricultural areas and in some cases even was over the limit value.

In Hungary, the maximum limit for Pb in feeds ranges 5.0-30.0 mg/kg DM; the lowest value is related to concentrates for adult ruminants, while the highest one is for raw materials containing >8% of phosphorus (CODEX PABULARIS HUNGARICUS, 1990).

2.2.3. Lead in human and animals (metabolism)

2.2.3.1. Uptake and absorption. As it is mentioned above, the daily Pb intake is taken through the respiratory tract in minor amount. However, human occupational Pb intoxications very often result from the inhalation of Pb vapours or Pb-containing dust.

Pulmonary Pb absorption depends on the state of substances (gas, solid particles), particle size of the Pb-containing dust, respiratory volume, concentration in the air, and distribution within the respiratory tract (REICHLMAYR-LAIS and KIRCHGESSNER, 1984; QUARTERMAN, 1987). Young and active animals will inhale air in greater amounts and more deeply than older, less active ones, and are therefore likely to get more Pb from air. Particles of 0.5 µm or less are retained in the nasopharynx and tracheobronchial tree, including the terminal bronchioles. Larger particles are removed in 30-70% by the activity of the ciliated cells of the respiratory epithelium. Lead retained in the non-ciliated regions of the lung, that is mainly the alveoli, is believed to be absorbed completely. Although, a portion of inhaled Pb may be cleared by pulmonary macrophages.

The maximum tolerable dietary level for Pb is considered to be 30 mg/kg for most species (NRC, 1980b). The extent to which orally administered Pb is absorbed in small amount compared with that for other toxic metals such as mercury. According to the literature, the absorbability of Pb falls into the range of 1-15% (REICHLMAYR-LAIS and KIRCHGESSNER, 1984; QUARTERMAN, 1987).

Factors influencing the bio-availability of Pb have been studied extensively. The gastrointestinal absorption of Pb depends on many factors, such as the amount of intake, chemical form, species, age and sex of animal as well as dietary composition, intestinal interactions with other dietary constituents and presence of bile acids.

When rats were given a semi-purified diet containing 10% Pb, the retention of Pb in the tissues was from 5 to 50 times higher than in rats given a commercial feed (QUARTERMAN, 1987).

With most toxic metals the extent to which they are absorbed, and consequently their toxicity, is markedly influenced by the water solubility of the material ingested (DEMICHELE, 1984; REICHLMAYR-LAIS and KIRCHGESSNER, 1984). HUMPHREYS (1991) points out that in case of Pb there is comparatively little difference between the degree of absorption of its soluble salts (e.g. $Pb(NO_3)_2$, Pb acetate), water insoluble salts (e.g. $PbCO_3$, $PbCrO_4$, PbO, PbS) and the metal. However, Pb administered in the form of the acetate, phosphate, oxide, basic carbonate is absorbed to a comparable degree. Lead given as its sulphide or as the metal itself is absorbed to a lesser extent.

There are considerable differences in the susceptibility of different species to Pb ingestion. Water deprivation increased the susceptibility of mice to Pb poisoning. The pig can tolerate high levels of Pb in the food (QUARTERMAN, 1987). Ruminants are more resistant to the harmful effects of Pb than most monogastric animals (Table 4).

Table 4 Toxic oral doses of Pb acetate in mg/kg
(LORGUE *et al*., 1996)

Species	LD (single exposure)
Cattle	600-800
Calves	200-400
Horses	400-600
Pigs	800-1000
Dogs	800-1000
Poultry	200-600
Ducks	1 g/animal

This difference is reflected in the variation in the level of Pb found in the post-mortem tissues from Pb-intoxicated animals (HUMPHREYS, 1991).

In case of young individuals, Pb absorption is increased. Children, up to the age of 8 years at least, absorbed and retained more orally ingested Pb than adults, up to 50%. The absorption of Pb, as of other metals, is very high (approximately 50%) in suckling animals. It decreases rapidly at weaning and steadily thereafter. In adult sheep, only 1-2% of orally administered Pb is absorbed from different compounds. Young, milk-fed calves may absorb some 10% of orally administered Pb. In human beings, only 5% of dietary Pb is absorbed, but with soluble Pb salts, the uptake is about 10%. In suckling mice some Pb is absorbed by pinocytosis. It is evident that Pb absorption in adults, in contrast to suckling, suggests that pinocytic absorption is less extensive in the mature mice (REICHLMAYR-LAIS and KIRCHGESSNER, 1984; HUMPHREYS, 1991).

In rats, there has been a greater retention and higher toxicity of Pb in males than females, and in sheep, castrates and females have been less severely affected than intact males. Human males have higher blood Pb concentrations than females in the same environment (QUARTERMAN, 1987).

Nutritional factors are thought to play an important role in Pb absorption, and consequently in toxic effect. Total food/feed intake, percent dietary protein, and fat and dietary intakes of Ca, P, Fe, Zn, Cu, Se, vitamin E, and vitamin D are known to influence Pb absorption.

In humans, gastrointestinal absorption of Pb increased from 10% to 35% when Pb compounds are ingested in absence of food. Feed restriction in rats also increased the efficiency of Pb absorption and retention in the carcass (DEMICHELE, 1984).

Organic components of the diet have a significant effect. Diets low in protein resulted in an elevated absorption of Pb. Amino acids with sulfhydryl groups improve the solubility and hence the absorbability of Pb compounds in weanling rats (LEVANDER, 1979; SAS, 1981; RAGAN, 1983; QUARTERMAN, 1987). Lead absorption is dependent upon both the quantity and type of dietary fat. When dietary fat was increased from 50 to 200 g/kg, Pb retention in the tissues of rats and mice was increased from two- to sevenfold. In animals fed different fats, butterfat caused the greatest increases in Pb absorption whereas fats containing large proportions of polyunsaturated fatty acids (rapeseed and sunflower oils) had little effect. In addition, high corn oil content (40%) of a diet also resulted in an increased retention of Pb in several tissues (LEVANDER, 1979; DEMICHELE, 1984; QUARTERMAN, 1987).

Of the many mineral compositions of a diet that have any importance in the absorption of Pb, Ca has the greatest effect. A decrease in Pb absorption, and retention in response to increased Ca intake and, conversely, an increase in Pb absorption, and retention in response to

reduced Ca intake have been demonstrated repeatedly. These effects are attributed mainly to interactions at the site of absorption. Lead may also be attached to the Ca-binding sites (e.g. of the erythrocytes). Calcium ions, above certain concentrations, can again displace Pb from its binding sites in erythrocytes. This displacement, however, does not become effective until Pb concentrations reach a certain level. Phosphorus (P) appears to play a role in this as well. Similarly, the Pb absorption into the tissues of rats was inversely related to the dietary content of P. Lead absorption was low in vitamin D-deficient rats and was markedly increased by vitamin D dosing. Vitamin D supplementation, however, induces a rise in the concentration of Ca-binding proteins in the intestinal mucosa and, therefore, an increase in Pb absorption (LEVANDER, 1979; SAS, 1981; RAGAN, 1983; DEMICHELE, 1984; REICHLMAYR-LAIS and KIRCHGESSNER, 1984; QUARTERMAN, 1987; HUMPHREYS, 1991).

Rats have been found more sensitive to Pb intoxication if they are Fe deficient. Increased tissue levels of Pb in Fe-deficient rats appear to be due to increased gastrointestinal absorption of Pb. The effect of dietary Fe deficiency on Pb absorption is potentially as great as that of calcium. Conversely, the intraluminal presence of Fe diminishes Pb absorption. These findings point to a competition between Pb and Fe for receptors. Lead is bound by ferritin and transferrin and by hemosiderin like compounds in liver, as well as by erythrocyte membranes, haemoglobin, and other components of blood. In all cases Pb binding is competitive with that of Fe (RAGAN, 1983; DEMICHELE, 1984; REICHLMAYR-LAIS and KIRCHGESSNER, 1984; QUARTERMAN, 1987).

Diets low or deficient in Zn increase Pb absorption and tissue Pb concentrations (LEVANDER, 1979; RAGAN, 1983; DEMICHELE, 1984; REICHLMAYR-LAIS and KIRCHGESSNER, 1984; QUARTERMAN, 1987; HUMPHREYS, 1991).

Vitamin E might have a protective effect against Pb toxicity. Vitamin E decreased the coproporphyrinuria and anaemia in rabbits suffering from subacute Pb poisoning. On the contrary, vitamin E deficiency increased the anaemia and basophilic stippling caused by Pb in rabbits. Lead can also react directly with certain membrane components of red cell, such as the phosphate groups of phospholipids, to disrupt the structure of the lipid bilayer producing changes in erythrocyte fragility. However, there was no significant interaction of Pb and Se in rats and Japanese quails since Se had no effect on the reduced ALAD (delta-aminolevulinic acid dehydratase) caused by Pb poisoning (LEVANDER, 1979; DEMICHELE, 1984; QUARTERMAN, 1987).

The bile stimulates the transport of Pb across the intestinal mucosal cells and, subsequently, the transport of this mucosal Pb into the body (REICHLMAYR-LAIS and KIRCHGESSNER, 1984).

In experiments in which different parts of the gut in rats were isolated *in situ* by ligation, the absorption of Pb was found to occur in duodenum where Pb entered the epithelial mucosal cells. Uptake of Pb by the mucosa is rapid and is followed by slower transport into the tissues reaching a maximum after 2 to 3 h. In the adult rat, Pb absorption from the intestinal lumen appears to proceed by both passive diffusion and active transport. The rate of transport usually increases with increasing concentration of Pb in the lumen. Although, a 100-fold increase in the Pb dose (from 0.05 to 5 µmol) given to rats resulted in only a 20-fold increase in Pb absorption. There appears to be a relative mucosal block for Pb with increasing intraluminal doses. There is apparently no feedback mechanism limiting the absorption of Pb, since the body burden of Pb does not influence its absorption (RAGAN, 1983; DEMICHELE, 1984; QUARTERMAN, 1987).

A large fraction of absorbed Pb (85-90% in sheep and 63-70% in cattle) is transported in association with erythrocyte membranes. The remainder is bound to serum albumin and less than 1% of blood Pb is present in an unbound form (HUMPHREYS, 1991).

2.2.3.2. Lead in tissues. Studies concerning the levels of Pb in man and the domestic animals have suggested a "natural" range of 90-400 mg/kg in the tissues, and 0.15-0.40 mg/kg in the blood. The distribution of Pb within tissues is influenced by its route of administration and chemical form. Parenterally administered Pb enters the mononuclear phagocyte system represented by the bone marrow, spleen and liver. The highest concentrations of orally administered Pb are usually found in bones, and somewhat lower levels are found in kidneys and liver. The kidney, and especially the kidney cortex contains an even higher concentration of Pb than the liver. Reasonable quantities of the Pb can be also stored in aorta and lung (1-2 mg/kg fresh weight), while the lowest are in muscle, adipose tissues, and brain (less than 0.1 mg/kg). Of the different parts of skeleton, Pb has been found in highest concentration in the teeth (35-40 mg/kg fresh weight) and the in the long bones (20-25 mg/kg). In pigs the highest concentration of Pb in bone occurred in the primary spongiosa, and greatly decreased in maturing bone tissue (QUARTERMAN, 1987). The concentrations of Pb in all tissues rise with increased intakes of Pb, particularly in the bones, liver and hair, and lesser amounts in the brain and muscles. The Pb content in hairs does not appear to be a sensitive or reliable index of the Pb status of individuals (DEMICHELE, 1984).

Lead concentration in blood of dogs kept under urban conditions was 0.13 mg/kg, while those kept in the green belt was 0.10 mg/kg (FELKAI *et al*., 1992). Although the number of cases of Pb poisoning in small companion animals (i.e. dogs) appears to be declining (MORGAN, 1994), the significant difference in Pb content between the living areas has called the attention to the pollution of air in cities, with special regard to its human and animal health aspects.

In case of dietary Pb supplementation of pregnant ewes, due to the penetration of Pb through the placenta significant concentrations of Pb could be detected in the indicator organs (i.e. liver, kidney, rib, and muscle) of new-born lambs (RÓZSA *et al*., 2002).

Compared to Cd, the influence of age on the Pb content of the organs of horses is unimportant and insignificant in all investigated parts of the body (i.e. metatarsal bone, cerebrum, and skeleton muscle) and independent of Pb supply (KOŚLA *et al*., 1989). The normal Pb concentrations of organs (i.e. rib, kidney, and liver) of Hungarian horses are higher by 24-195% than of horses from other Central European countries (REGIUSNÉ *et al*., 1990b; REGIUSNÉ, 2001).

2.2.3.3. Excretion. Lead can be excreted via faeces, urine, sweat, and saliva. Since ingested Pb is poorly absorbed, faecal excretion is particularly high and may average around 0.2 mg/day. By comparison, urinary excretion amounts to an average of 30 μg/day. The excretion through sweat, to average 60 μg/day, appears to be considerable. Absorbed Pb gets to the liver by the portal system and is partly eliminated via the bile. Accordingly, the Pb is assumed to recycle to the liver (enterohepatic circulation). Bile, thus, is an important route of excretion in the gut (LACZAY, 1995). The Pb which is absorbed enters the blood and reaches the bones and soft tissues of the body, including the liver, from which it is gradually excreted via the bile into the small intestine and from there it is eliminated in the faeces (RAGAN, 1983; DEMICHELE, 1984; REICHLMAYR-LAIS and KIRCHGESSNER, 1984).

2.2.4. Health effects

2.2.4.1. Lead toxicity. Depending on duration and severity of exposure, the effects range from non-specific gastrointestinal symptoms (anorexia, constipation, nausea and vomiting) to central-nervous symptoms (headache, nervousness, tremor, lethargy or coma) and death. All clinical symptoms result from the toxic effects of Pb, which are manifested mainly in the

blood (anaemia), nervous system (encephalopathy and neuropathy), and kidneys (renal dysfunction), the three major sites of Pb intoxication.

The anaemia results from diminished haemoglobin synthesis, haemolysis of immature erythrocytes, and direct haemolysis of mature erythrocytes with a shortened life span. The reduced haemoglobin synthesis following Pb intoxication may be attributed primarily to an inhibition of heme synthesis. Toxic Pb doses adversely affect several enzymes of the heme synthesis: ALAS (delta-aminolevulinic acid synthetase), ALAD, heme synthetase or ferrochelatase, and uroporphyrinogen I synthetase. Nevertheless, ALAD is the enzyme most responsive to Pb. The enzyme catalyses the dehydration of ALA (delta-aminolevulinic acid) to porphobilinogen. Heme synthetase or ferrochelatase catalyses the incorporation of Fe into protoporphyrin IX (REICHLMAYR-LAIS and KIRCHGESSNER, 1984; QUARTERMAN, 1987).

Due to the defective heme formation, impaired synthesis of haemoglobin must be expected also in the case of enzymes possessing heme as the prosthetic group. For example, the activity of the cytochrome P-450 complexes has been shown to be reduced, especially in acute Pb poisoning, while there was only a mild inhibition in chronic cases (DEMICHELE, 1984).

Functional, metabolic, and morphological abnormalities of the kidneys are very frequent consequences of Pb intoxication. The vessels and tubules are affected especially. In acute poisoning, tubular lesions dominate, while in the case of chronic poisoning vascular lesions occur, with development of atrophy and fibrosis. Lead accumulates in the proximal convoluted tubule cells of the renal cortex. These morphological changes bring about impairment of the reabsorptive mechanism, with the effect of porphyria, aminoaciduria, glucosuria, hyperphosphaturia and acidosis (DEMICHELE, 1984; REICHLMAYR-LAIS and KIRCHGESSNER, 1984; QUARTERMAN, 1987; KANEKO, 1989).

Cardiomyopathy also occurs as a result of both acute and chronic Pb exposure, with abnormal electrocardiograph.

Another characteristic phenomenon of Pb poisoning is the occurrence of intranuclear inclusion bodies that have been detected in the liver cord cells, proximal tubular cells of the kidney, in the osteoclasts and brain glia cells. The formation of these inclusion bodies is considered to be a protective mechanism of the body, which maintains a relatively low Pb concentration in the cytoplasm, preventing deleterious effects (DEMICHELE, 1984; REICHLMAYR-LAIS and KIRCHGESSNER, 1984; QUARTERMAN, 1987).

2.2.4.1.1. Zinc Protoporphyrin (ZPP) Assay. Despite the widespread use of the blood Pb concentration as a monitor of occupational exposure, its use has a weakness: it attempts basically to assess the body burden rather than toxicity. Some indications have been obtained that the toxic effects of Pb – before such effects become serious – would provide a far more valuable method for monitoring exposed industrial population. Because of dissatisfaction with blood Pb as a monitor of exposure, attention has been increasingly directed toward measuring some of the metabolites known to be affected by Pb absorption, as possible substitutes for blood Pb analyses. Lead interferes with the synthesis of heme and causes alteration, both quantitatively and qualitatively, in some of the intermediates involved in this synthesis. Among such metabolites are ALA, ALAD, coproporphyrin and PP (protoporphyrin). ALAD activity in blood, the ALA and coproporphyrin excretion in urine and the EPPs (erythrocyte protoporphyrins) are recommended for the assessment of Pb exposure of the organism (JOSELOW and FLORES, 1977; DEMICHELE, 1984; GRÜN *et al*., 1986).

Measurement of ALA is difficult, and the results lack sensitivity for low level Pb exposure, therefore, instead of its substrate ALA, erythrocyte ALAD is more commonly assayed. ALAD has been already inhibited at blood Pb concentrations of 10-15 µg/100 ml (0.50-0.70 µmol/L). This is to be a highly sensitive and reliable index of the blood Pb

concentration. The increased Pb level of blood of Pb exposed calves and cows resulted in an inhibited ALAD activity. There was a close negative correlation between the Pb level of blood and ALAD activity (GRÜN *et al*., 1986; KANEKO, 1989; WADA *et al*., 1993).

In the final step of heme synthesis, Fe is inserted into protoporphyrin. When hem synthesis is limited by the availability of Fe, PP accumulates and Zn substitutes for Fe. The Zn chelate or ZPP is stable and remains in the erythrocytes largely bound to globin throughout their life spans (HART *et al*., 1980; LABBÉ and RETTMER, 1989; SMITH, 1989; LABBÉ, 1992).

In RBCs from Pb-poisoned people, PP has been shown to exist as the ZPP. Iron deficiency anaemia is also associated with an increased level of ZPP in the RBC, while free (unchelated) PP is produced in the RBCs of patients with erythropoietic protoporphyria (HART *et al*., 1980; LABBÉ and RETTMER, 1989).

An average of about 10% of the total EPP is "free" in normal human, but about one-third or more is usually "free" in the blood of normal cows, dogs, rats and rabbits (SCHWARTZ *et al*., 1980).

Zinc protoporphyrin, the predominant abnormal metabolite that appears in whole blood as a result of Pb absorption or Fe deficiency, can be assayed haematofluorometrically in whole blood with great sensitivity. Zinc protoporphyrin has its own characteristic fluorescence spectrum from 500 nm to 700 nm with peak high of 594 nm (JOSELOW and FLORES, 1977; SCHWARTZ *et al*., 1980).

Zinc protoporphyrin might be substituted for blood Pb as a preferred biological index of exposure. Several advantages would be offered: ZPP more directly reflects the metabolic damage caused by Pb and is thus a more useful indicator of the effects of Pb absorption; ZPP assays are simplier, less costly, and more rapidly performed than blood Pb (JOSELOW and FLORES, 1977; SCHIFMAN *et al*., 1982).

Numerous reports have stated that the Zn-protoporphyrin is specifically elevated in Pb poisoning. At lower blood Pb concentrations, up to about 50 µg/100 ml, there was relatively little elevation of the ZPP content with increasing blood Pb concentration. At higher Pb levels, of about 50-55 µg/100 ml, the ZPP content increased rapidly (JOSELOW and FLORES, 1977). Although, SMITH (1989) found that the ZPP increased when Pb concentrations were at 15-18 µg/100 ml (0.75-0.85 µmol/L).

Unfortunately, ZPP is insensitive for the screening of blood Pb below 1.2 µmol/L, and gives frequent false-negative results (LEUNG *et al*., 1993). In addition, organic Pb compounds may follow a somewhat different course through the body, and may not exert their major toxic effects on the haematopoetic system. Hence, a biochemical index such as ZPP, may not be appropriate as a monitor for the effects of organic Pb absorption. In some other disease states (e.g. erythropoietic protoporphyria or severe Fe deficiency anaemia), the use of ZPP may also not be suitable, since elevated ZPP levels can occur in such conditions without Pb absorption (JOSELOW and FLORES, 1977).

The effects of Pb intoxication on EPP were studied in rats, rabbits, and guinea pigs. In humans, EPP levels show a logarithmic relationship to Pb levels. On an average, a 4-fold elevation of Pb level is associated with a 10-fold elevation in EPP. The extremely high levels of ZPP which accompany human Pb poisoning were not found in the animal species studied. Actually, guinea pigs showed no PP response to subcutaneous Pb (total dose 1.5 mg/kg). Dietary Pb (600 mg/kg) produced no more than four times the baseline EPP in rats. Even in those rabbits in which a 17-fold elevation of blood Pb was induced by administration of a lethal dose of Pb (total dose 14-18 mg/kg), only a 7-fold increase in EPP was accomplished (HART *et al*., 1980).

A significant positive correlation was obtained between the Pb concentrations and free EPP concentrations in the blood of calves. (WADA *et al*., 1993).

For the prediction of sub clinical Pb toxicosis in sheep, the most appropiate method has been the determination of the blood's ZPP concentration (SAS, 1980).

2.2.4.2. Lead essentiality. Despite its remarkably high toxicity, Pb had been classically administered as a treatment for variety of illnesses. Indications of a stimulatory effect of Pb on metabolic processes and the high affinity of Pb toward biological ligands gave rise to the evidence in recent years that Pb might also be an essential element.

The essentiality of Pb has been demonstrated in model studies by growth depression, biochemical changes, and clinical symptoms occurring in the progeny of rats fed a Pb-depleted diet. Very low levels of Pb (20-45 µg/kg) significantly depressed growth, decreased liver Fe store, reduced haematocrit and haemoglobin, changed the activity of some enzymes including alkaline phosphatase, and produced hypochromic microcytic anaemia. These results show that Pb is needed for the full functioning of metabolism (REICHLMAYR-LAIS and KIRCHGESSNER, 1984; QUARTERMAN, 1987).

2.3. MERCURY (Hg)

Mercury (Hg) was named for Mercury, the Roman god of commerce and theft. Mercury is not considered an essential element for living organisms. In 1953 a severe neurological disorder was first recognized among persons living in the Minamata Bay, Japan and consuming local fish suffered methylmercury poisoning. The *Minamata* disease, most ending fatally or with permanent severe disability, is characterized by cell degenerations in the central nervous system (KURLAND, *et al*., 1960; TEDESCHI, 1982. Other epidemics including also Japan (1964 to 1965) and Iraq (1971 to 1972) were caused by the consumption of home-made bread prepared from wheat treated with methylmercury fungicide (WARREN, 1989).

Mercury is a liquid, being the only metal to exist in this state at room temperature. It is extremely volatile. Toxicologically, Hg is one of the more interesting and dangerous metals. It has two characteristics which combine to give very powerful toxicological properties. First, its volatibility leads to high localized atmospheric concentrations. Second, although metallic Hg and inorganic Hg compounds are themselves toxic, bio-tansformation into short-chain alkyl Hg compounds, by bacteria, occurs readily in aquatic environments, yielding far more toxic substances. Methyl forms do not occur in animal cells and tissues in significant amounts, unless ingested or injected as such. In other words, the animal body has an extremely limited capacity to convert inorganic and various organic forms of Hg into the more toxic methyl forms (WILLIAMS, 1981).

2.3.1. Mercury in nature

Mercury occurs widely in the biosphere (CLARKSON, 1987). The most important ores of Hg, cinnabar (HgS) and calomel (Hg_2Cl_2), have been mined for 2,300 years and from which it is extracted either by precipitation with aluminium in alkaline solution or by roasting in air. The element is not particularly abundant, the usual levels in soils being 100 µg/kg, and a really high concentration would be 10 mg/kg. The calcareous chernozem soils of experimental fields of MTA TAKI in Nagyhörcsök naturally contain 0.31 mg/kg of Hg (KÁDÁR, 1991).

Modern technological developments involving the use of Hg compounds are responsible for the discharge of large and variable amounts of the element into the environment. The metal itself has widespread industrial use in Hg vapour lamps, electrolytic cells in chloralkali industry, and in different scientific apparatus. Amalgam (mixture of metallic Hg and a silver-tin alloy) has been used in dentistry for over 100 years. Uses of inorganic Hg are largely dependent on its antifouling and antibacterial action, including in the paint, agricultural, cosmetic, and pharmaceutical industries; at one time these Hg compounds were used in the treatment of syphilis and skin diseases. Due to the similar effects on microorganism, a number of different organic Hg compounds (e.g. alkyl, aryl, and alkoxyalkyl) are used as seed dressings, fungicides, algacides, and diuretics. It is apparent that Hg can enter the biosphere from the variety of man-made sources and also from the burning of fossil fuels.

2.3.2. Mercury in human foods and animal feeds

Mercury can enter the food chain through the activity of microorganisms with the ability to methylate the element (bio-methylation) in sediments of waters.

Nearly all foods contain Hg with the mean level of 0.03 mg/kg (ranging from 0.005 to 0.25 mg/kg). Thus, the daily intake in a human subject on a normal diet would be of the order of 20 µg.

Fish often contain more Hg (0.5 mg/kg) than other foods; it is mainly present as highly toxic methylmercury. Therefore, in certain fish-eating populations its intake may be higher, even up to 31 µg/day. In different fish species in Hungary 80-100 per cent of total Hg content was methylmercury varying between 0.12–0.6 mg/kg (FUNDÁK *et al*., 1983). Consequently, fish and fish products are the dominant source.

In Hungary, the maximum limit for Hg in human foods ranges 0.01-0.05 mg/kg (w/w); the lowest value is related to fresh vegetables and fruits, while the highest one is for dried vegetables and fruits (ÁGAZATI MŰSZAKI IRÁNYELVEK, 1990).

The total Hg intake in humans should not exceed 5 µg/kg BW/week with not more than 3.3 µg/kg BW/week as methylmercury (WHO, 1996). The suggested maximum tolerable dietary level for domestic animals, therefore, is 2 mg/kg Hg for both inorganic and organic forms. Animals, however, can tolerate higher dietary quantities of the inorganic compounds, but the maximum tolerable level for this form is not increased because of the possibility of elevated tissue levels of the element. Studies with rats and mice support the proposed tolerance level for domestic animals, but with mink suggests that this species is much more sensitive to Hg (NRC, 1980c).

Although man-made Hg pollution of natural waters can greatly increase the Hg levels in freshwater fish, it should be taken into consideration that the Hg levels are high in wide-ranging ocean fish where no such pollution could have occurred. In the marine mammals the Hg was not present largely as methylmercury as it is in fish, and was highly correlated with the selenium levels. In fact, in marine mammals a 1:1 Hg-Se ratio and almost perfect linear correlation between Hg and Se were found. It was suggested that marine mammals are able to detoxify methylmercury by a specific mechanism in which selenium is involved.

In foods of plant origin little or none of the Hg is normally present in this form, while in meat and dairy products the low levels of Hg can include a small proportion of methylmercury, probably from residues in feeds containing fish meal or treated cereal grains. The Hg content of cow's milk can range from 3 to 10 µg/kg.

Little is known of normal Hg intakes by grazing or even by stable-fed farm animals. A mean Hg concentration for commercial fish meals (0.18 mg/kg) is well above the Hg levels in ordinary cereal grains or protein supplements of plant origin.

In Hungary, the maximum limit for Hg in feeds ranges 0.1-0.5 mg/kg DM; the lowest value is related to all concentrates, except for pet foods having maximum level of 0.4 mg/kg; the highest one is for fish meals (CODEX PABULARIS HUNGARICUS, 1990).

2.3.3. Mercury in human and animals (metabolism)

The metabolic behaviour of Hg varies greatly with the chemical form (inorganic or organic) in which it is presented to the animal, the extent other elements, with which it interacts, are present in the diet.

Selenium has been reported to decrease Hg toxicity. It seems reasonable that in areas naturally low in Se, individuals would be at greater risk of Hg poisoning than those in areas of high Se status (CLARKSON, 1987).

2.3.3.1. Uptake and absorption. Mammals and birds can be exposed to Hg pollution by two main routes: inhalation of Hg vapour and ingestion of polluted food. Perhaps Hg compounds are readily absorbed by the skin, as well. This route may represent a significant way of entry on industrial exposure and in the use of cosmetic and pharmaceutical preparations (WILLIAMS, 1981).

The contribution of Hg inhaled from the air is negligible compared with intake from the food, except the occupational exposure. The maximum recommended concentration in

industrial atmospheres is 0.05 mg/m^3, although this is often exceeded, at 0.2 mg/m^3, in the atmosphere near areas of high soil because of its extreme volatility. Mercury vapour is almost completely absorbed through the alveolar membrane; a range of 75 to 100% for the absorption of Hg in the rat following inhalation has been found.

The water supply is also a relatively insignificant source of Hg, except when contaminated from industrial or geological sources. Typically, 1% of the total intake of inorganic Hg is derived from drinking water and 84% from the diet.

Absorption in the gastrointestinal tract is very much dependent on the chemical form of the Hg. Absorption of elemental Hg is extremely limited, amounting to less than 0.01% of the administered dose. Inorganic Hg salts are poorly absorbed, varying from 5 to 15% of an oral dose in both humans and animals, whereas that of organic Hg compounds is much higher, about 90-95% of the administered dose in human (WHO, 1996).

2.3.3.2. Mercury in tissues. In the blood there is a tendency for the metallic Hg to be oxidized. However, this is a relatively slow process so that unoxidized vapour remains dissolved in the blood long enough for it to reach the blood-brain barrier. The brain, therefore, takes up more Hg in this way than when equivalent doses of inorganic Hg salts are injected.

Comparing the retention, distribution, and excretion between equivalent amounts of inorganic and organic Hg compounds, approximately 100 times less Hg was found in the blood of $HgCl_2$ treated rats, 10 times less in the brain, and half as much in the liver. Mercury vapour is retained to an extent of 80% of the inhaled amount; its levels in RBCs are usually somewhat higher than those in plasma, but the difference is not so high as in the case of methylmercury.

The methylmercury content of edible fish also distributes to the RBCs. People who never eat fish have very low levels of Hg in the RBCs (2-5 µg/kg). Persons with moderate fish consumption probably have mean Hg levels about 10 µg/kg. High fish consumers, especially of shark, tuna, and swordfish, have values as high as 400 µg/kg. In particular, methylmercury is able to cross the blood-brain barrier very readily.

Mercury levels range from 0.05 mg/kg to 0.3 mg/kg in most tissues of normal subjects who had not been exposed to the element, but the highest levels, at 2.7 mg/kg, were also found in the kidneys. Part of the body exposed to the atmosphere such as skin, hair (wool), and nails (horns, hooves) tend to give higher values, quite up to 5.5 mg/kg, because of exogenous contamination.

In individuals not exposed to airborne Hg, the Hg levels in hair also relate to fish consumption. In a comprehensive worldwide evaluation, the average hair Hg concentration was 1.4 mg/kg for men consuming fish once a month or less; for men consuming fish once every 2 weeks it was 1.9 mg/kg; and for once a day, it was 11.6 mg/kg up to 50 mg/kg (WILLIAMS, 1981; CLARKSON, 1987).

In contrast to organic Hg, inorganic Hg accumulates in the kidneys where it is largely bound to metallothioneins. Retention in the kidneys, therefore, is longer than in the rest of the body (FATHI, 1983).

A Polish survey indicates that the pork, beef, and horse meat as well as hen eggs do not have any serious risk to human health because of Hg concentrations being well below FAO/WHO proposed tolerance level of 0.05 mg/kg (JUSZKIEWICZ and SZPRENGIER, 1986).

The metabolic differences between methylmercury and inorganic Hg are shown also in placental transfer. The placenta presents an effective barrier against the transfer of inorganic Hg in rats. Transfer of methylmercury to the foetus is greatly accomplished.

2.3.3.3. Biological half-time. In case of inhalation of Hg vapour, the whole body half-time in humans is about 50 days. The biological half-time of methylmercury in the whole body in humans is approximately 70 days as compared to 40 days for inorganic Hg after oral doses. Although methylmercury is converted into inorganic Hg, the rate of decomposition is much slower. Furthermore, there is complete reabsorption when excreted in the bile, colonic glands and urinary excretion is low. All these factors contribute to the long biological half-time which is 70 days in man.

2.3.3.4. Excretion. It is generally agreed that excretion is greater in the faeces (approximately 10 µg/day) than the urine (approximately 5 µg/day) and that lesser routes such as exhalation, saliva, sweat, hair, and milk also play a part.

Faecal excretion predominates initially after exposure to inorganic Hg; renal excretion is increasing with time.

Methylmercury is secreted in bile, most of which is subsequently reabsorbed from the intestinal tract and redistributed to the tissues, thus contributing to the high retention of methylmercury. Little of the methylmercury in the intestinal tract is broken down to inorganic Hg by intestinal flora and the latter, being poorly reabsorbed, is excreted in the faeces.

Biliary secretion and demethylation of micro flora are greatly diminished in suckling animals, thus explaining the slow rate of excretion before weaning.

Urine Hg concentrations have been reported to be <0.5 mg/kg in 80% of a normal population. Although urine levels of groups of workers tend to reflect general body burdens, it is difficult to correlate levels with exposure in individual subjects since the Hg content of urine is highly variable.

2.3.4. Health effects

2.3.4.1. General. The toxicity of Hg is largely due to the high affinity to sulfhydryl (-SH) groups. These –SH groups are found in some diffusible low molecular weight substances such as cysteine (Cys), CoA (coenzyme-A), but more importantly are located in most proteins. Thus, although the Hg compounds are highly specific for the sulfhydryl group, they are highly non-specific in their targets because of the wide distribution of this group. They can disturb almost all functions in which proteins are involved and especially can inhibit most enzymes if present in sufficient concentration.

In spite of this large range of potential sites of action, the toxicological effects of Hg are, in fact, highly specific, with well-defined symptoms and progress for the different chemical forms of the trace element (OSWEILER, 1996).

2.3.4.2. Metallic mercury. Acute poisoning by metallic Hg is relatively rare and toxicity is more often seen as a chronic effect, usually in workers (e.g. goldsmith, mirror makers) exposed to Hg vapour over a long period of time. The effects of inhaled Hg vapour vary as a function of the time of exposure. With acute exposure, the lung is the critical organ (i.e. pulmonary oedema in mice). With sub-acute exposure, the kidney becomes the critical organ while with prolonged exposure, the brain becomes the critical organ (i.e. moderate histopathological changes in rabbits). These changes in critical target organ arise because of the distribution of Hg; only a small amount of the absorbed Hg accumulates in the brain, but once this has been reached, elimination is very slow.

Symptoms of chronic Hg poisoning in dogs arising from the inhalation of vapour include gingivitis, diarrhoea, anorexia, loss of weight, and disturbance to the central nervous system.

2.3.4.3. Inorganic mercury. The target organ for inorganic mercurials is very clearly the kidney. The common expression "*mad as a hatter*" (WILLIAMS, 1981), however, derives from symptoms of central nervous system disturbances (e.g. severe psychological and emotional problems) shown by workers applying solution of $Hg(NO_3)_2$ to rabbit fur in hat industry. Fortunately, exposure to high levels of inorganic Hg nowadays is extremely rare, although chronic effects arising from prolonged occupational exposure may occur.

Mercuric chloride ($HgCl_2$) is soluble and fairly readily absorbed. It is about 13 times more toxic for humans than the corresponding iodide (FURST, 1987). Ingestion of $HgCl_2$ leads to an ashen-grey appearance of the mouth due to precipitation of protoplasm of the mucous membranes, followed by gastroenteritis with abdominal pain and nausea, then by renal dysfunction with proteinuria, anuria, and uraemia. Death is usually due to the renal lesions, especially those of the tubular epithelium (WILLIAMS, 1981).

Feeding both 2 mg/kg and 20 mg/kg $HgCl_2$ to day-old chicks produced only Hg accumulation in the liver and kidney in 20 days and 5 days, respectively. Chickens given 250 or 500 mg/kg Hg as $HgCl_2$ in drinking water for 98 days showed decreased growth and feed intake, a death rate of 48 and 100 percent, respectively.

Pigs were not affected by 0.5 mg/kg Hg as $HgCl_2$ for 27 days, but 5 mg/kg increased tissue Hg, and level of 50 mg/kg produced "fatty" livers.

In Japanese quail dietary levels from 2 to 4 mg/kg $HgCl_2$ had no adverse effects, but 4 and 8 mg/kg decreased egg fertility.

Daily consumption of 1 mg/kg $HgCl_2$ by mice for life did not affect health and longevity, but 5 mg/kg was toxic (NRC, 1980c). Chronic Hg exposure (200 mg/kg $HgCl_2$ for 160 days) induced simultaneous functional and structural alterations in kidneys, testicles, and cardiovascular system of rats (CARMIGNANI *et al*., 1991).

2.3.4.4. Organic mercury. Organic compounds with the shortest carbon chain are the most toxic since they can pass across membranes more readily. The toxicity and target organs for methylmercury vary with animal species. In man, other primates and horse the target is the brain, particularly the posterior cortex, while in lower mammals mostly the peripheral nervous system may be affected. Symptoms include depression, ataxia, incoordination, visual disturbances and difficulty in speaking (WILLIAMS, 1981; OSWEILER, 1996).

2.4. NICKEL (Ni)

Nickel is both essential and toxic for humans, animals and microbes. Nickel is required for growth and iron (Fe) absorption (KIRCHGESSNER and SCHNEGG, 1980; EIDELSBURGER and KIRCHGESSNER 1995). There is evidence that Ni is never deficient under practical conditions and adding Ni to animal diets is not needed. The biological importance of this element is rather its toxicity. Long-term ecological or anthropogenic exposure of humans to Ni via the food chain or work place can lead to chronic diseases or further to mutagenic or carcinogenic changes. Nickel dermatitis (parakeratosis-like damage) is a relatively common form of Ni toxicity in humans. After the filling up of the Ni depots (skeleton, muscle, kidneys, liver), the Ni eczema develops at places where the skin gets contact with Ni-containing jewellery, bracelets, etc.. At present, 10-15% of humans are Ni sensitive and can suffer from Ni-hand eczema. In this aspect, the incorporation of Ni into the food chain of soil, plant, animal, and man is of particular importance (ANKE *et al*, 1983a).

2.4.1. Nickel in nature

Nickel is found widely in the uppermost layer of the earth's crust at 80 mg/kg and its content in the sea-waters amounts to 0.24 µg/kg (PAIS, 1998). It occurs in several ores (chalcopyrite, pyrrhotite, nicocolite). Nickel is used in alloys, storage batteries, for electroplating. During industrial Ni processing, Ni can get into the environment, and therefore into the food chain, both via soil, air and water. Nickel in various forms is relatively non-toxic when consumed orally, however, workers exposed to airborne Ni have an increased incidence of respiratory disease, including cancer (NRC, 1980d).

2.4.2. Nickel in human foods and animal feeds

Plant foods are generally higher in Ni than foods of animal origin. Thus foods that usually contain high concentrations (>0.3 mg/kg) of Ni include nuts, legume seeds, cacao products (chocolate), hydrogenated solid shortenings, and shellfish. Grains, vegetables, and cured meats are generally of intermediate (0.1-0.3 mg/kg) Ni content. Foods of animal origin, such as fish, milk, and eggs, are generally low (<0.1 mg/kg) in Ni. The canning of some fruits and vegetables apparently increases their Ni content; for example, plums and fresh tomatoes contained 0.2 and 0.09 mg/kg Ni, respectively, whereas the canned products contained 0.4 and 0.5 mg/kg, respectively. Canned pineapple contained 0.9 mg/kg Ni (NRC, 1980d; ANKE *et al*., 1983a; NIELSEN, 1987).

Nickel concentrations in plants can be influenced by a number of factors including: plant species, stage of maturity, soil Ni concentration and availability of Ni in the soil. Legumes are generally higher in Ni than grasses when grown under similar conditions. Nickel in forages decreases with maturity. Nickel concentrations in young-vegetative-stage forages range from 1.05 to 3 mg/kg. At a mature flowering stage, the same forages had Ni levels ranging from 0.35 to 0.6 mg/kg. The availability of soil Ni for plant uptake is highly dependent on soil pH. Nickel forms stable complexes at a neutral pH. However, at pH lower than 6.5, Ni is released from these compounds and increased movement into plants subsequently occurs (SPEARS, 1984). Most of the microelement contents of the soils increases with the pH up to a value of 6.5 pH, but it decreases with any further increase in pH value (in case of Ni, Pb, Cu, Zn, Fe, Mo, Mn, Co). With the increase of the pH, the contents of Cd, Hg, and Se increase, too (PATÓCS *et al*., 1989). Furthermore, flora of alkaline volcanic soils (e.g. andesite soil) has a Ni content of >1.0 mg/kg DM, while the Ca-rich soils (e.g. loess) contain <1.0 mg/kg DM of Ni (REGIUSNÉ *et al*., 1982). Nickel-accumulating plants grow on Mg-rich

sites. Therefore, the Ni-Mg-interactions might be less important in the herbivores of these areas (ANKE *et al*., 1995a).

Most animal foods, because they are plant based, contain relatively high levels of Ni. Low quality roughage or fibre sources (e.g. alfalfa meal, cottonseed meal) are usually lower in Ni (1.3 and 0.6 mg/kg, respectively) than high quality roughages such as corn silage (1.4 mg/kg of Ni). Common pasture plants contain 0.5-3.5 mg/kg DM of Ni. Nickel content of some grains including wheat, corn, oats, barley varies, ranging from 0.08 to 1.00 mg/kg; whereas that of protein sources including linseed, soybean, and sunflower cake varies from 5 to 8 mg/kg (KRONEMANN *et al*., 1980; ANKE *et al*., 1995a, 1995b, 2000).

The Hungarian vegetations have significantly higher Ni content (Table 5) in comparison with the values found in East Germany (SZENTMIHÁLYI *et al*., 1980; REGIUSNÉ *et al*., 1982).

Table 5 Ni (mg/kg in DM) in forages and crops growing in Hungary and East Germany

Species	Hungary	East Germany
Red clover	1.4	1.0
Lucerne	1.5	1.3
Wheat	0.9	0.4
Rye	0.8	0.4

Milk products and meat meals used as protein supplements are apparently poor Ni sources; fish meal was found to contain 0.7-2.8 mg/kg Ni (SPEARS *et al*., 1986). Commercial diets for dogs, rats, dairy cows, layers, and swine contain 2.1, 3.3, 2.59, 1.32, and 1.76 mg/kg Ni, respectively (NRC, 1980d; SPEARS, 1984).

The Ni content of drinking water is typically very low; Ni could not be detected or its concentration is <10 µg/kg. Higher concentrations may occur in the water of industrial areas (STRAIN *et al*., 1980; NRC, 1980d).

The appropriate concentration of Ni found in human foods and animals feeds indicate that Ni-deficiency symptoms have not yet been found in human beings and animals under natural conditions (NIELSEN, 1987; ANKE *et al*., 1995a).

2.4.3. Nickel in human and animals (metabolism)

2.4.3.1. Uptake and absorption. Total dietary Ni intakes in humans vary greatly with the amount and proportion of foods of animal (Ni-low) and plant origin (Ni-high) consumed; Ni intake probably is in the range of 150-700 µg/day. However, vegetarians (ovo lacto vegetarians) consume highly significantly more (approximately double) Ni than people with mixed diets (ANKE *et al*., 2002).

Most ingested nickel remains unabsorbed in the gastrointestinal tract and is excreted in the faeces. Typically <10% of ingested nickel is absorbed, with no significant retention in the body. However, a higher percentage may be absorbed in an Fe-deficient or gravid state; for example, pigs absorbed >20% of Ni ingested from day 21 of gravidity until parturition (NIELSEN, 1987).

2.4.3.2. Nickel in tissues. The transport of Ni across the mucosal epithelium of small gut is an active transport rather than simple diffusion, and the Ni ions use the Fe transport system.

Nickel occurs in low concentrations in all animal tissues. If animals are not fed extremely high or low levels of Ni, their tissue Ni concentrations are similar to humans:

generally <1 mg/kg on DM basis (Table 6). In contrast to human, where women's hair contains more Ni, the sex of a guinea pig did not affect the hair Ni content being approximately 4 mg/kg. However, the Ni content of animal organs is markedly affected by age or Ni exposure.

Table 6 Mean Nickel Concentrations (µg/kg) in Animal Tissues (NIELSEN, 1987)

Tissue	Goat	Minipig	Pig
Bone	907	603	---
Heart	591	351	174
Kidney	1209	1176	645
Liver	1115	564	127
Lung	764	304	236
Milk	288	110	230
Muscle	526	259	---

Goat, minipig, and pig tissues are all on dry matter basis, except milk samples, which are on fresh basis

Bones (i.e. ribs, carpals), kidneys, liver and cardiac muscle reflect the Ni status significantly with decreasing security in adult goats. Independent of the maternal Ni status foetuses and their organs stored considerable Ni amounts intrauterine. These Ni-reserves are necessary during the lactation period since milk is poor in Ni (216-288 µg/kg). This is apparently true not only for goats but also for human beings (ANKE *et al*., 1980b).

In the blood, serum albumin is the principal Ni^{2+}-binding protein in human, bovine, rabbit, and rat. Canine and porcine albumins, because of their chemical structure (i.e. there is Tyr instead of His in the chain), have less affinity for Ni^{2+} than albumins from other species. Two other proteins in serum that might influence Ni transport or metabolism are His-rich glycoprotein and nickeloplasmin isolated from human and rabbit serum (SPEARS, 1984).

Serum Ni varies among the species but comparatively little within species if Ni exposure is not excessively altered. Its content (µg/L) normally ranges between 1.5 and 5 for humans, 2 and 4 for dogs, 1.5 and 6 for cats, 4 and 5 for pigs, 3 and 4 for goats, 1 and 4 for rats, 6.5 and 14 for rabbits, and 2 and 7 for guinea pigs (NIELSEN, 1987).

However, abnormal serum Ni concentration occurs in response to some pathological conditions. Serum Ni is elevated in patients with acute myocardial infarction, stroke and burns (>25% body surface) as well as in women with pregnancy toxaemia. The increased serum Ni level may lead to decreased coronary blood flow and oxidative metabolism in rats and dogs. In both rats and pigs with hemorrhagic shock or myocardial infarction, the Ni content in the heart is reduced. The experimental findings, thus, indicate that Ni may have an important role in cardiovascular pathology (BALOGH and RUBÁNYI, 1980; GERGELY *et al*., 1980; KOLLER *et al*., 1980; LIGETI *et al*., 1980; RUBÁNYI and KOVÁCH, 1980).

Otherwise, significantly diminished Ni concentrations could be found in patients with hepatic cirrhosis or chronic uraemia (NIELSEN, 1987).

2.4.3.3. Excretion. Dogs and rats excreted 90% of ingested Ni in the faeces and 10% in the urine, while calves excreted >20 times as much Ni in faeces as in the urine (NRC, 1980d; ANKE *et al*., 1983a).

The kidney is the primary route of excretion for absorbed Ni but a small percentage of it may be excreted via the bile, whereas Ni can be found in the bile of rats and rabbits. Moreover, under conditions of excessive sweating, dermal losses of Ni could be relatively high (NIELSEN, 1987).

2.4.3.4. Nickel functions and nickel requirements. In the late 1960's, the essentiality for the "new" trace elements began with tin and followed by nickel (MERTZ, 1993). The essentiality of Ni has been shown in rats, chicks, pigs, goats, and sheep. Nickel functions either as a cofactor or structural component in specific metalloenzymes (e.g. urease, hydrogenases, factor F_{430}), or as a bioligand cofactor facilitating the intestinal absorption of the ferric ion. Ni-containing ligands convert Fe^{3+} to Fe^{2+} for efficient absorption (DIEKERT *et al*., 1980; SPEARS, 1984; NIELSEN, 1987; ANKE *et al*., 1983b, 1984, 1995a, 1995b).

Ruminal urease activity in growing bulls was increased by Ni regardless of protein/N source (i.e. soybean meal, blood meal, urea) (SPEARS *et al*., 1986; REGIUSNÉ, 1991a).

Nickel requirement of humans would be 25-35 µg daily. Ruminants have a dietary Ni requirement in the range of 300 to 350 µg/kg DM, probably because some rumen bacteria use Ni as part of their urease enzyme, and monogastric animals have a dietary Ni requirement of <200 µg/kg DM; the dietary Ni requirement of chicks and growing rats is estimated to be 50-80 µg/kg (NIELSEN, 1987; PAIS, 1989, ANKE *et al*., 2002).

2.4.4. Health effects

2.4.4.1. Nickel deficiency. A Ni-poor nutrition of <0.1 mg/kg (DM) lead to Ni deficiency. Deficiencies of Ni have been produced in chicks, pigs, goats, sheep, and rats. The following signs appear representative of Ni deficiency as reduced feed intake, growth depressions (even of intrauterine development), impaired Fe utilization (decreased haemoglobin level), lesions on skin and hair, disturbed Zn (i.e. its resorption is actually reduced), Ca (i.e. its renally excretion is higher), and Mg metabolism, worse reproduction and milking performance and higher mortality (HOFFMANN *et al*., 1983; ANKE *et al*., 1984, 1995a).

Other possible signs, in Ni-deprived chickens are the depressed haematocrit, less yellow lipochrome pigment in the skin and ultrastuctural abnormalities in the rough endoplasmic reticulum of the liver; the depressed ruminal urease activity in cows and lambs (SUNDERMAN *et al*., 1972; NIELSEN *et al*., 1975; NRC, 1980d; SPEARS, 1984; NIELSEN, 1987).

Very severe Ni deficiency has caused reproductive problems in swine and goats. Signs of Ni deprivation include delayed sexual maturity (oestrus) and elevated perinatal mortality characterized by scaly and crusty skin in piglets or a rough coat and dermatitis in young goats besides the general signs (ANKE *et al.*, 1980a).

Biochemical parameters (e.g. AST, ALT, CK) were found to be lower in the Ni-deficient goats. The Ni concentrations in the kidneys, liver, and heart of these animals were significantly decreased. Nickel deficiency has caused changes in the metabolism of heart that may result in decreased enzyme activities (SZILÁGYI *et al*., 1981, 1989, 1991). Furthermore, alpha-amylase activity is also reduced in the tissue of liver and pancreas (KIRCHGESSNER and SCHNEGG, 1980; ANKE *et al.*, 1984).

2.4.4.2. Nickel Toxicity. Toxicity of Ni through oral intake is low, probably because of homeostatic control mechanisms that regulate the absorption and excretion of Ni, thus preventing tissue accumulation of this element. Nickel salts exert their toxic action mainly by gastrointestinal irritation and not by inherent toxicity. The kidney appears to play a key role in homeostasis of absorbed Ni and kidney damage has been observed in animals fed high levels of Ni. In birds, the kidney is the first known organ to accumulate Ni when increasing dietary Ni levels are fed (SPEARS, 1984).

There is no sharp line between levels of dietary Ni that produce minimal or no adverse effects and those that produce marked adverse effects. Generally, ≥250 mg/kg Ni in a diet is required to produce signs of Ni toxicity in rats, mice, chicks, dogs, cows, rabbits, pigs, ducks, and monkeys (NIELSEN, 1987).

Many of the apparent signs are the result of the reduced feed intake, partially caused by reduced palatability. Observed signs of oral Ni toxicity are depressed growth, coarse hair, and diarrhoea in piglets fed 375 or 500 mg/kg Ni; diminished feed intake and depressed growth in rats fed 1,000 mg/kg Ni as $NiCl_2$. Dogs fed 2,500 mg/kg Ni vomited and salivated excessively. The health, feed consumption, milk production, and milk composition of dairy cows are unaffected by dietary supplements of Ni at 250 mg/kg.

In rats, i.p. Ni administration at a level of 4 mg/kg as $NiCl_2$ inhibited the insulin secretion, reduced Ca and increased Zn concentration in pancreatic tissues because of the competitive interaction between Ca and Zn (BASAGOITI *et al*., 1986). The exogenous administration of Ni into broilers stimulates secretion of glucagons. Reductions in the insulin to glucagons ratio are associated with decreased fat deposition by chickens, suggesting that carcass composition and quality may be influenced by plasma Ni concentrations (CÁRTANA and AROLA, 1992; OSCAR and MITCHELL, 1995).

Ducks fed 800 mg/kg Ni exhibited only black faeces and elevated tissue Ni concentrations. Experiments with chicks indicate a lower Ni tolerance in this species, furthermore, nickel chloride may be somewhat more toxic to chicks than nickel sulphate or acetate (SPEARS, 1984). Chicks fed 300 mg/kg Ni, as $NiCl_2$, namely, exhibit depressed growth, reduced feed intake, impaired energy metabolism, and a significantly increased Ni content in kidney (WEBER and REID, 1968). Mortality and anaemia were observed in chicks receiving 1,100 mg/kg Ni. Kidney Ni content tended to plateau at the higher dietary levels of Ni, ranging from 300 to 1,100 mg/kg, when other tissues started to show substantial amounts of Ni accumulation (LING and LEACH, 1979; NIELSEN, 1987).

Signs of Ni toxicity in hens fed 1,000 mg/kg Ni decreased the egg production and egg weight and increased the mortality and malformation rate (ANKE *et al*., 1995a, 1995b). Feathers as well as liver and kidney contained much more Ni than other organs and tissues. The reduced laying performance resulted from lower feed intake. The embryotoxic (teratogenic) effect of Ni is caused by the significantly reduced Zn content in the egg (TRÜPSCHUCH *et al*., 1996).

The high Ni offers do not significantly affect the P, K, Na, Mn, Cu and Fe metabolism of goat, mini-pig, hen and rat. However, the Ni exposure has a significant effect on the Mg status in these species; skeleton and the egg have significantly reduced Mg content after supplementation of 1,000 mg/kg Ni (ANKE *et al*., 1995a).

2.4.4.2.1. Factors influencing nickel toxicity. The manifestation of high dietary Ni can be affected by the dietary levels of several nutrients. Nickel and Fe or Cu, or Zn are interrelated competitively. Iron-deficient chicks are more susceptible to Ni toxicity. Nickel absorption is increased during Fe deficiency, suggesting a (partly) common intestinal transport system. On the other hand, high dietary levels of Fe have been shown to prevent Ni toxicity in the chick.

Nickel toxicity in chicks could be also prevented by increased dietary Zn. Zinc was found to depress Ni absorption in chicks. The Cu-deficiency signs of depressed growth and lower blood haemoglobin are exacerbated when dietary Ni is 225 mg/kg (SPEARS, 1984; NIELSEN, 1987).

The addition of Co at 100 mg/kg to the Ni-containing diet resulted in a further depression in growth rate and blood haemoglobin concentration. The Ni content of the kidney was also significantly increased by the Co treatment. These results suggested that Co enhanced Ni toxicity. The growth depression observed with addition of Co appears to be due to the toxicity of Co independent of an effect upon Ni toxicity. The toxicity of Co and Ni appeared to be additive (LING and LEACH, 1979).

In addition to trace elements, dietary vitamin C and protein could possibly influence Ni toxicity. High dietary vitamin C alleviated Ni toxicity in rats; increasing dietary protein from 10 to 30% also decreased the toxicity of Ni in chicks (NIELSEN, 1987).

3. MATERIALS AND METHODS

All experiments (*Experiment 1-6*) were carried out in the animal facilities of the Institute of Animal Breeding, Nutrition and Laboratory Animal Science, Faculty of Veterinary Science, *Szent István* University.

3.1. ANIMALS

New Zealand White rabbits (NZW) deriving from a state-registered conventional outbreed rabbitry in Hungary (LAB-NYÚL BT, Gödöllő) were involved into the trials. A total of 20 NZW, at 2300±87 g body weight (BW), of 16 NZW, at 2796±115 g BW, of 16 NZW, at 2807±171 g BW, of 16 NZW, at 3010±230 g BW, and of 15 NZW, at 4501 ±571 g BW (Exp. 1, Exp. 2, Exp. 3, Exp. 4 and Exp. 6, respectively) were used. There were males rabbits in *Experiment 1-3*, and only females in *Experiment 4* and *Experiment 6*.

For *Experiment 5*, a total of 600 ARBOR feather-sexed cockerels were obtained from a commercial hatchery (Bábolna Rt.) at 1 day of age.

3.2. HOUSING

For the experiments, the rabbits were individually kept in wire mesh (so called metabolic) cages. The temperature was 20±2°C and the humidity between 60-65% in the animal room. The controlled lighting period was applied (16 hrs light : 8 hrs dark). The rabbits were allowed to drink *ad libitum* tap water.

Cockerels were placed in 3-floor batteries (type: D-116; manufactured by Delta Ltd., Tatabánya), using 60 birds/m^2 in the beginning. After weighing the chicks at 14 d of age, 120 birds (40 per treatment) were randomly distributed in the cages to obtain the identical average weights with minimum variance at density of 10 birds per cage (15 birds/m^2). The temperature in the broiler house was 31^{o}C when the one-day-old chickens were received, and it was reduced by 1^{o}C during every 3rd day of the experiment up to 21^{o}C. Twenty-three hours lightening was applied with 1 hour of dark pauses. The relative humidity of the air was regulated with a humidifier.

During the experiments, the animals were considered clinically healthy on the basis of the veterinary investigation.

3.3. NUTRITION

Rabbits were fed, generally, commercial pelleted concentrates (Purina-Hage Ltd) and washed, whole carrots (*Exp. 1*), potatoes (*Exp. 2*), and beetroots (*Exp. 3*). Moreover, the daily ration was supplemented with pelleted alfalfa meal in *Experiment 2*. The rabbits received only concentrate (Table 7) in *Experiment 4* and *Experiment 6*. The pellet size was 5x10-12 mm in each case.

Root and tuber samples were obtained from the exposure tolerance experiments conducted by the MTA TAKI. Extremely high doses (270 mg/kg soil) of Cd as $CdSO_4 \cdot 8H_2O$, Pb as $Pb(NO_3)_2$ and Hg as $HgCl_2$ were applied onto the calcareous chernozem soil with about 20% clay, 3% humus and 5% $CaCO_3$ of Experimental Station (Nagyhörcsök) and the rate of accumulation of the above listed heavy metals in plants was determined.

Carrot samples contained in DM **2.30** mg/kg **Cd**, **4.01** mg/kg **Pb**, and **30.00** mg/kg **Hg**.
Potato samples contained in DM **2.12** mg/kg **Cd**, **4.1** mg/kg **Pb**, and **3.44** mg/kg **Hg**.
Beetroot samples contained in DM **4.72** mg/kg **Cd**, **3.03** mg/kg **Pb**, and **6.75** mg/kg **Hg**.

Table 7 Ingredients of the concentrate fed to rabbits

Ingredients	**%**
Barley	38.6
Wheat	10.0
Maize	15.0
Wheat bran	5.7
Sunflower meal (solvent, 37% CP)	16.5
Alfalfa meal (22% CP)	11.7
Lysine-HCl	0.2
Limestone	1.5
Salt	0.3
Vitamin-mineral premix	0.5
Total	100.0

Nutrient content	
DE[1], MJ/kg	11.20
Crude protein, %	16.80
DCP[2], %	13.45
Crude fibre, %	13.20

Mineral content	
Ca, %	0.95
P, %	0.52
Na, %	0.25
Fe, mg/kg	426
Cu, mg/kg	26.9
Zn, mg/kg	166
Mn, mg/kg	135
Cd, mg/kg	0.14
Pb, mg/kg	1.58
Hg, mg/kg	0.00
Ni, mg/kg	5.8

[1]DE: digestible energy; [2]DCP: digestible crude protein

Cockerels were fed a starter diet until 14 d of age and then a corn-soybean based grower diet between 14 and 49 d of age (Table 8).

Table 8 Composition of the basal diets for broiler chickens, %

Ingredients	Starter	Grower
Corn	42.95	42.50
Wheat	22.00	14.00
Soybean meal (48% CP)	25.00	29.80
Fish meal (65% CP)	3.00	---
Meat meal (54% CP)	---	3.80
Animal fat (40% EE)	4.00	6.60
Limestone	1.35	---
MonoCalciumPhosphate	0.90	---
Salt	0.30	---
Vitamin-mineral premix	0.50	---
Complete premix[1]	---	3.30
Total	100.00	100.00
Nutrient content		
AME[2], MJ/kg	12.68	13.24
Crude protein, %	21.33	20.15

[1]Composition of complete premix: MCP, 24.24%; limestone, 45.45%; salt, 9.09%; premix, 15.15%; Lysine, 4.55%; Methionine, 1.52%;
[2]AME: apparent metabolizable energy

3.3.1. Feeding processes, treatments. To determine the apparent digestibility of major nutrients in carrots, potatoes, and beetroots, there were two stages in feeding. First, there was the "basal diet" period. Second, there was the "mixture ration" period.

3.3.1.1. Experiment 1 (Feeding of carrots)

"Basal diet" period: Rabbits (n = 20) were given concentrate *ad libitum* for 14 days
"Mixture ration" period: Concentrate of order of 50 g and carrots containing Cd or Pb or Hg, or uncontaminated were fed *ad libitum* to rabbits (n = 5/treatment) for 14 days.

3.3.1.2. Experiment 2 (Feeding of potatoes)

"Basal diet" period: Rabbits (n = 16) were given concentrate at 50 g and 100 g of pelleted alfalfa meal for 14 days.
"Mixture ration" period: Both concentrate and alfalfa meal were restricted to 25 and 50 g, respectively and potato samples containing Cd or Pb or Hg, or uncontaminated were fed *ad libitum* to rabbits (n = 4/treatment) for 14 days.

3.3.1.3. Experiment 3 (Feeding of beetroots)

"Basal diet" period: Rabbits (n = 16) were given concentrate *ad libitum* for 14 days.
"Mixture ration" period: Concentrate of order of 50 g and beetroots containing Cd or Pb or Hg, or uncontaminated were fed *ad libitum* to rabbits (n = 4/treatment) for 14 days.

3.3.1.4. Experiment 4 (Rabbit model for heavy metal loading)

All rabbits (n = 16) were fed *ad libitum* exclusively with concentrate.

The animals were treated perorally with inorganic salts of Cd as $CdSO_4 \cdot 8H_2O$ or Pb as $Pb(NO_3)_2$ or Hg as $HgCl_2$ (n = 4/treatment). The concentrations used were 2.3 mg/kg of Cd (in DM of the daily ration), 4.01 mg/kg of Pb and 30 mg/kg of Hg. Stock solutions of toxic metals were prepared and the daily application of 0.2 ml occurred through a metal catheter for 28 days. The control animals recived daily 0.2 distilled water through a metal catheter for 28 days.

3.3.1.5. Experiment 5 (Broiler model for supplemental Ni)

For the 5-week long feeding trial, the grower diet was supplemented with 0, 50 or 500 mg/kg Ni from $NiCl_2 \cdot 6H_2O$ between 14 and 49 days of age.

3.3.1.6. Experiment 6 (Rabbit model for supplemental Ni)

The commercial pellet for rabbit supplemented with 0, 50 or 500 mg/kg Ni from $NiCl_2 \cdot 6H_2O$ were fed *ad libitum* for 24 days.

3.4. INVESTIGATED PARAMETERS and PROCEDURES

3.4.1. Body weight, feed intake and FCE (feed conversion efficiency). All experimental rabbits were weighed weekly. Feed intake of animals was also measured weekly. In case of broilers (*Experiment 5*), the BW and the feed intake were recorded weekly, and the FCE was calculated.

3.4.2. Digestibility of nutrients. After the 10-day preliminary adjustment periods the metabolic balance trials were carried out (*Experiment 1-3* and *Exp. 6*), in which both the daily feed intake and faeces were measured. The digestibility of nutrients for each sample was calculated by the formula of differential experiment (FEKETE and GIPPERT, 1983).

To calculate the digestibility of nutrients, the chemical composition of the diets fed in Experiments were determined by methods of AOAC (1990).

3.4.3. Histopathology. In order to determine the influences of toxic metals on the selected organs (*Experiment 1-3* and *6*), at the end of the trials, after weighing the euthanasia of all rabbits was carried out by overdosing of intraperitoneal pentobarbital injection (Nembutal inj. A.U.V., Sanofi-Phylaxia, Budapest).

At 49 days of age, euthanasia of 5 cockerels per treatments was carried out by inhalation of CO_2 to perform their histopathological examinations.

Procedures

For histopathological investigation (Central Veterinary Institute, Budapest), hearts, lungs, livers, spleens, kidneys, gonads (ovaries or testicles), and digestive tracts were completely recovered and weighed. Appropriate samples were taken from the femoral muscles and ribs, too. All samples were fixed in phosphate-buffered 10% formaldehyde solution, stained with haematoxylin and eosin and Fat-Red, and were examined by light microscopy.

For enzyme analysis, the pancreas of rabbits was completely recovered and frosted.

3.4.4. Trace elements in tissues and fluids of the rabbits. The concentration of Cd, Pb, Hg, and Ni was detected in the above-mentioned organs as well as in adipose tissue, hair, blood, urine, and faeces.

Procedures

Appropriate samples of animal tissues, hair, and faeces (approximately 1 g), dried at 105°C, were digested in teflon bombs at 80°C for 4 hrs with a mixture of 10 ml of 65% (m/m) HNO_3 and 2 ml 30% (m/m) H_2O_2. The digesta was diluted to 50 ml with distilled water. Having extremely low amounts of animal tissues (e.g. testicle, ovary) the quantity of the materials (acid, peroxide, distilled water) were reduced proportionally. Approximately 3.0 g of bone was digested 3 times with HNO_3 + H_2O_2 as mentioned above. The final volume of the digesta was 150 ml. From the urine samples, 150-200 ml was concentrated to 20 ml, digested with 10 ml HNO_3 and 2 ml H_2O_2, and diluted to 50 ml.

The chemical analysis (*Exp. 1-3*) was performed by using a plasma emission spectrometer (ICP), type JY 24 (Joben Yvon), in sequential mode at the following wavelengths: 228.802 for Cd, 220.353 for Pb, and 194.227 for Hg.

In *Experiment 4* all samples were analysed by atomic absorption spectrometry (equipment of Perkin-Elmer, Model 5000 AAS), using graphite furnace atomisation for Cd, Pb and Ni (type HGA-500), and cold vapour technique for Hg (type MHS-10).

3.4.5. Haematology and Zn-protoporphyrin (ZPP). For the determination of haematological values (*Experiment 4*) including WBC (white blood cell), RBC (red blood cell), HGB (blood haemoglobin), HCT (haematocrit), MCV (mean cell volume), MCH (mean cell haemoglobin), MCHC (mean cell haemoglobin concentration), and number of PLT (platelets) as well as for the ZPP (zinc protoporphyrin) assay, the blood (3 ml) was taken from the marginal ear veins of rabbits before and after the ingestion of trace elements (0 and 28 days). Blood samples were placed in plastic tubes containing K-EDTA as anti-coagulant.

Procedures

The haematological values were measured (SzIU Fac. Vet. Sci., Dept. and Clinic of Internal Medicine, Budapest) by instrument of hem automat (Model Abacus).

The ZPP concentrations were measured by haematofluorometry of plasma (Institute of Public Health, Budapest). The instrument used (Model AVIV) measures the ratio of ZPP fluorescence to heme absorption, and the results are reported as µmol ZPP/mol heme.

3.4.6. Serum biochemistry. For the measurement of following biochemical parameters, after clotting and centrifugation, serum (2 ml) of each rabbit and of 12 random broilers per treatment was used. In case of *Experiment 1,* the activity of AST (aspartate aminotransferase), ALT (alanine aminotransferase), GGT (gamma-glutamyltransferase), ALP (alkaline phosphatase), CHE (cholinesterase), CK (creatine kinase), as well as the concentration of CREA (creatinine), CHOL (cholesterol), TRIG (triglycerides) and GSHPx (glutathione peroxidase) were measured while in *Experiment 4*, activity of AST, ALT, CREA and UREA (urea). In both *Experiment 5* and *6* GGT, AST, CHE, CHOL, TRIG, CREA and ALP or ALT levels were determined.

Procedures

Measurement of the biochemical parameters was based on the recommendation of Deutsche Gessellschaft für Klinische Chemie, the International Society for Animal Clinical Biochemistry and of the European Committee for Clinical Laboratory Standards (SZILÁGYI, 1990). Both optimized and kinetical methods were preferred. Optimised standard-method was

used for the determination of AST (EC 2.6.1.1.) and ALT (EC 2.6.1.2.) activity according to BERGMEYER *et al*. (1978), of CK (EC 2.7.3.2.) according to BRUNS *et al*. (1976) and of ALP (EC 3.1.3.1.) activity according to KAWADE (1964). Kinetic colorimetric method was used for the determination of GGT (EC 2.3.2.2.) activity, while the concentration of CREA was measured by photometric colorimetric test with deproteinisation (*Jaffé*-reaction). End-point direct assay (MATKOVICS *et al*., 1988) was used for determination of GSHPx: (EC 1.11.1.9.) activity. Enzimatic colorimetric method was used for measurement of both CHOL and TRIG. UV kinetic determination was used for the UREA concentration. The biochemical parameters were measured (ÁTK, Herceghalom) by liquid reagent photometry (equipment of Eppendorf ACP-5040) using tests (Clinisotest, Hungary).

3.4.7. Pancreatic enzymes. Changes in activity of amylase, trypsin, protease and lipase in pancreatic tissues and that of intestinal content were also determined (*Experiment 4*). For analysis both the pancreas and the contents of total small intestine were obtained.

Procedures

For enzyme analysis, 1-2 g were cut from the defrosted pancreas. The pancreas samples were homogenized in 4 ml distilled water using a *Potter-Elvehjem* instrument, than were centrifuged (5000/min) for 5 min, and the supernatant was decanted for the determination of enzyme activities. In case of content of small intestine, approximately 4 ml defrosted homogenized samples were centrifuged (5000/min) for 5 min, and the supernatant was decanted and vortexed thoroughly for analysis. Alpha-amylase activity was determined by the method of CESKA *et al*. (1969) using *Phadebas* Test (Pharmacia Diagnostics AB, Sweden;). Trypsin activity was measured by calorimetry (KAKADE *et al*., 1969), and lipase activity as proposed by SCHÖN *et al*. (1961). Protein content of the samples was assayed by the method of *Lowry-Folin* (HERD, 1971), with bovine albumin used as reference standard. Unit enzyme activity was defined as the quantity of enzyme require to split 10^{-6} M substrate in 1 min in the given test condition. The specific activity values are related to 1 mg tissue protein.

3.5. ETHICAL ISSUE

The experiments were approved (No. 15/2001 for Exp. 4) by the Animal Use and Care Administrative Advisory Committee of the Municipal Veterinary Service for Animal Protection and it is in agreement with the Ethical Codex of the Hungarian Association of Laboratory Animal Science.

3.6. STATISTICAL ANALYSIS

Results have been presented as mean and standard deviation (SD) in each experiment. To make comparison between two population variances, it was examined by *F* distribution. Assume that the population distribution is normal, an unpaired two-tailed *Student*'s *t* test was used. Significance tests were analysed by the software procedure of SPSS vers. 3.0 (NORUŠIS, 1988). Statistical significance was accepted if $P < 0.05$.

In case of digestibilty, means of toxic metal-exposed potato and beetroot samples were compared to that of both uncontaminated samples and basal diet.

In case of biochemical results after the 24-day-long Ni burden, the means of 0 day and 24 day were analysed in each treatment. The insignificant changes ($P > 0.05$) recived the same marks in upper index (i.e. a-a), while the significant changes ($P < 0.05$) were marked with different letters (i.e. a-b).

In case of relative organ weight, the means of toxic metal-exposed samples were compared to that of uncontaminated samples.

4. RESULTS

4.1. BODY WEIGHT and FEED INTAKE

Body weight (BW). At the end of the 14-day-feeding trials, the body weight of rabbits consumed carrots, potatoes or beetroots (*Experiment 1-3*) was smaller than that of animals (as controls) received only basal diet (Table 9). Nevertheless these differences were not significant (P>0.05).

Table 9 Body weight (kg) of rabbits fed with basal diet and carrots, potatoes or beetroots, (mean and ±SD)

Body weight	**Basal diet**	**Carrot** (n=5)			
		uncontaminated	**Cd**	**Pb**	**Hg**
Initial ±SD	2.45 ±0.17	2.40 ±0.16	2.34 ±0.12	2.35 ±0.11	2.28 ±0.17
Final ±SD	2.62 ±0.34	2.52 ±0.19	2.45 ±0.10	2.45 ±0.15	2.35 ±0.16
Body weight	**Basal diet**	**Potato** (n=4)			
		uncontaminated	**Cd**	**Pb**	**Hg**
Initial ±SD	2.63 ±0.58	2.88 ±0.49	2.73 ±0.46	2.60 ±0.43	2.87 ±0.42
Final ±SD	2.94 ±0.52	2.86 ±0.47	2.83 ±0.47	2.64 ±0.43	2.91 ±0.42
Body weight	**Basal diet**	**Beetroot** (n=4)			
		uncontaminated	**Cd**	**Pb**	**Hg**
Initial ±SD	2.82 ±0.19	2.94 ±0.13	2.86 ±0.16	2.87 ±0.16	2.92 ±0.15
Final ±SD	2.92 ±0.19	2.78 ±0.07	2.79 ±0.10	2.82 ±0.14	2.83 ±0.08

At 14 days of age, the initial average body weight of cockerels fed the diet supplemented 0, 50 or 500 mg/kg Ni (*Experiment 5*) were 287.9±41.0, 287.7±41.5, and 287.8±41.4 g, respectively (Table 10). Supplemental 500 mg/kg Ni significantly (P<0.05) reduced the weight gain (1803.1±251.4 g), thus, the BW of broilers was lower compared to 0 mg/kg Ni (2090.8 vs 2176.4 g). Nevertheless 50 mg/kg Ni slightly, but not significantly improved the BW gain in comparison with control (2045.9±232.1 *vs* 1987.6±196.3 g). Therefore as a consequence, the BW was higher (2334.6±249.1 *vs* 2276.4±205.2 g).

In case of rabbits (*Experiment 6*), supplemental 500 mg/kg Ni insignificantly (P>0.05) reduced the weight gain (Table 11) compared to 0 or 50 mg/kg Ni (0.15±0.22 kg *vs* 0.19±0.17 and 0.31±0.14 kg, respectively). The supplemental Ni of 50 mg/kg resulted in higher, but an insignificant increase in average daily weight gain.

Table 10 Growth performance of broiler chickens in Experiment 5 (*Broiler model for supplemental Ni*)

Item	Supplemental Ni		
	0 mg/kg	**50 mg/kg**	**500 mg/kg**
Growth performance:			
Initial BW[1], g	287.9[a]	287.7[a]	287.8[a]
±SD	41.0	41.5	41.3
CV%[5]	14.3	14.4	14.4
Sx[6]	0.86	0.86	0.86
n	48	48	48
Final BW[2], g	2176.4[a]	2334.6[a]	2090.8[b]
±SD	205.2	249.1	267.5
CV%	9.01	10.7	12.8
Sx	4.56	5.79	5.69
n	45	43	47
Weight gain[3], g	1987.6[a]	2045.9[a]	1803.1[b]
±SD	196.3	232.1	251.4
CV%	9.87	11.3	13.9
Sx	4.36	5.4	5.35
n	45	43	47
Feed intake[3], g	4251[a]	4364[a]	4084[a]
±SD	316.8	422.5	333.8
FCE[4], g/g	2.14[a]	2.13[a]	2.26[b]
±SD	0.18	0.24	0.19

BW: body weight at [1]14 d of age, [2]49 d of age; [3]14 to 49 d of age; [4]FCE: feed conversion efficiency; [5]SD/mean *x* 100; [6]SD/n; a-a: P>0.05; a-b: P<0.05.

Table 11 Growth performance of rabbits in Experiment 6
(*Rabbit model for supplemental Ni*)

Item	Supplemental Ni		
	0 mg/kg	**50 mg/kg**	**500 mg/kg**
Growth performance:			
Initial BW[1], kg	4.48[a]	4.51[a]	4.67[a]
±SD	0.59	0.47	0.54
CV%[2]	13.3	10.4	11.6
Sx[3]	0.12	0.094	0.11
n	5	5	5
Final BW, kg	4.66[a]	4.82[a]	4.82[a]
±SD	0.49	0.45	0.63
CV%	10.6	9.32	13.0
Sx	0.1	0.09	0.13
n	5	5	5
Weight gain, kg	0.19[a]	0.31[a]	0.15[a]
±SD	0.17	0.14	0.22
CV%	91.4	43.5	149.8
Sx	0.03	0.03	0.04
n	5	5	5
Feed intake, kg/day	0.15[a]	0.16[a]	0.15[a]
±SD	0.03	0.03	0.03

[1]body weight; [2]SD/mean *x* 100; [3]SD/n; a-a: P>0.05

Feed intake. The heavy metals (Cd, Pb and Hg) ingested from roots and tubers (i.e. carrots, potatoes and beetroots) or by peroral treatment directly (*Experiment 1-4*) did not reduce the appetite and the feed intake of rabbits. Therefore they have eaten their daily ration, mainly the basal diets. There was covered 39, 26 and 40% of the daily dry matter requirements by carrots, potatoes and beetroots, respectively (Table 12-15).

The feed intake of cockerels (*Exp. 5*) was insignificantly decreased by supplemental 50 or 500 mg/kg Ni in comparison with control (4364±422.5 and 4084±333.8 *vs* 4251±316.8 g, respectively). FCE (2.26±0.19) was significantly (P<0.05) worse by 500 mg/kg Ni (Table 10).

In *Experiment 6*, either 50 or 500 mg/kg Ni failed to reduce the feed intake of rabbits compared to control (Table 11).

Table 12 Daily feed and nutrient intake (g) in Experiment 1 (*Feeding of carrots*)

Treatment	Feeds	g	DM	OM	CP	CF	EE	NFE
Control (n=5)	Basal diet	150	137.7	27.2	22.8	18.0	2.7	83.7
	Carrot	---	---	---	---	---	---	---
	Total	**150**	**137.7**	**127.2**	**22.8**	**18.0**	**2.7**	**60.5**
Uncontaminated (n=5)	Basal diet	50	45.9	42.4	7.6	6.0	0.9	27.9
	Carrot	106.6	18.3	15.4	2.0	1.2	0.3	11.8
	Total	**156.6**	**64.2**	**57.8**	**9.6**	**7.2**	**1.2**	**39.7**
Cd (n=5)	Basal diet	50	45.9	42.4	7.6	6.0	0.9	27.9
	Carrot	188.9	38.9	33.5	4.0	1.9	0.7	27.0
	Total	**238.9**	**84.8**	**75.9**	**11.6**	**7.9**	**1.6**	**54.9**
Pb (n=5)	Basal diet	50	45.9	42.4	7.6	6.0	0.9	27.9
	Carrot	174.1	37.0	31.7	3.6	1.7	0.8	25.5
	Total	**224.1**	**82.9**	**74.1**	**11.2**	**7.7**	**1.7**	**53.4**
Hg (n=5)	Basal diet	50	45.9	42.4	7.6	6.0	0.9	27.9
	Carrot	182.5	35.5	29.8	3.9	2.0	0.8	23.1
	Total	**232.5**	**81.4**	**72.2**	**11.5**	**8.0**	**1.7**	**51.0**

DM: dry matter, OM: organic matter, CP: crude protein, CF: crude fibre, EE: ether extract, NFE: N-free extract; Basal diet: concentrate for rabbits

Table 13 Daily feed and nutrient intake (g) in Experiment 2 (*Feeding of potatoes*)

Treatment	Feeds	g	DM	OM	CP	CF	EE	NFE
	Diet	*50*	*46.1*	*40.6*	*10.5*	*5.3*	*1.8*	*23.0*
Basal diet 1	*Alfalfa*	*100*	*91.8*	*81.5*	*21.4*	*18.7*	*3.9*	*37.5*
	Total	***150***	***137.9***	***122.1***	***31.9***	***24.0***	***5.7***	***60.5***
	Diet	*25*	*23.0*	*20.3*	*5.2*	*2.6*	*0.9*	*11.5*
Basal diet 2	*Alfalfa*	*50*	*45.9*	*40.7*	*10.7*	*9.4*	*1.9*	*18.7*
	Total	***75***	***68.9***	***61.0***	***15.9***	***12.0***	***2.8***	***30.2***
Control (n=4)	Basal diet 1	150	137.9	122.1	31.9	24.0	5.7	60.5
	Potato	---	---	---	---	---	---	---
	Total	**150**	**137.9**	**122.1**	**31.9**	**24.0**	**5.7**	**60.5**
Uncontaminated (n=4)	Basal diet 2	75	68.9	61.0	15.9	12.0	2.8	30.2
	Potato	112.4	25.7	24.2	3.5	0.3	0.1	20.3
	Total	**187.4**	**94.6**	**85.2**	**19.4**	**12.3**	**2.9**	**50.5**
Cd (n=4)	Basal diet 2	75	68.9	61.0	15.9	12.0	2.8	30.2
	Potato	130.2	23.4	22.1	3.0	0.8	0.3	18.0
	Total	**205.2**	**92.3**	**83.1**	**18.9**	**12.8**	**3.1**	**48.2**
Pb (n=4)	Basal diet 2	75	68.9	61.0	15.9	12.0	2.8	30.2
	Potato	128.9	26.4	25.0	3.1	0.9	0.3	20.8
	Total	**203.9**	**95.3**	**86.0**	**19.0**	**12.9**	**3.1**	**51.0**
Hg (n=4)	Basal diet 2	75	68.9	61.0	15.9	12.0	2.8	30.2
	Potato	127.5	24.0	22.7	3.1	0.8	0.2	18.6
	Total	**202.5**	**92.9**	**83.7**	**19.0**	**12.8**	**3.0**	**48.8**

DM: dry matter, OM: organic matter, CP: crude protein, CF: crude fibre, EE: ether extract, NFE: N-free extract; Diet: concentrate for rabbits, Alfalfa: pelleted alfalfa meal

Table 14 Daily feed and nutrient intake (g) in Experiment 3 (*Feeding of beetroots*)

Treatment	Feeds	g	DM	OM	CP	CF	EE	NFE
Control (n=4)	Basal diet	150	136.2	124.8	22.5	22.8	3.9	75.9
	Beetroot	---	---	---	---	---	---	---
	Total	**150**	**136.2**	**124.8**	**22.5**	**22.8**	**3.9**	**75.9**
Uncontaminated (n=4)	Basal diet	50	45.4	41.6	7.5	7.6	1.2	25.3
	Beetroot	236.3	28.7	25.3	3.3	1.1	0.2	20.6
	Total	**286.3**	**74.1**	**66.9**	**10.8**	**8.7**	**1.4**	**45.9**
Cd (n=4)	Basal diet	50	45.4	41.6	7.5	7.6	1.2	25.3
	Beetroot	228.8	33.7	28.1	5.3	1.7	0.4	20.6
	Total	**278.8**	**79.1**	**69.7**	**12.8**	**9.3**	**1.6**	**45.9**
Pb (n=4)	Basal diet	50	45.4	41.6	7.5	7.6	1.2	25.3
	Beetroot	227.4	33.1	28.0	4.9	1.7	0.2	21.2
	Total	**277.4**	**78.5**	**69.6**	**12.4**	**9.3**	**1.4**	**46.5**
Hg (n=4)	Basal diet	50	45.4	41.6	7.5	7.6	1.2	25.3
	Beetroot	211.8	27.9	23.2	4.5	1.5	0.3	16.8
	Total	**261.8**	**72.3**	**64.8**	**12.0**	**9.1**	**1.5**	**42.1**

DM: dry matter, OM: organic matter, CP: crude protein, CF: crude fibre, EE: ether extract, NFE: N-free extract; Basal diet: concentrate for rabbits

Table 15 Daily feed intake (g as fed) in Experiment 4 (*Rabbit model for heavy metal loading*), (mean and ±SD)

Treatment	g
Cd (n=4)	165.5 ±24.8
Pb (n=4	181.3 ±18.1
Hg (n=4)	163.7 ±42.9
Control (n=4)	172.2 ±27.9

4.2. DIGESTIBILITY

Experiment 1 (*Feeding of carrots*)

The digestibility of carrots for each nutrient tended to be no lower than that of the basal diet. In fact, except of CP the digestibility of nutrients in carrots was significantly higher compared to basal diet. The digestibility of OM was higher by 6-11%. The digestibility of CF improved by 33-60%, while the digestibility of NFE by 6-8%. The digestibility of CP in the samples did not alter.

Comparing the carrot samples, in case of uncontaminated samples the significantly lowest digestibility of DM (75%) and EE (65%), and the highest digestibility of OM (83.5%), particularly of CF (46%) and NFE (88%) were observed. The digestibility of CF and NFE in uncontaminated samples was higher by 14-17 and 1-2 per cent, respectively that in contaminated samples. No differences were found in CP digestibility.

Experiment 2 (*Feeding of potatoes*)

The data presented in the Table 16 provide evidence that except the DM and NFE both in Pb- and Hg-containing potatoes, the digestibility of nutrients in potatoes tended to be the same or significantly higher than that of the basal diet.

Table 16 Nutrients' digestibility (%) of the basal diet and of potato tubers, (mean and ±SD)

Nutrients	**Basal diet**	**Potato** (n=4)			
		uncontaminated	**Cd**	**Pb**	**Hg**
DM	62.16[a]	68.67[b]	66.15[b]	64.73[b]	65.53[b]
±SD	1.59	6.11	1.63	5.72	1.52
OM	61.59[a]	68.67[b]	66.67[b]	51.69[c]	53.96[c]
±SD	1.54	6.11	1.80	7.79	2.40
CP	73.39[a]	72.63[a]	71.26[a]	86.25[b]	85.94[b]
±SD	1.77	8.84	2.71	2.25	1.19
CF	28.71[a]	38.19[abc]	36.71[b]	50.24[c]	48.62[c]
±SD	0.72	9.35	3.23	6.03	6.72
EE	70.55[a]	74.92[ab]	75.41[b]	78.25[b]	76.9[b]
±SD	2.04	2.52	1.09	3.27	2.00
NFE	67.56[a]	74.27[a]	72.27[a]	46.97[b]	51.78[b]
±SD	2.53	5.00	4.77	10.97	3.55

DM: dry matter; OM: organic matter; CP: crude protein; CF: crude fibre, EE: ether extract; NFE: nitrogen-free extract; a-b-c: $P<0.05$; a-a, b-b, and c-c: $P>0.05$

Comparing the potato tubers, the Pb and Hg content of potatoes significantly ($P<0.05$) decreased the digestibility of OM and NFE, but at the same time improved the digestibility of CP and CF. The highest values in digestibility of OM and NFE (66.67 and 72.27%), although the worst digestibility of CP and CF (71.26 and 36.71%) were found at potato tubers containing high level of Cd. The digestibility of nutrients of uncontaminated (so called "healthy") potatoes did not differ from other potato tubers.

Experiment 3 (*Feeding of beetroots*)

The data presented in the Table 17 show that except the CF, the digestibility of nutrients in beetroot samples tended to be lower than that of the basal diet. But the digestibility of CF was significantly (P<0.05) better in case of beetroot samples treated with Cd or Pb.

Uncontaminated ("healthy") beetroot had lower digestibility for nutrients than the basal diet. Beetroot samples of high Pb and Hg content had significantly (P<0.05) lower digestibility of OM, CP, EE, and NFE than those of basal diet.

Table 17 Nutrients' digestibility (%) of the basal diet and of beetroots, (mean and ±SD)

Nutrients	Basal diet	Beetroot (n=4)			
		uncontaminated	Cd	Pb	Hg
DM	86.41[a]	75.71[ab]	83.07[a]	59.82[b]	66.52[b]
±SD	3.21	6.43	3.27	9.97	10.02
OM	86.87[a]	77.23[ab]	84.24[a]	61.57[b]	68.41[b]
±SD	3.09	6.21	2.94	9.19	9.53
CP	86.11[a]	75.33[ac]	82.08[a]	56.88[b]	64.98[bc]
±SD	4.15	6.81	4.05	9.68	11.17
CF	38.08[a]	54.32[ab]	67.93[b]	64.68[b]	46.81[ab]
±SD	13.95	15.32	6.22	12.96	12.55
EE	91.69[a]	78.50[ab]	82.42[ab]	64.29[b]	69.75[b]
±SD	2.62	6.67	4.15	9.31	12.35
NFE	88.96[a]	82.56[a]	87.53[a]	69.57[b]	75.02[b]
±SD	2.62	4.69	2.24	7.98	6.89

DM: dry matter; OM: organic matter; CP: crude protein; CF: crude fibre, EE: ether extract; NFE: nitrogen-free extract; a-b: P<0.05

Experiment 6 (Rabbit model for supplemental Ni)

Both 50 and 500 mg/kg Ni-addition decreased the digestibility of CP and CF compared to the control (72.77±1.41, 18.5±2.81% and 71.9±1.8, 19±2.4% *vs* 73.51±2.97, 23.59±5.3%, respectively). These alterations, however, were insignificant (Table 18).

Table 18 Nutrients' digestibility (%) of the rabbit pellet in Experiment 6 (*Rabbit model for supplemental Ni*), (mean and ±SD)

Nutrient	Supplemental Ni		
	0 mg/kg	50 mg/kg	500 mg/kg
DM	65.72 ±1.98[a]	63.55 ±0.96[a]	64.28 ±0.36[a]
EE	86.92 ±1.40[a]	87.78 ±1.28[a]	87.89 ±0.58[a]
CF	23.59 ±5.30[a]	18.50 ±2.81[a]	19.02 ±2.45[a]
CP	73.51 ±2.97[a]	72.77 ±1.41[a]	71.90 ±1.80[a]
NFE	76.54 ±1.44[a]	74.56 ±1.05[a]	76.25 ±0.58[a]

DM: dry matter; CP: crude protein; CF: crude fibre, EE: ether extract; NFE: nitrogen-free extract; a-a: P>0.05

4.3. TRACE ELEMENT RETENTION

The average daily ingestion of Cd was 0.103 mg (Table 19). About 38% of the ingested Cd excreted from the body almost exclusively via the faeces (Figure 1). Total retention observed in rabbits was about 62% of the ingested Cd.

Table 19 Daily intake, excretion and retention of trace elements in rabbits (mg)

Trace element	Intake	Excretion			Retention
		via faeces	via urine	total	
Cd (n=5)	0.103	0.039	0.000	0.039	0.064
Pb (n=5)	0.241	0.157	0.003	0.160	0.081
Hg (n=5)	1.160	0.548	0.002	0.550	0.610

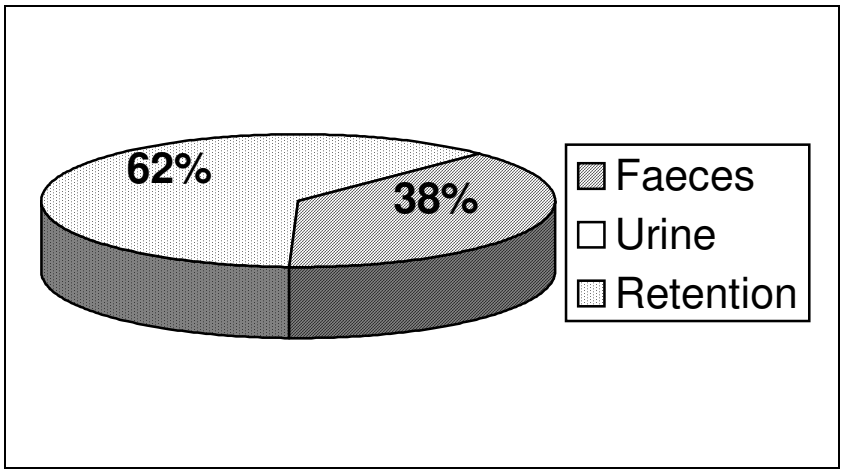

Figure 1 Cadmium excretion and retention in percentage of intake

The average daily ingestion of Pb was 0.241 mg (Table 19). About 66% of the ingested Pb excreted from the body; about 65% via the faeces and about 1% via the urine (Figure 2). Total retention observed in rabbits was about 34% of the ingested Pb.

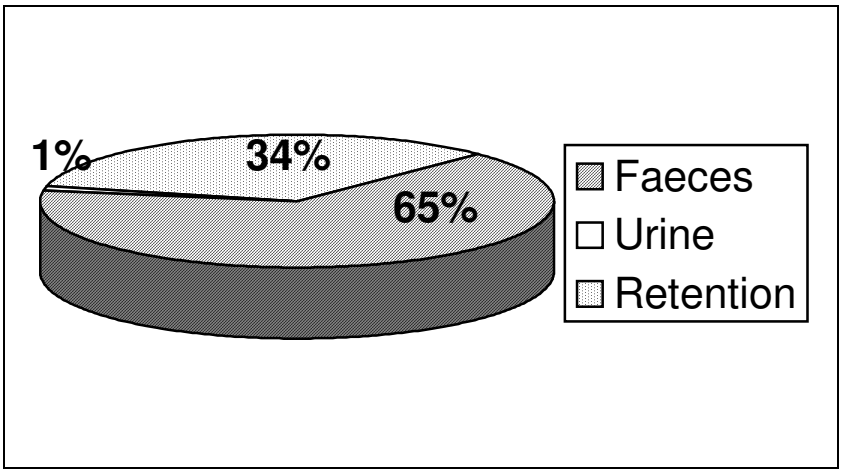

Figure 2 Lead excretion and retention in percentage of intake

The average daily ingestion of Hg was 1.16 mg (Table 19). About 47.4% of the ingested Hg excreted from the body almost exclusively via the faeces (47.2%) and only about 0.2% via the urine (Figure 3). Total retention observed in rabbits was about 52.6% of the ingested Hg.

Cadmium was built into the tissues in the largest amount followed by Hg and Pb (Table 19).

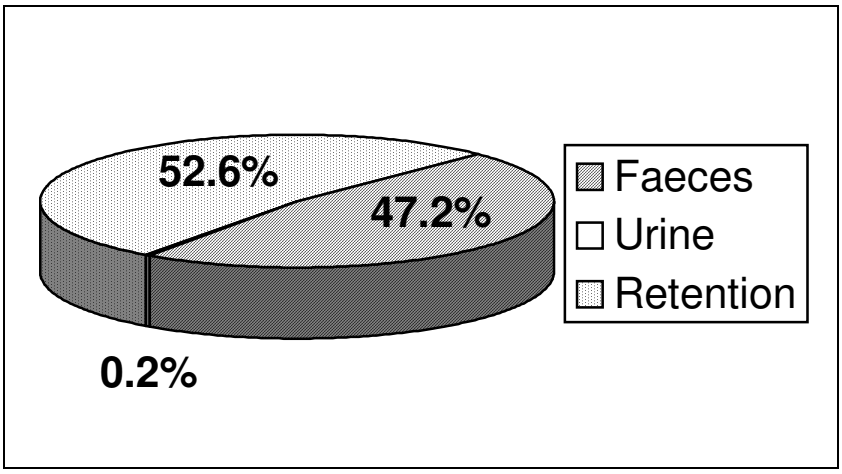

Figure 3 Mercury excretion and retention in percentage of intake

Experiment 1-3 (Feeding of carrots, potatoes and beetroots)

Cadmium accumulated (Table 20), in case of consuming Cd-exposed carrots, highly in the kidneys (2.59 mg/kg), followed by the liver (0.722 mg/kg). Cadmium could not be detected in the other organs and tissues (i.e. heart, lung, spleen, testicles, adipose tissue, muscle, ribs, hair); its concentrations were below the detection limit (0.1 mg/kg). The Cd content was approximately 6-fold in the liver and doubled in the kidneys compared to the normal ones (Figure 4).

In case of consuming Cd-contaminated potatoes, Cd was retained mainly also in the kidneys and liver (2.85 and 0.459 mg/kg). The Cd content was approximately 4-fold and 3-fold in the liver and kidneys, respectively, compared to the uncontaminated samples (Figure 5). Detectable amount of Cd could be detected in the testicles (0.196 mg/kg) and ribs (0.131 mg/kg). The testicular Cd content was twice higher than that of the normal organ. Cadmium could not be detected in the other organs and tissues (i.e. heart, lung, spleen, adipose tissue, muscle, hair); its concentrations were below the detection limit (0.1 mg/kg).

In case of consuming Cd-contaminated beetroots, the highest Cd contents were found in the kidneys (1.48 mg/kg) and the liver (0.265 mg/kg). The Cd content doubled in the kidneys compared to control samples (Figure 6). Cadmium could not be detected in other organs and tissues (i.e. heart, lung, spleen, testicles, adipose tissue, muscle, ribs, hair); its concentrations were below the detection limit (0.1 mg/kg).

Lead accumulated (Table 21), in case of consuming Pb-exposed carrots, in the kidneys and liver (4.664 and 1.846 mg/kg). Detectable amount of Pb was also found in ribs (0.649 mg/kg). The Pb content in the kidneys was about 10-fold the value found in the normal organ, while the Pb content in both liver and the rib bones was as much as in normal samples (Figure 7). Lead could not be detected in the other organs and tissues (i.e. heart, lung, spleen, testicles, adipose tissue, muscle, hair); its concentrations were below the detection limit (0.5 mg/kg).

In case of consuming Pb-contaminated potatoes, the markedly elevated concentrations of Pb were found in the kidneys (0.608 mg/kg) and the spleen (0.919 mg/kg, which was twice the value in normal spleen (Figure 8). There was not detectable amount of Pb in the other organs and tissues (i.e. heart, lung, liver, testicles, adipose tissue, muscle, ribs, hair).

In case of consuming Pb-contaminated beetroots, the Pb could not be detected in any organs and tissues. Their Pb content was below the detection limit (0.5 mg/kg).

Mercury accumulated (Table 22), in case of consuming Hg-exposed carrots, in the kidneys (50.48 mg/kg) and the liver (3.53 mg/kg). Mercury could not be detected in other organs and tissues (i.e. heart, lung, spleen, testicles, adipose tissue, muscle, ribs, hair); its concentrations were below the detection limit (0.5 mg/kg). The Hg content was approximately 100-fold in the kidneys and 7-fold in the liver compared to the normal organs (Figure 9).

In case of consuming Hg-contaminated potatoes, Hg was retained only in the kidneys (8.708 mg/kg).

In case of consuming Hg-contaminated beetroots, Hg could be also only detected in the kidneys (11.802 mg/kg).

Table 20 Cadmium content of the different organs (mg/kg in dry matter) of rabbits after feeding of normal (uncontaminated) and contaminated carrots, potatoes and beetroots (mean and ±SD)

Organ/tissue	Carrots	
	Normal (n=5)	**Contaminated** (n=5)
Heart	< 0.1	< 0.1
Lung	< 0.1	< 0.1
Liver	0.119±0.096	0.722±0.142
Kidneys	1.119±0.378	2.590±0.597
Spleen	< 0.1	< 0.1
Testicles	< 0.1	< 0.1
Adipose tissue*	< 0.1	< 0.1
Muscle	< 0.1	< 0.1
Ribs	< 0.1	< 0.1
Hair	< 0.1	< 0.1

Organ/tissue	Potatoes	
	Normal (n=4)	**Contaminated** (n=4)
Heart	< 0.1	< 0.1
Lung	< 0.1	< 0.1
Liver	0.129±0.030	0.459±0.128
Kidneys	0.947±0.288	2.850±1.017
Spleen	< 0.1	< 0.1
Testicles	< 0.1	0.196±0.076
Adipose tissue*	< 0.1	< 0.1
Muscle	< 0.1	< 0.1
Ribs	< 0.1	0.131±0.055
Hair	< 0.1	< 0.1

Organ/tissue	Beetroots	
	Normal (n=4)	**Contaminated** (n=4)
Heart	< 0.1	< 0.1
Lung	< 0.1	< 0.1
Liver	0.183±0.008	0.265±0.117
Kidneys	0.854±0.221	1.480±0.453
Spleen	< 0.1	< 0.1
Testicles	< 0.1	< 0.1
Adipose tissue*	< 0.1	< 0.1
Muscle	< 0.1	< 0.1
Ribs	< 0.1	< 0.1
Hair	< 0.1	< 0.1

*taken from abdominal fat

Cd contents
in the normal carrots: 0.1 mg/kg DM, in the contaminated carrots: 2.3 mg/kg DM;
in the normal potatoes: 0.1 mg/kg DM, in the contaminated potatoes: 2.12 mg/kg DM;
in the normal beetroots: 0.4 mg/kg DM, in the contaminated beetroots: 4.72 mg/kg DM

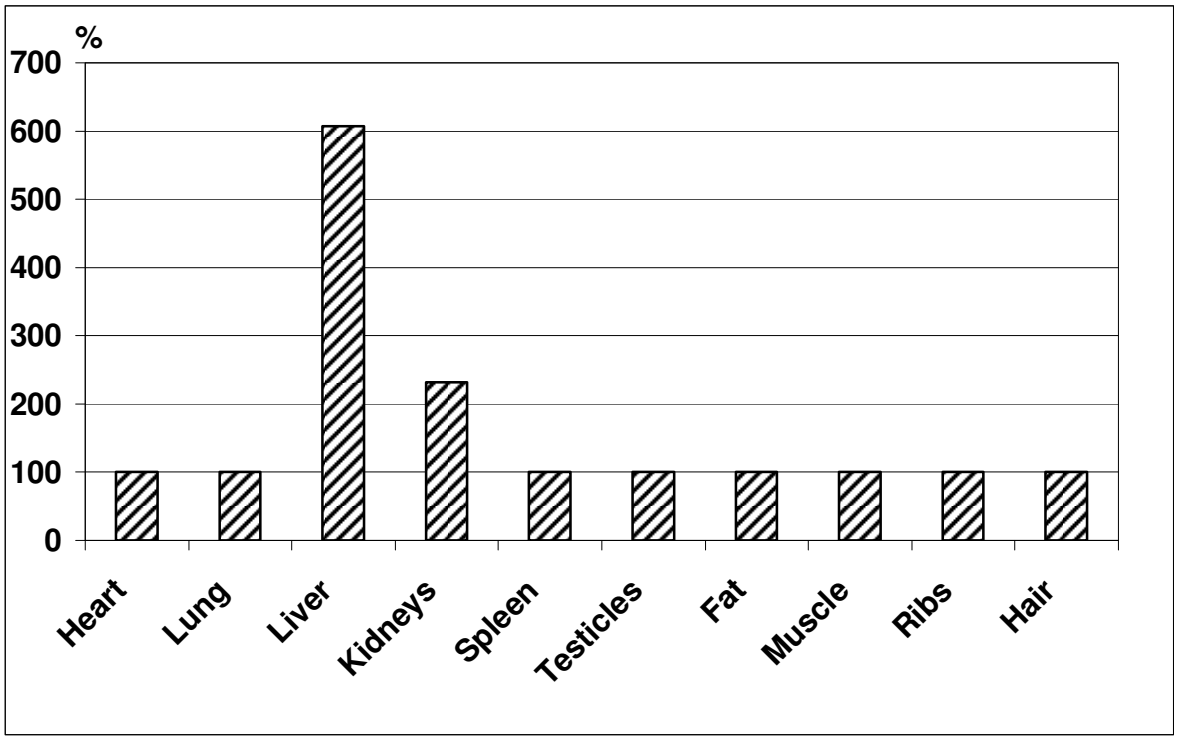

Figure 4 Relative cadmium content (%) in organs of the rabbits consuming carrots produced on Cd-polluted (270 mg/kg) soil in comparison to controls (control = 100%)

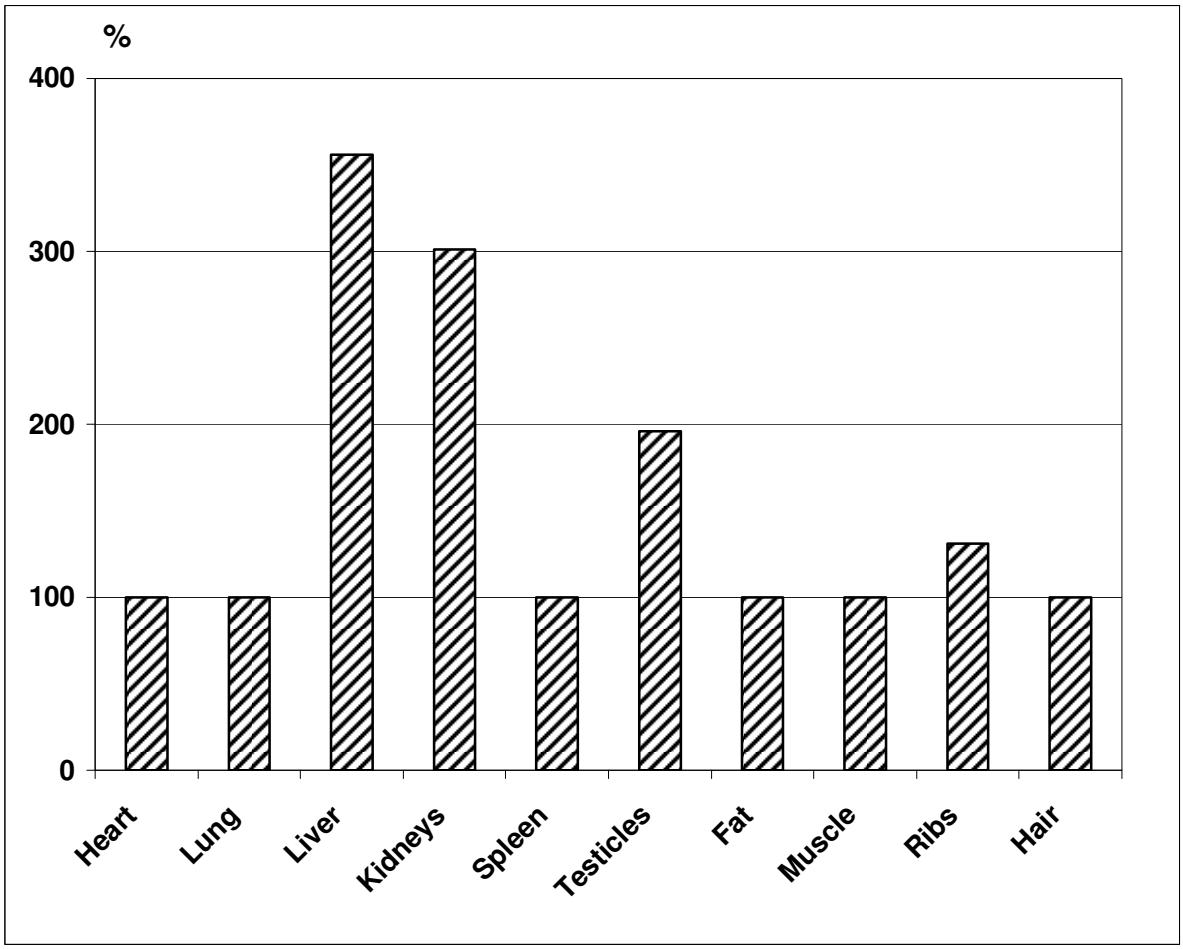

Figure 5 Relative cadmium content (%) in organs of the rabbits consuming potatoes produced on Cd-polluted (270 mg/kg) soil in comparison to controls (control = 100%)

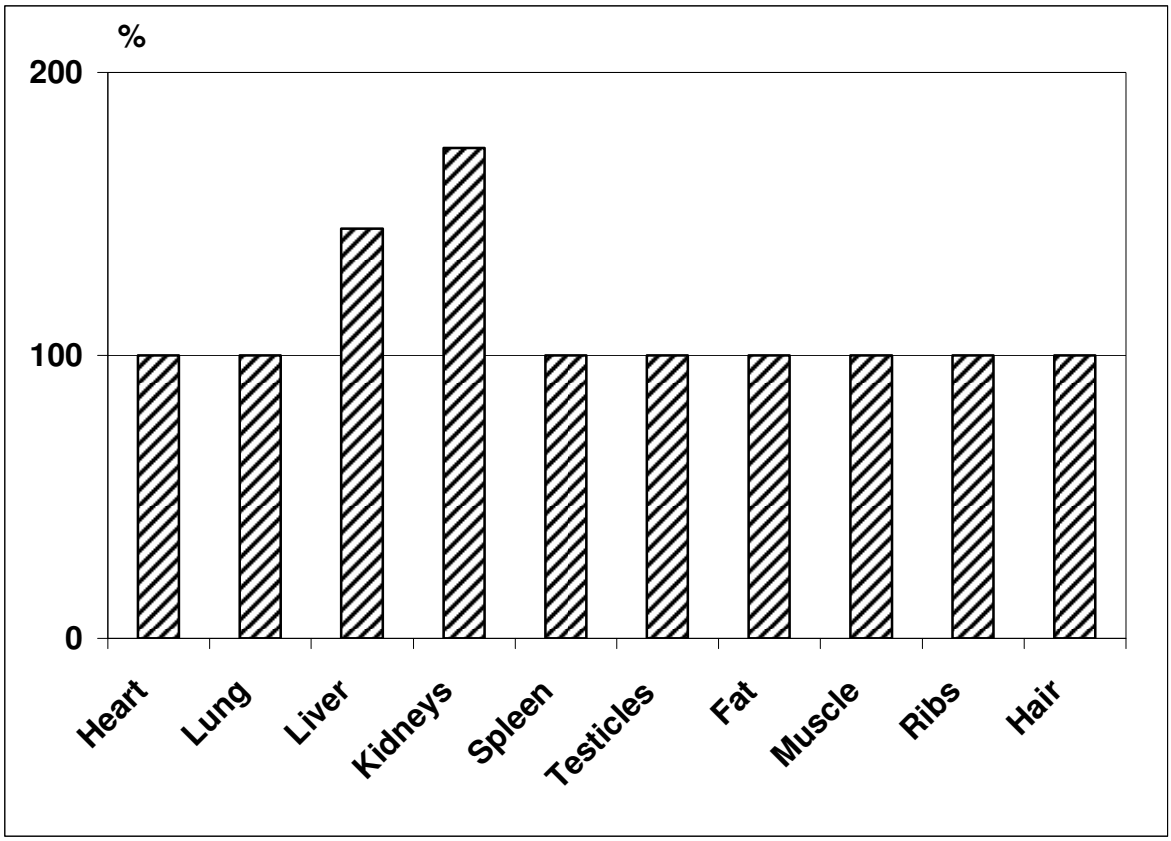

Figure 6 Relative cadmium content (%) in organs of the rabbits consuming beetroots produced on Cd-polluted (270 mg/kg) soil in comparison to controls (control = 100%)

Table 21 Lead content of the different organs (mg/kg in dry matter) of rabbits after feeding of normal (uncontaminated) and contaminated carrots, potatoes and beetroots (mean and ±SD)

Organ/tissue	Carrots	
	Normal (n=5)	**Contaminated** (n=5)
Heart	< 0.5	< 0.5
Lung	< 0.5	< 0.5
Liver	1.717±1.052	1.846±0.244
Kidneys	< 0.5	4.664±0.983
Spleen	< 0.5	< 0.5
Testicles	< 0.5	< 0.5
Adipose tissue*	< 0.5	< 0.5
Muscle	< 0.5	< 0.5
Ribs	< 0.5	0.649±0.212
Hair	< 0.5	< 0.5

Organ/tissue	Potatoes	
	Normal (n=4)	**Contaminated** (n=4)
Heart	< 0.5	< 0.5
Lung	< 0.5	< 0.5
Liver	< 0.5	< 0.5
Kidneys	< 0.5	0.608±0.085
Spleen	< 0.5	0.919±0.283
Testicles	< 0.5	< 0.5
Adipose tissue*	< 0.5	< 0.5
Muscle	< 0.5	< 0.5
Ribs	< 0.5	< 0.5
Hair	< 0.5	< 0.5

Organ/tissue	Beetroots	
	Normal (n=4)	**Contaminated** (n=4)
Heart	< 0.5	< 0.5
Lung	< 0.5	< 0.5
Liver	< 0.5	< 0.5
Kidneys	< 0.5	< 0.5
Spleen	< 0.5	< 0.5
Testicles	< 0.5	< 0.5
Adipose tissue*	< 0.5	< 0.5
Muscle	< 0.5	< 0.5
Ribs	< 0.5	< 0.5
Hair	< 0.5	< 0.5

*taken from abdominal fat

Pb contents
in the normal carrots: 1.9 mg/kg DM, in the contaminated carrots: 4.01 mg/kg DM;
in the normal potatoes: 0.6 mg/kg DM, in the contaminated potatoes: 4.1 mg/kg DM;
in the normal beetroots: <0.5 mg/kg DM, in the contaminated beetroots: 3.03 mg/kg DM

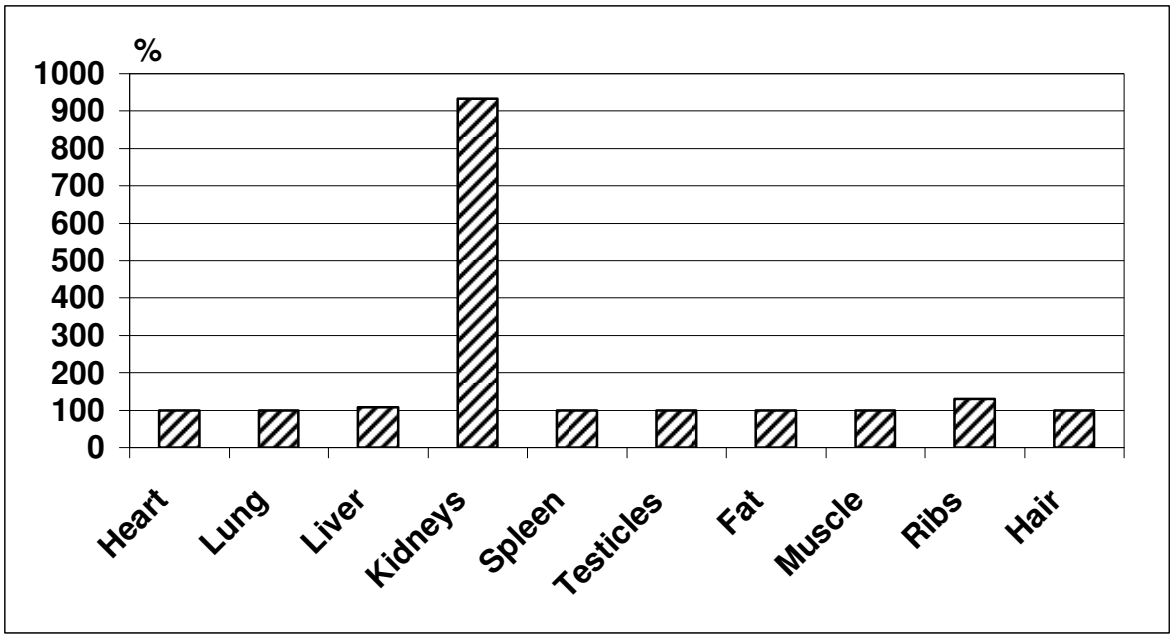

Figure 7 Relative lead content (%) in organs of the rabbits consuming carrots produced on Pb-polluted (270 mg/kg) soil in comparison to controls (control = 100%)

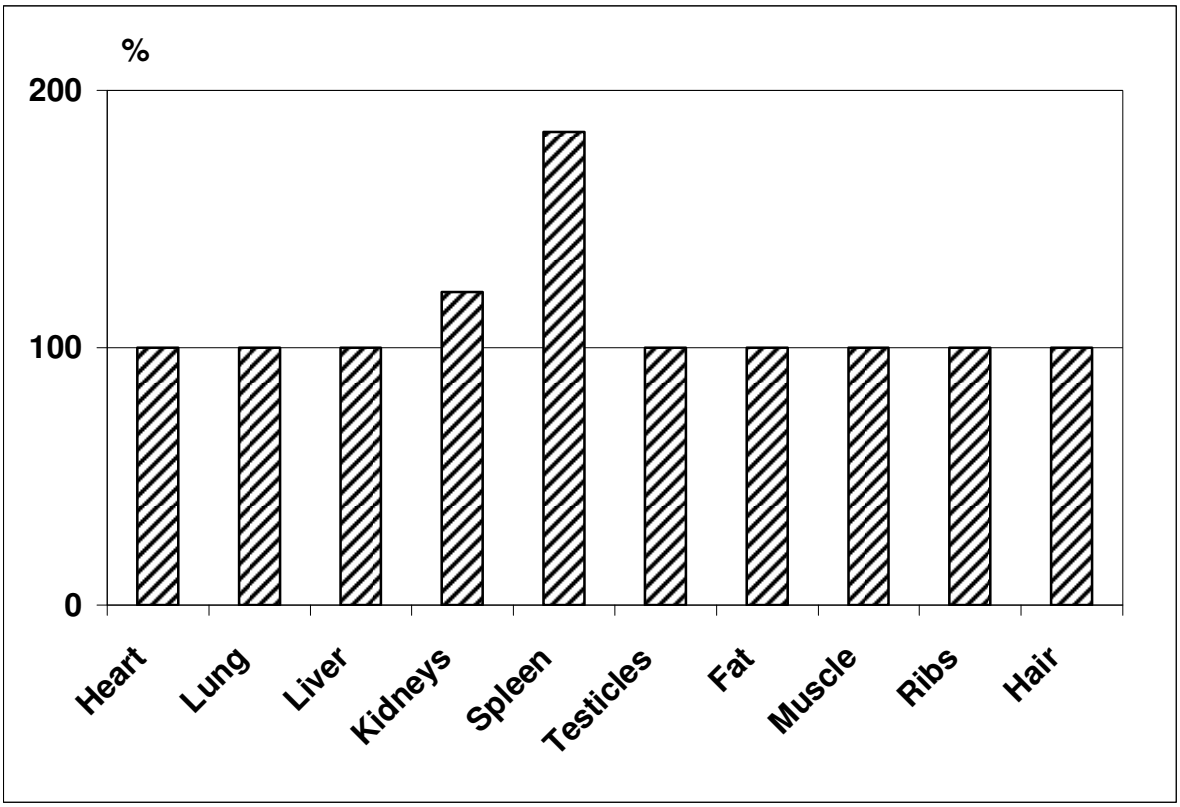

Figure 8 Relative lead content (%) in organs of the rabbits consuming potatoes produced on Pb-polluted (270 mg/kg) soil in comparison to controls (control = 100%)

Table 22 Mercury content of the different organs (mg/kg in dry matter) of rabbits after feeding of normal (uncontaminated) and contaminated carrots, potatoes and beetroots (mean and ±SD)

Organ/tissue	Carrots	
	Normal (n=5)	**Contaminated** (n=5)
Heart	< 0.5	< 0.5
Lung	< 0.5	< 0.5
Liver	< 0.5	3.530±2.027
Kidneys	< 0.5	50.480±42.583
Spleen	< 0.5	< 0.5
Testicles	< 0.5	< 0.5
Adipose tissue*	< 0.5	< 0.5
Muscle	< 0.5	< 0.5
Ribs	< 0.5	< 0.5
Hair	< 0.5	< 0.5

Organ/tissue	Potatoes	
	Normal (n=4)	**Contaminated** (n=4)
Heart	< 0.5	< 0.5
Lung	< 0.5	< 0.5
Liver	< 0.5	< 0.5
Kidneys	< 0.5	8.708±2.936
Spleen	< 0.5	< 0.5
Testicles	< 0.5	< 0.5
Adipose tissue*	< 0.5	< 0.5
Muscle	< 0.5	< 0.5
Ribs	< 0.5	< 0.5
Hair	< 0.5	< 0.5

Organ/tissue	Beetroots	
	Normal (n=4)	**Contaminated** (n=4)
Heart	< 0.5	< 0.5
Lung	< 0.5	< 0.5
Liver	< 0.5	< 0.5
Kidneys	< 0.5	11.802±3.065
Spleen	< 0.5	< 0.5
Testicles	< 0.5	< 0.5
Adipose tissue*	< 0.5	< 0.5
Muscle	< 0.5	< 0.5
Ribs	< 0.5	< 0.5
Hair	< 0.5	< 0.5

*taken from abdominal fat

Hg contents
in the normal carrots: <0.5 mg/kg DM, in the contaminated carrots: 30.00 mg/kg DM;
in the normal potatoes: <0.5 mg/kg DM, in the contaminated potatoes: 3.44 mg/kg DM;
in the normal beetroots: <0.5 mg/kg DM, in the contaminated beetroots: 6.75 mg/kg DM

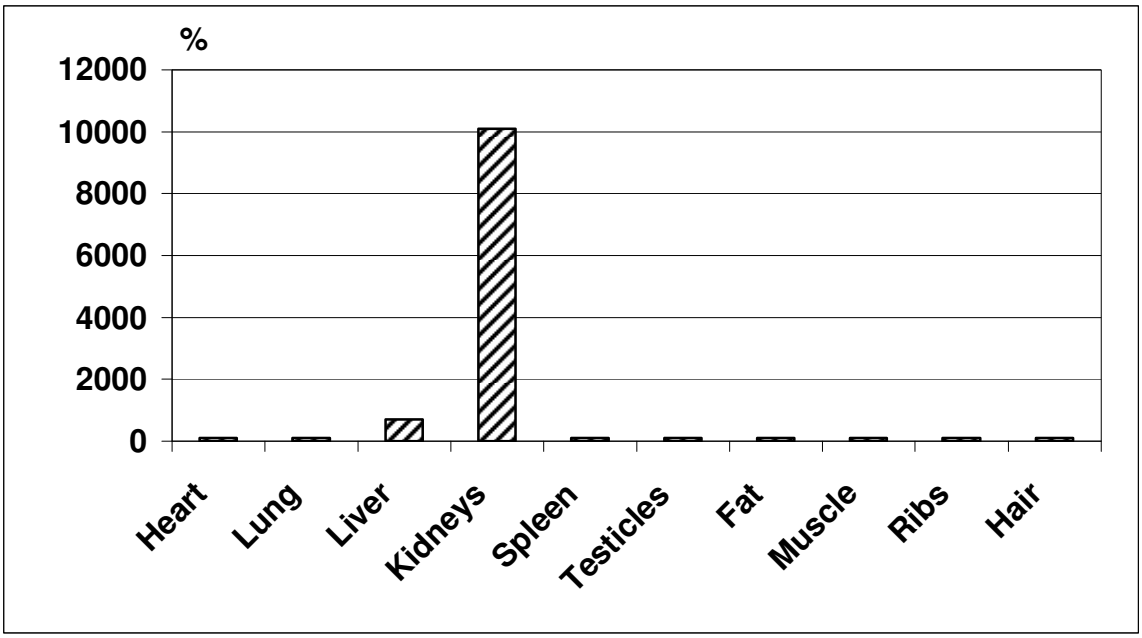

Figure 9 Relative mercury content (%) in organs of the rabbits consuming carrots produced on Hg-polluted (270 mg/kg) soil in comparison to controls (control = 100%)

Experiment 4 (Rabbit model for heavy metal loading)

After oral treatments (Table 23), the data of Cd concentration in tissues of rabbits demonstrate that kidneys and liver contain the highest Cd level (3.575 and 1.45 mg/kg) and the muscle the lowest (0.007 mg/kg). Considerable Cd concentrations were detected in ovaries (0.022 mg/kg), bones (0.028 mg/kg) and hair (0.075 mg/kg). The Cd content was approximately 7-fold and 5-fold in the liver and kidneys, respectively, compared to the controls (Figure 10). The Cd content in the lung, spleen, ovaries and bone was 2-3-fold, while in hairs it was 10-fold that of controls. The detectable amount of Cd in muscle (heart and skeletal) was as much as in controls.

Lead concentrations observed in heart (0.095 mg/kg), liver (0.535 mg/kg), lung (0.163 mg/kg), muscle (0.238 mg/kg) and ribs (0.355 mg/kg) being 2-5 times higher than the Pb concentrations in these tissues of control animals (Table 23; Figure 11).

Mercury content was increased in all tissues examined (Table 23), even in the fat depot (0.01 mg/kg). Markedly elevated concentration of Hg (Figure 12) was observed in the kidneys (32.95 mg/kg), liver (20.35 mg/kg), ovaries (2.93 mg/kg) and hair (0.943 mg/kg). Mercury could be detected in muscle (0.225 mg/kg) and ribs (0.39 mg/kg).

Considerable concentrations of trace elements were also found in soft faeces (Table 23).

Table 23 Cd, Pb, and Hg content of the different organs, faeces and soft faeces (mg/kg in dry matter) of rabbits after oral treatment, (n=4)

Organ/tissue	Cd		Pb		Hg	
	Control	Treated	Control	Treated	Control	Treated
Heart	0.006	0.007	<0.05	0.095	<0.005	0.605
Lung	0.010	0.035	<0.05	0.163	<0.005	2.538
Liver	0.198	1.45	0.267	0.535	0.011	20.350
Kidneys	0.678	3.575	0.207	0.288	0.011	32.950
Spleen	0.029	0.064	0.405	0.495	<0.005	0.278
Ovaries	0.009	0.022	<0.05	0.08	<0.005	2.930
Adipose tissue*	<0.005	<0.005	<0.05	<0.05	<0.005	0.01
Muscle	0.007	0.007	<0.05	0.238	<0.005	0.225
Ribs	0.009	0.028	0.355	0.600	<0.005	0.390
Hair	0.007	0.075	0.03	0.03	0.009	0.943
Faeces	0.415	5.898	1.875	8.575	0.023	59.225
Soft faeces	0.424	5.418	1.037	10.050	0.030	38.250

*taken from abdominal fat

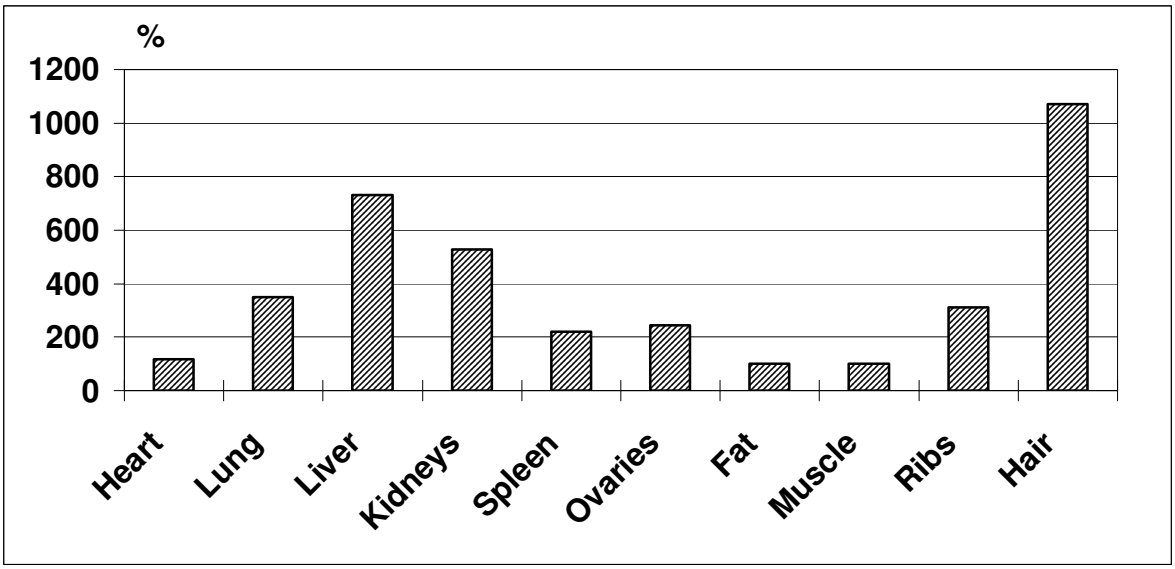

Figure 10 Relative cadmium content (%) in organs of Cd-treated rabbits compared to controls (control = 100%)

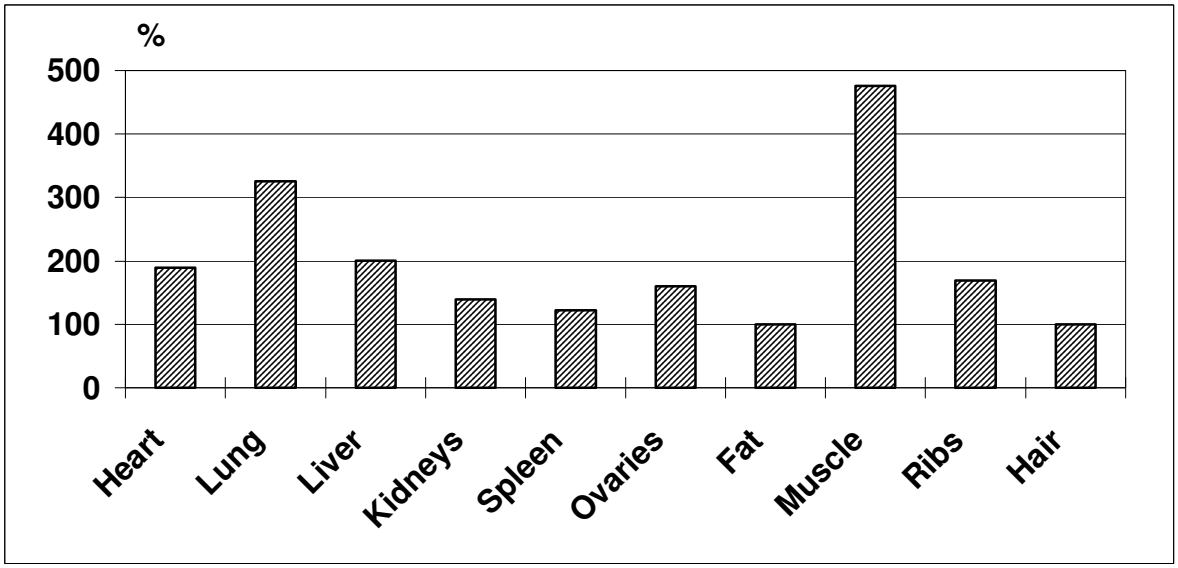

Figure 11 Relative lead content (%) in organs of Pb-treated rabbits compared to controls (control = 100%)

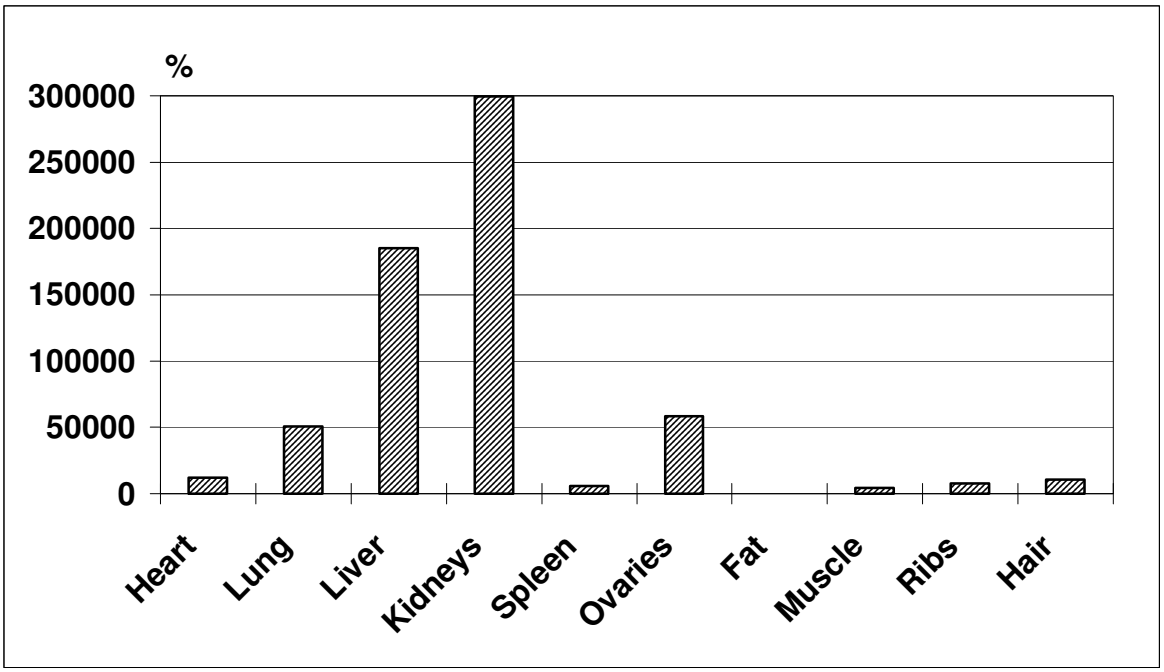

Figure 12 Relative mercury content (%) in organs of Hg-treated rabbits compared to controls (control = 100%)

Experiment 6 (*Rabbit model for supplemental Ni*)

Rabbits ingested 6.2±1.2, 59.3±9.5 and 506.5±120.4 mg/kg of Ni in form of $NiCl_2$ (Table 24). In case of Ni-burden, approximately 98% of Ni was eliminated from the body via faeces and 0.5-1.5% with the urine, and approximately 1% was retained in the body.

Table 24 Ingestion, excretion via faeces and urine, and retention of Ni in rabbits (mean and ±SD)

Item	**Supplemental Ni**		
	0 mg/kg	**50 mg/kg**	**500 mg/kg**
Ingestion, mg	6.2 ±1.2 100%	59.3 ±9.5 100%	506.5 ±120.4 100%
Faeces, mg	5.3 ±1.1 86.6%	57.9 ±9.4 97.6%	497.6 ±102 98.2%
Urine, mg	0.3 ±0.02 4.5%	1.0 ±0.2 1.7%	3.3 ±0.7 0.6%
Deposition	8.9%	0.7%	1.2%

With increasing the Ni-load, the Ni content of the organs was significantly increased (Table 25). Nickel accumulated in the kidneys (4.9±0.5 or 17.1±3.1 mg/kg DM), the ribs (10.3±0.4 or 10.4±0.6 mg/kg DM), the heart (1.4±0.2 or 2.4±0.4 mg/kg DM), the liver (1.3±0.1 or 2.2±0.2 mg/kg DM), and in the lung (0.9±0.2 or 1.6±0.4 mg/kg DM). The ovaries had relatively high concentration of Ni (0.3 or 0.4 mg/kg DM, 50 and 500 mg/kg Ni, respectively), being 3-4 times higher than the Ni concentrations in these tissues of control animals. With increasing the Ni-load, the Ni content of muscle was significantly increased (0.02±0.01, 0.07±0.01 and 0.15±0.02 mg/kg DM, 0, 50 and 500 mg/kg Ni, respectively).

Table 25 Nickel content of organs and tissues in female rabbits, mg/kg dry matter (mean and ±SD)

Organ/tissue	**Supplemental Ni**		
	0 mg/kg	**50 mg/kg**	**500 mg/kg Ni**
Heart	1.0 ±0.1^{a}	1.4 ±0.2^{b}	2.4 ±0.4^{c}
Lung	0.7 ±0.1^{a}	0.9 ±0.2^{a}	1.6 ±0.4^{b}
Liver	0.9 ±0.05^{a}	1.3 ±0.1^{b}	2.2 ±0.2^{c}
Kidneys	1.9 ±0.3^{a}	4.9 ±0.5^{b}	17.1 ±3.1^{c}
Spleen	0.07 ±0.03^{a}	0.12 ±0.05^{ab}	0.12 ±0.03^{b}
Ovaries	0.1	0.3	0.4
Fat	0.01 ±0.008^{a}	0.02 ±0.003^{b}	0.05 ±0.014^{c}
Muscle	0.02 ±0.01^{a}	0.07 ±0.01^{b}	0.15 ±0.02^{c}
Ribs	9.1 ±0.6^{a}	10.3 ±0.4^{b}	10.4 ±0.6^{b}
Hair	0.04 ±0.01^{a}	0.04 ±0.01^{ab}	0.06 ±0.01^{b}

a-a, b-b: $P>0.05$; a-b-c: $P<0.05$

4.4. HISTOPATHOLOGY

Experiment 1-4 (*Feeding of carrots, potatoes or beetroots and Rabbit model for heavy metal loading*)

According to pathological and histopathological investigation of all rabbits, carrot samples containing high Cd, Pb or Hg resulted in a significant ($P<0.05$) reduction of kidney and spleen weight as compared to organs of rabbits fed uncontaminated samples. The changes in relative weight of other organs (i.e. heart, liver, lung and testicles) were negligible (Table 26).

The Pb-loaded potato tubers significantly ($P<0.05$) increased the relative weight of the liver, while the Cd and Hg loading decreased significantly ($P<0.05$) the relative weight of the heart. Due to the heavy metal loading, the relative weight of the kidneys tended to be lower, particularly in case of Hg (Table 26).

The high content of Cd, Pb or Hg in beetroots increased the relative weight of some organs (i.e. heart, liver and lung); although only the Cd caused a significant ($P<0.05$) increase in the liver and lungs (Table 26). The relative weight of testicles tended to be markedly lower because of the Pb burden in comparison with value found in rabbits fed uncontaminated beetroots.

Table 26 Relative organ weight of rabbits (%) after feeding of carrots, potatooes or beetroots (mean and ±SD)

Organs	**Carrots** (n=5)			
	uncontaminated	**Cd**	**Pb**	**Hg**
Heart	0.22 ±0.02[a]	0.24 ±0.02[a]	0.25 ±0.03[a]	0.25 ±0.02[a]
Liver	2.78 ±0.18[a]	2.53 ±0.33[a]	2.69 ±0.30[a]	3.27 ±0.47[a]
Lung	0.54 ±0.09[a]	0.56 ±0.11[a]	0.53 ±0.08[a]	0.56 ±0.1[a]
Kidneys	0.79 ±0.11[a]	0.60 ±0.13[b]	0.61 ±0.08[b]	0.59 ±0.08[b]
Testicles	0.14 ±0.04[a]	0.12 ±0.06[a]	0.13 ±0.05[a]	0.15 ±0.04[a]
Spleen	0.08 ±0.01[a]	0.06 ±0.01[b]	0.05 ±0.01[b]	0.06 ±0.01[b]

Organs	**Potatoes** (n=4)			
	uncontaminated	**Cd**	**Pb**	**Hg**
Heart	0.22 ±0.01[a]	0.19 ±0.01[b]	0.22 ±0.04[a]	0.18 ±0.02[b]
Liver	2.49 ±0.12[a]	2.59 ±0.23[a]	3.01 ±0.31[b]	2.26 ±0.11[a]
Lung	0.48 ±0.04[a]	0.50 ±0.07[a]	0.47 ± 0.05[a]	0.53 ±0.14[a]
Kidneys	0.59 ±0.06[a]	0.51 ±0.03[a]	0.58 ±0.04[a]	0.49 ±0.07[a]
Testicles	0.16 ±0.04[a]	0.13 ±0.01[a]	0.17 ±0.04[a]	0.16 ±0.02[a]
Spleen	0.03 ±0.02[a]	0.04 ±0.01[a]	0.04 ±0.02[a]	0.03 ±0.01[a]

Organs	**Beetroots** (n=4)			
	uncontaminated	**Cd**	**Pb**	**Hg**
Heart	0.23 ±0.03[a]	0.24 ±0.06[a]	0.21 ±0.01[a]	0.21 ±0.02[a]
Liver	2.58 ±0.17[a]	3.18 ±0.45[b]	3.03 ±0.51[a]	2.84 ±0.18[a]
Lung	0.41 ±0.02[a]	0.48 ±0.0[b]	0.43 ±0.04[a]	0.46 ±0.07[a]
Kidneys	0.44 ±0.12[a]	0.55 ±0.03[a]	0.52 ±0.06[a]	0.62 ±0.23[a]
Testicles	0.20 ±0.04[a]	0.16 ±0.01[a]	0.10 ±0.03[a]	0.17 ±0.01[a]
Spleen	0.04 ±0.01[a]	0.04 ±0.03[a]	0.04 ±0.01[a]	0.06 ±0.03[a]

a-a: $P>0.05$; a-b: $P<0.05$

Histological examination in each experiment revealed that the rate of spermatogenesis in the testis was reduced in the Cd- and Pb-loading groups compared to rabbits fed uncontaminated samples (Figure 13). In case of consuming Hg-contaminated carrots, the rate of spermatogenesis in the testes was also reduced. A large number of syncytial giant cells and degenerated cells indicating abnormal meiosis were found among the spermatogenic cells.

Figure 13 Histological structure of rabbit testicle (HE, x200)

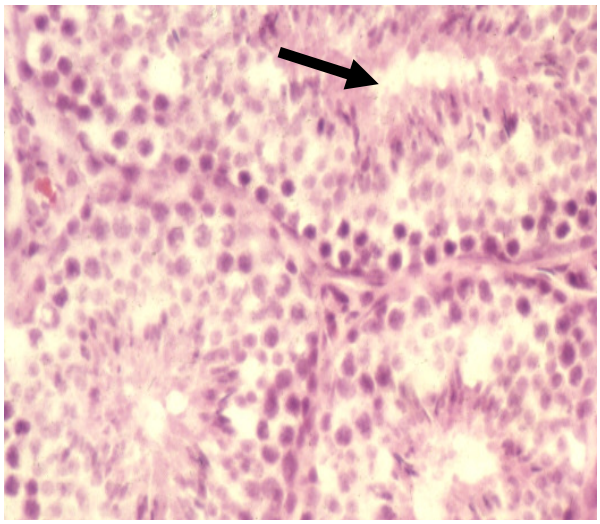

Figure 13a Seminipherous tubules of a control rabbit showing 4-5 layers of germ cells and mature spermatocytes (arrow)

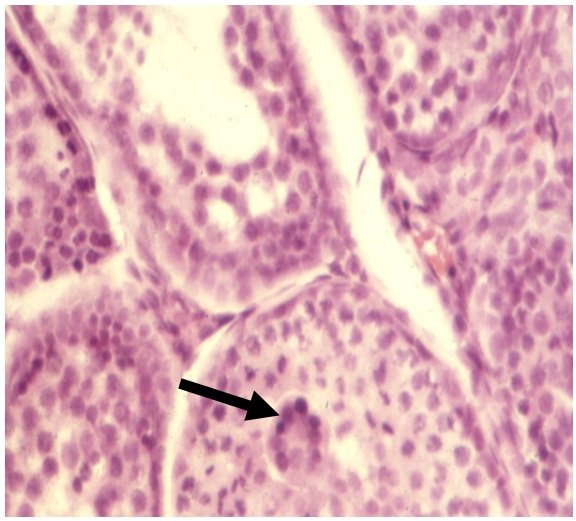

Figure 13b Seminipherous tubules of a Cd-exposed rabbit showing a reduction in germ cells and mature spermatocytes with syncytial giant cell (arrow)

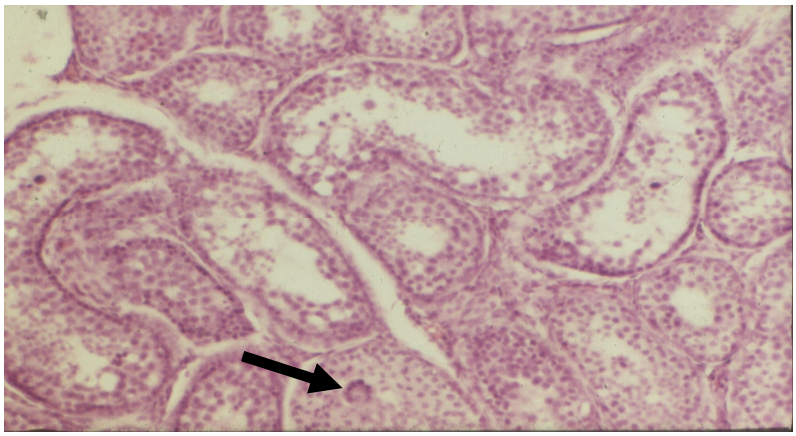

Figure 13c Seminipherous tubules of a Pb-exposed rabbit showing a reduction in germ cells and mature spermatocytes with syncytial giant cell (arrow)

In case of does, ovarian follicles containing healthy ova at different developmental stages were found, but signs of actual ovulation (*corpus luteum*) were not found (Figure 14).

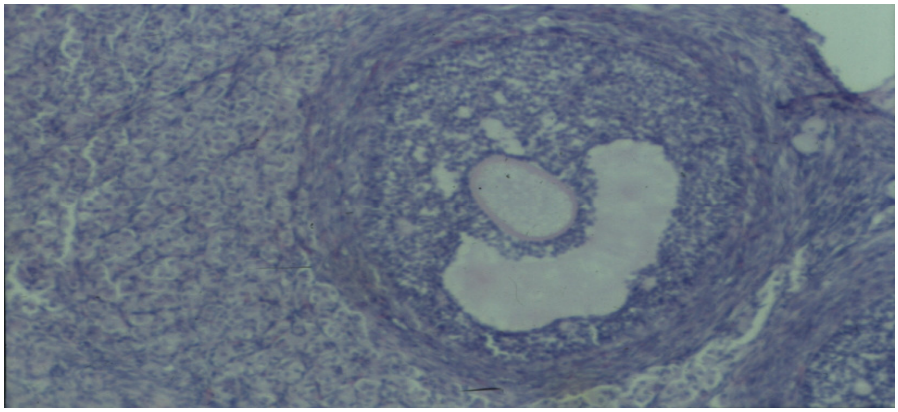

Figure 14 Tercier ovarian follicle of a toxic metal-exposed female rabbit (HE, x200)

After ingestion Cd, Pb and Hg, lesion of liver parenchyma such as focal fatty infiltration was detected in *Experiment 4* (Figure 15)

Figure 15 Histological structure of rabbit livers (Fat Red, x100)

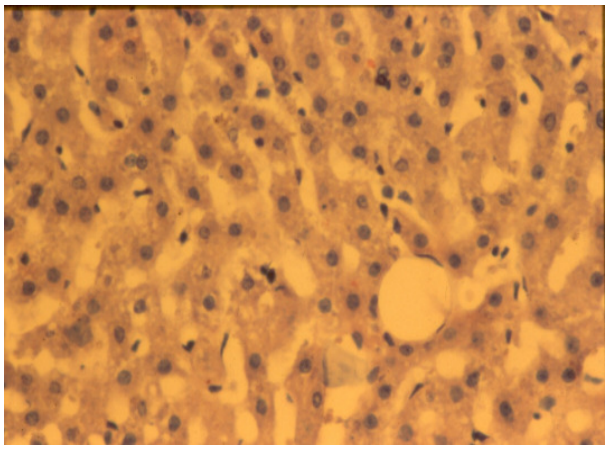

Figure 15a Liver cells of a control rabbit showing normal histological structure without fatty infiltration

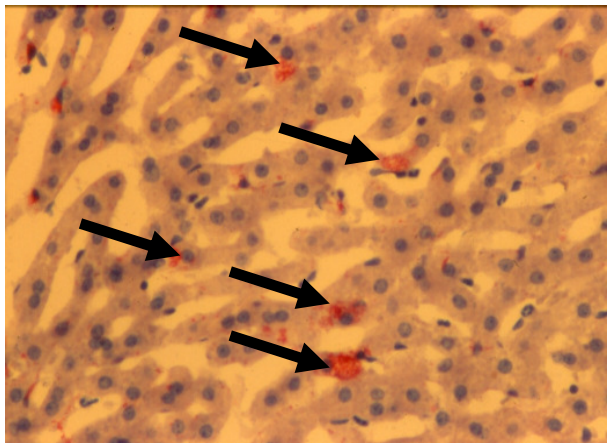

Figure 15b Liver cells of a Cd-exposed rabbit showing fatty infiltration (arrows)

Orally administered toxic metals (i.e. Cd, Pb, Hg) caused slight tubulonephrosis in rabbits (Figure 16).

Figure 16 Histological structure of rabbit kidneys (HE, x200)

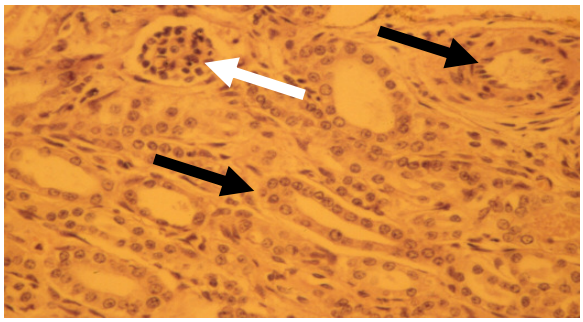

Figure 16a Renal tubules (black arrows) and glomerulus (white arrow) of a control rabbit showing normal histological structure

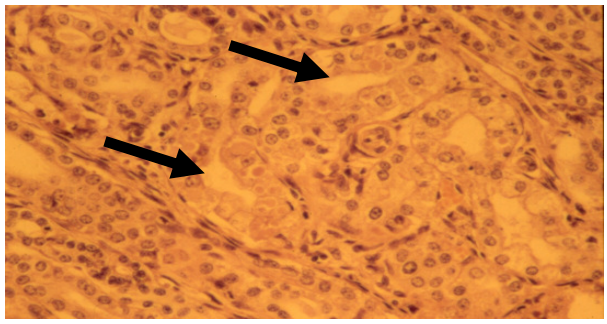

Figure 16b Renal tubules (arrows) of a Cd-exposed rabbit showing tubulonephrosis

Histological examination of bones estimated that any toxic metal burdens of rabbit body failed to damage the function of the bone marrow and did not alter the *erythropoiesis*.

Experiment 5 (*Broiler model for supplemental Ni*)

Supplemental $NiCl_2$ did not alter mortality. The relative weight of cockerel livers and testicles were significantly ($P<0.05$) decreased (Table 27) by Ni loading compared to the control group (1.66, 0.03 and 2.09, 0.05 vs 2.57, 0.12, respectively). The changes in relative weight of heart and spleen were negligible.

Mild or moderate form of pathological focal fatty infiltration caused by 50 or 500 mg/kg Ni was found in liver samples.

Table 27 Absolute (g) and relative (%) weight of organs in 49-day-old cockerels

Organ		Supplemental Ni		
		0 mg/kg	**50 mg/kg**	**500 mg/kg**
Heart	absolute	11.75	10.06	10.41
	relative	0.48[a]	0.38[a]	0.49[a]
Liver	absolute	62.80	43.87	44.29
	relative	2.57[a]	1.66[b]	2.09[b]
Spleen	absolute	2.73	2.33	3.18
	relative	0.11[a]	0.09[a]	0.15[a]
Testicles	absolute	2.82	0.72	1.15
	relative	0.12[a]	0.03[b]	0.05[b]

a-a: $P>0.05$; a-b: $P<0.05$

Experiment 6 (*Rabbit model for supplemental Ni*)

There were insignificant (P>0.05) changes in relative weight of organs caused by 50 mg/kg dietary Ni supplementation (Table 28).

The 500 mg/kg Ni supplementation significantly (P<0.05) reduced the relative weight of liver, kidneys and ovaries, compared to the lower addition (Table 28). The changes in relative weight of other organs (i.e. heart, lung and spleen) were negligible.

Moreover, the histopathological investigations indicate that the activity of ovaries were reduced by adding 500 mg/kg Ni.

Table 28 Absolute (g) and relative (%) weight of organs in female rabbits

Organ		Supplemental Ni		
		0 mg/kg	**50 mg/kg**	**500 mg/kg**
Heart	absolute	9.7	10.46	9.19
	relative	0.19[a]	0.19[a]	0.17[a]
Lung	absolute	19.9	19.59	18.57
	relative	0.4[a]	0.36[a]	0.34[a]
Liver	absolute	94.8	122.12	86.31
	relative	1.9[a]	2.26[a]	1.60[b]
Spleen	absolute	2.1	2.17	1.72
	relative	0.04[a]	0.04[a]	0.03[a]
Kidneys	absolute	19.2	22.41	16.39
	relative	0.37[a]	0.41[a]	0.30[b]
Ovaries	absolute	0.35	0.40	0.32
	relative	0.007[a]	0.007[a]	0.006[b]

a-a: P>0.05; a-b: P<0.05

Nickel burden of body failed to affect the Cu, Zn, Fe, and Mn concentration of the different organs in rabbit, except of liver, spleen and ribs (Table 29). In these organs, however, significantly (P<0.05) reduced concentrations of Cu were observed.

Table 29 Interactions of Ni with Cu, Zn, Fe and Mn in rabbit tissues after the 24-day-long supplementation of Ni (n=5; mean and ±SD)

Organ/tissue	Cu	Zn	Fe	Mn	Supplemental Ni
			(mg/kg)		
Heart	11.6 ±3.8	56.8 ±5.3	215.6 ±14.1	1.2 ±0.2	0 mg/kg
	11.5 ±2.8	56.6 ±5.9	212.8 ±13.1	1.2 ±0.2	50 mg/kg
	12.2 ±1.7	54.4 ±4.8	210.0 ±17.4	1.2 ±0.2	500 mg/kg
Lung	5.2 ±1.6	54.2 ±5.9	204.8 ±17.1	0.8 ±0.07	0 mg/kg
	5.0 ±0.9	51.4 ±5.7	198.6 ±16.5	0.8 ±0.09	50 mg/kg
	5.3 ±1.4	53.4 ±5.0	205.0 ±14.6	0.8 ±0.09	500 mg/kg
Liver	13.5 ±1.2[a]	95.6 ±7.8	310 ±31.8	6.9 ±0.4	0 mg/kg
	10.5 ±1.3[b]	97.0 ±8.1	303 ±24.4	6.9 ±0.5	50 mg/kg
	12.4 ±1.0[a]	98.4 ±6.7	299.4 ±23.5	6.7 ±0.4	500 mg/kg
Kidneys	10.3 ±0.5	94.2 ±5.6	177.7 ±14.7	7.2 ±0.4	0 mg/kg
	10.3 ±0.7	90.2 ±7.9	177.2 ±10.8	7.2 ±0.5	50 mg/kg
	10.5 ±0.4	93.2 ±5.6	169.0 ±12.6	7.3 ±0.6	500 mg/kg
Spleen	6.8 ±1.4[a]	78.4 ±6.3	560.6 ±85.6	1.4 ±0.3	0 mg/kg
	4.9 ±0.8[b]	80.6 ±6.8	570.4 ±46.7	1.4 ±0.3	50 mg/kg
	6.0 ±0.6[ab]	81.2 ±8.5	556.4 ±66.5	1.4 ±0.4	500 mg/kg
Ovaries	3.5	64	72	2	0 mg/kg
	3.4	57	68	2	50 mg/kg
	3.8	61	66	2.2	500 mg/kg
Fat	< 0.2	2.2 ±0.3	5.0 ±0.3	< 0.25	0 mg/kg
	< 0.2	2.3 ±0.4	5.2 ±0.4	< 0.25	50 mg/kg
	< 0.2	2.2 ±0.4	5.0 ±0.4	< 0.25	500 mg/kg
Muscle	1.6 ±0.2	31.8 ±3.1	22.0 ±2.8	0.5 ±0.09	0 mg/kg
	1.6 ±0.2	32.4 ±3.2	21.2 ±3.1	0.5 ±0.07	50 mg/kg
	1.6 ±0.2	30.2 ±3.8	22.2 ±3.1	0.5 ±0.06	500 mg/kg
Rib bones	4.1 ±0.3[a]	100.8 ±8.0		3.4 ±0.4	0 mg/kg
	3.7 ±0.1[b]	100.2 ±8.2		3.7 ±0.3	50 mg/kg
	3.7 ±0.3[b]	98.4 ±9.3		3.5 ±0.4	500 mg/kg
Hair	11.4 ±0.7	12.7 ±1.7	206.4 ±18.1	0.8 ±0.11	0 mg/kg
	11.3 ±0.7	14.3 ±2.6	207.0 ±16.0	0.8 ±0.09	50 mg/kg
	11.6 ±0.5	12.8 ±2.4	211.2 ±18.8	0.7 ±0.06	500 mg/kg

a-a: $P>0.05$; a-b: $P<0.05$

4.5. HAEMATOLOGY, ZPP, SERUM BIOCHEMISTRY and PANCREATIC ENZYMES

4.5.1. Haematology and ZPP. *Experiment 4* (*Rabbit model for heavy metal loading*)

Initial concentrations of Cd, before ingestion, in serum of rabbits proved to be less than 0.1 µg/L (Table 30). After 4 weeks of Cd ingestion, the Cd concentration significantly ($P<0.001$) increased to 0.13 µg/L (range 0.11-0.16 µg/L).

Initial concentrations of Pb, before ingestion, in serum of rabbits ranged from 17-28 µg/L with a mean value of 23.22 µg/L (Table 30). After 4 weeks of Pb ingestion, the Pb concentration significantly ($P<0.001$) increased to 40-51 µg/L with an average of 46.5 µg/L.

Initial concentrations of Hg, before ingestion, in serum of twelve rabbits was less than 1.0 µg/L (Table 30). After 4 weeks of mercury ingestion, the Hg concentration significantly ($P<0.001$) increased to the average of 97.58 µg/L (range 72-123 µg/L).

Table 30 Concentration of heavy metals (µg/L) in the serum of rabbits treated perorally with Cd, Pb or Hg, (mean and ±SD, and range)

Treatment (n=4)	**Concentration**	
	d 0	**d 28**
Cd	$< 0.1^{a}$	0.13 ± 0.02^{b} (0.11-0.16)
Pb	23.22 ± 3.31^{a} (17-28)	46.50 ± 4.80^{b} (40-51)
Hg	$< 1.0^{a}$	97.58 ± 21.12^{b} (72-123)

a-b: $P<0.001$

The ingestion of Cd did not cause any changes in the haematological values (Table 31).

The initial mean of RBC, HGB, and HCT ($6.56\pm0.82 \times 10^{12}$/L, 120.95±11.39 g/L, and 37.41±4.51%, respectively) were significantly ($P<0.05$) decreased ($5.04\pm2.74 \times 10^{12}$/L, 96.75±49.30 g/L, and 29.55±16.00%, respectively) as a result of Pb exposure (Table 31). However, the initial mean of MCV, MCH, and MCHC (57.08±1.63 fl, 18.53±0.91 pg, and 324.32±11.63 g/L, respectively) were significantly ($P<0.05$) increased (59.10±1.12 fl, 20.03±2.17 pg, and 341.50±35.33 g/L) at the 4-week ingestion of Pb.

Mercury exposure significantly ($P<0.001$) decreased the initial mean of WBC (Table 31) as well as Pb did ($8.69\pm2.34 \times 10^{9}$/L *vs* 4.03±3.58 and $3.80\pm2.03 \times 10^{9}$/L).

Table 31 Haematological values in the rabbits before and after the 28-day-long peroral heavy metal treatment (n=4/treatment; mean and ±SD)

Treatment	WBC (10^9/L)	RBC (10^{12}/L)	HGB (g/L)	MCH (pg)	HCT (%)	MCV (fl)	MCHC (g/L)	PLT (10^9/L)
Initial value	8.69[a]	6.56[a]	120.95[a]	18.53[a]	37.41[a]	57.08[a]	324.32[a]	382.00[a]
±SD	2.34	0.82	11.39	0.91	4.51	1.63	11.63	121.31
Cd	7.20[a]	7.23[a]	125.50[a]	18.80[a]	41.45[a]	57.40[a]	327.50[a]	405.00[a]
±SD	2.77	0.58	5.26	0.82	2.67	1.21	9.54	8.44
Pb	4.03[c]	5.04[b]	96.75[b]	20.03[b]	29.55[b]	59.10[b]	341.50[b]	268.67[a]
±SD	3.58	2.74	49.30	2.17	16.00	1.12	35.33	144.35
Hg	3.80[c]	6.10[a]	116.25[a]	19.10[a]	34.98[a]	57.53[a]	332.50[a]	272.75[a]
±SD	2.03	1.07	17.35	1.02	5.33	2.18	5.80	122.68

WBC: white blood cells, RBC: red blood cells, HGB: haemoglobin, MCH: mean cell haemoglobin, HCT: haematocrit, MCV: mean cell volume, MCHC: mean cell haemoglobin concentration, PLT: platelets; a-a: P>0.05, a-b: P<0.05, a-c: P<0.001

The initial ZPP concentration was insignificantly changed as a consequence of Pb-ingestion (106.00±19.78 μmol/mol hem *vs* 114.57±37.80 μmol/mol hem).

4.5.2. Serum biochemistry. *Experiment 1* (*Feeding of carrots*)

Cadmium loading did not change the activity of both AST and ALT (Table 32) but significantly (P<0.01) decreased the GGT activity and increased the ALP activity compared to control (2±1 *vs* 8±5 U/L and 123±28 *vs* 55±31 U/L).

Uptake of Pb did not alter the AST activity but significantly (P<0.05) decreased the activity of both ALT (12±3 *vs* 22±4.5 U/L) and GGT (4±3 *vs* 8±5 U/L) and increased that of ALP (103±22 *vs* 55±31 U/L) compared to control (Table 32).

Mercury loading also did not alter the activity of both AST and ALT (Table 32) but significantly decreased the GGT activity (P<0.01) and increased the ALP activity (P<0.05) compared to control (1±0.3 *vs* 8±5 U/L and 96±7 *vs* 55±31 U/L).

Cadmium, Pb or Hg burden considerably reduced the activity of both CHE and GSHPx and increased the CK level (Table 32).

Table 32 Serum biochemistry values in rabbits feeding carrots (n=5; mean and ±SD)

Treatment	AST (U/L)	ALT (U/L)	GGT (U/L)	ALP (U/L)	CHE (U/L)	CK (U/L)	GSHPx (U/g protein)
Control	4[a]	22[a]	8[a]	55[a]	735[a]	364[a]	20[a]
±SD	1	4.5	5	31	365	90	8
Cd	4[a]	18[a]	2[c]	123[c]	429[a]	623[a]	13[a]
±SD	1.5	13.5	1	28	224	211	4
Pb	4[a]	12[b]	4[b]	103[b]	385[a]	646[a]	17[a]
±SD	1	3	3	22	267	260	6
Hg	5[a]	18[a]	1[c]	96[b]	339[a]	523[a]	14[a]
±SD	1	7	0.3	7	309	83	10

AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyltransferase, ALP: alkaline phosphatase, CHE: cholinesterase, CK: creatine kinase, GSHPx: glutathione peroxidase; a-a: P>0.05, a-b: P<0.05, a-c: P<0.01

Experiment 4 (*Rabbit model for heavy metal loading*)

Lead and Hg loading significantly (P<0.01) increased AST activities (Table 33) compared to base-line (41±15 and 43±13 U/L *vs* 20±7 U/L, respectively).

ALT activities (Table 33) were also significantly increased (P<0.01) by both Cd and Hg exposure (61±13 U/L and 64±17 *vs* 49±8 U/L, respectively).

The concentration of CREA was significantly increased by only Cd loading (116±7 *vs* 95±18 µmol/L).

UREA concentrations were not changed by any toxic metal burdens (Table 33).

Table 33 Serum biochemistry values in the rabbits before and after the 28-day-long heavy metal treatment (n=4; mean and ±SD)

Treatment	AST (U/L)	ALT (U/L)	CREA (µmol/L)	UREA (mmol/L)	UREA/CREA
Initial value	20^{a}	49^{a}	95^{a}	5.1^{a}	0.05^{a}
±SD	7	8	18	0.8	0.01
Cd	27^{a}	61^{c}	116^{b}	4.9^{a}	0.04^{a}
±SD	11	13	7	0.6	0.01
Pb	41^{c}	62^{a}	99^{a}	5.1^{a}	0.05^{a}
±SD	15	25	10	0.7	0.01
Hg	43^{d}	64^{c}	106^{a}	5.0^{a}	0.05^{a}
±SD	13	17	16	0.8	0.01

AST: aspartate aminotransferase, ALT: alanine aminotransferase,
CREA: creatinine; a-a: P>0.05, a-b: P<0.05, a-c: P<0.01, a-d: P<0.001

Experiment 5 (*Broiler model for supplemental Ni*)

The dietary supplementation of 50 mg/kg Ni in form of $NiCl_2$ significantly decreased the level of both CHOL and CREA compared to control (6.0±0.5 *vs* 8.0±3.0 mmol/L and 55±12 *vs* 136±121 mmol/L , repectively), whereas the activity of GGT, AST, CHE and ALP and the concentration of TRIG were not altered.

The dietary supplemental of 500 mg/kg Ni insignificantly increased the activity of AST and CHE (Table 34) in comparison with control (46±26 *vs* 43±17 U/L and 1285±293 *vs* 1233±250 U/L, respectively). The activity of GGT and ALP and the concentration of CHOL, TRIG and CREA were not altered.

Table 34 Activity of enzymes (U/L), and concentration of cholesterol, triglyceride and creatinine (mmol/L) in 49-day-old cockerel blood
(mean and ±SD)

Item	Supplemental Ni		
	0 mg/kg	50 mg/kg	500 mg/kg
GGT	17 ±9[a]	13 ±6[a]	11 ±5[a]
AST	43 ±17[a]	39 ±18[a]	46 ±26[a]
CHE	1233 ±250[a]	1047 ±204[a]	1285 ±293[a]
ALP	1797 ±396[a]	1564 ±320[a]	1742 ±424[a]
CHOL	8.0 ±3.0[a]	6.0 ±0.5[b]	7.5 ±1.7[a]
TRIG	1.3 ±1.2[a]	0.7 ±0.2[a]	1.0 ±0.6[a]
CREA	136 ±121[a]	55 ±12[b]	119 ±72[a]

GGT: gamma-glutamyltransferase, AST: aspartate aminotransferase, CHE: cholinesterase, ALP: alkaline phosphatase, CHOL: cholesterol, TRIG: triglyceride, CREA: creatinine; a-a: P>0.05, a-b: P<0.05

Experiment 6 (*Rabbit model for supplemental Ni*)

The 50 mg/kg dietary Ni significantly (P<0.05) increased the concentration of TRIG from 0.6±0.1 up to 1.3±0.3 mmol/L and caused an insignificantly (P>0.05) higher activity of CHE and ALT (Table 35). The AST activity was insignificantly (P>0.05) decreased from 25±16 to 11±8 U/L. The GGT activity and concentration of both CHOL and CREA particularly did not change.

The 500 mg/kg Ni in rabbit diet significantly (P<0.05) increased the GGT activity from 7±2 up to 12±1 U/L and the concentration of TRIG from 0.4±0.1 to 0.7±0.2 mmol/L, and decreased the activity of CHE from 777±413 to 396±404 U/L. The activity of both AST and ALT and concentration of both CHOL and CREA particularly were not altered (Table 35).

Table 35 Activity of enzymes (U/L), and concentration of cholesterol, triglyceride and creatinine (mmol/L) in rabbit blood before and after the 24-day-long peroral Ni burden
(mean and ±SD)

Item	Supplemental Ni					
	0 mg/kg		50 mg/kg		500 mg/kg	
	d 0	d 24	d 0	d 24	d 0	d 24
GGT	9 ±4[a]	10 ±5[a]	9 ±4[a]	9 ±9[a]	7 ±2[a]	12 ±1[b]
AST	9 ±5[a]	12 ±3[a]	25 ±16[a]	11 ±8[a]	9 ±4[a]	10 ±6[a]
CHE	813 ±635[a]	646 ±607[a]	472 ±312[a]	760 ±545[a]	777 ±413[a]	396 ±404[a]
ALT	22 ±10[a]	23 ±11[a]	19 ±12[a]	38 ±26[a]	23 ±12[a]	17 ±4[a]
CHOL	4 ±0.9[a]	3 ±0.8[a]	4 ±0.9[a]	3 ±1.2[a]	3 ±0.4[a]	3 ±0.5[a]
TRIG	0.7 ±0.1[a]	0.8 ±0.1[a]	0.6 ±0.1[a]	1.3 ±0.3[b]	0.4 ±0.1[a]	0.7 ±0.2[b]
CREA	200 ±38[a]	173 ±35[a]	191 ±25[a]	200 ±45[a]	201 ±59[a]	198 ±37[a]

GGT: gamma-glutamyltransferase, AST: aspartate aminotransferase, CHE: cholinesterase, ALT: alanine aminotransferase, CHOL: cholesterol, TRIG: triglyceride, CREA: creatinine; a-a: P>0.05, a-b: P<0.05

4.5.3. Pancreatic enzymes. *Experiment 4* (*Rabbit model for heavy metal loading*)

Amylase activity in pancreas tissue (Table 36) was significantly (P<0.001) reduced by Cd, Pb and Hg compared to the control (9.52±0.62, 5.87±1.16 and 4.51±0.55 *vs* 12.37±0.97 U/mg protein, respectively).

Trypsin activity in pancreas (Table 36) was also significantly reduced by Cd (P<0.05) and Hg (P<0.001) compared to control (5.01±0.36 and 3.58±0.72 *vs* 6.33±0.57 mU/mg protein, respectively).

Protease and lipase activities in pancreas were significantly reduced by Pb (P<0.05 and P<0.01, respectively) and Hg (P<0.01, in both cases) compared to controls (41.31±4.79, 175.38±10.72 and 36.74±6.74, 160.17±14.46 *vs* 51.68±5.71, 217.07±19.35 mU/mg protein, respectively). Cadmium burden insignificantly (P>0.05) reduced both the protease and lipase activities of pancreatic tissues (Table 36).

Table 36 Activity of amylase (U/mg protein), trypsin, total protease and lipase (mU/mg protein) in pancreatic tissue of peroral heavy metal treated rabbits (n=4; mean and ±SD)

Treatment	Amylase	Trypsin	Total Protease	Lipase
Control	12.37[a]	6.33[a]	51.68[a]	217.07[a]
±SD	0.97	0.57	5.71	19.35
Cd	9.52[d]	5.01[c]	44.39[a]	192.19[a]
±SD	0.62	0.36	6.21	17.65
Pb	5.87[d]	5.53[a]	41.31[b]	175.38[c]
±SD	1.16	0.98	4.79	10.72
Hg	4.51[d]	3.58[d]	36.74[c]	160.17[c]
±SD	0.55	0.72	6.74	14.46

a-a: P>0.05, a-b: P<0.05, a-c: P<0.01, a-d: P<0.001

In the content of the small intestine, activity of trypsin and lipase was significantly reduced by Cd, Pb, and Hg and activity of amylase and protease was reduced only by Pb and Hg. Cadmium loading caused only an insignificantly reduced activity of both amylase and protease (Table 37).

Table 37 Activity of amylase (U/mg protein), trypsin, total protease and lipase (mU/mg protein), in the small intestinal content of peroral heavy metal loaded rabbits (n=4; mean and ±SD)

Treatment	Amylase	Trypsin	Total Protease	Lipase
Control	0.62[a]	1.96[a]	21.76[a]	1.05[a]
±SD	0.09	0.16	1.57	0.14
Cd	0.50[a]	1.61[b]	19.30[a]	0.78[b]
±SD	0.09	0.14	2.04	0.11
Pb	0.44[b]	1.42[d]	18.14[c]	0.55[d]
±SD	0.05	0.10	0.77	0.10
Hg	0.31[d]	1.20[d]	15.81[d]	0.43[d]
±SD	0.05	0.14	1.04	0.07

a-a: P>0.05, a-b: P<0.05, a-c: P<0.01, a-d: P<0.001

5. DISCUSSION

Toxicity. Oral LD_{50} of elementary Cd for rats is 225 mg/kg BW, while that of $CdSO_4$ is 280 mg/kg BW. Maximum Tolerable Level (MTL) means that dietary level that will not impair animal performance and should not produce unsafe residues in human food derived from the animal. The MTL of dietary Cd for domestic animals (i.e. rabbit, cattle, sheep, swine, poultry and horse) is 0.5 mg/kg (NRC, 1980a). **The Cd content in root and tuber samples ranging from 2.21 to 47.2 mg/kg DM exceeded the MTL.**

Amount of Pb required to cause poisoning varies in different animal species (Table 38).

Table 38 Toxicity of Pb in different animal species (HUMPHREYS, 1991)

Animal species	Amount of Pb to cause poisoning	
	Acute	Chronic
Cattle	80-160 mg/kg BW*	3.0-4.0 mg/kg BW/day* for 6-8 weeks
	600-800 mg/kg BW**	
Horses	1.25-2.0 g/kg BW*	1.7 mg/kg BW/day
Sheep, goats	3.0-6.0 mg/kg BW*,	0.6-0.9 g/kg BW/day* for 3-5 weeks
	600-800 mg/kg BW**	
Pigs	160-420 mg/kg BW*	No data
Dogs	1.0-2.5 g/kg BW*	0.32 mg/kg BW/day for 6 months
Poultry	160-600 mg/kg BW**	170-350 mg/kg BW/day*

*from Pb acetate, **from other Pb salts

The MTL of dietary Pb for domestic animals (i.e. rabbit, cattle, sheep, swine, poultry and horse) is 30 mg/kg (NRC, 1980b). The level for rabbits was derived by interspecific extrapolation and based on human food residue considerations. The acute oral single (lethal) dose of Pb in various species is usually considered to be 400-800 mg/kg. The chronic oral lethal dose in different species is, generally, as low as 0.5-5 mg/kg for an exposure of several weeks or months (LACZAY, 1995). **The Pb content in root and tuber samples ranging from 3.03 to 4.1 mg/kg DM is considered to study the sub-acute Pb loading.**

The Toxic Dose of $HgCl_2$ in cattles is 4-8 g, in horses is 5-10 g, in sheep is 4 g and in dogs and cats 0.1-0.3 g (OSWEILER *et al*., 1985). Oral LD_{50} of $HgCl_2$ is 1 mg/kg BW. The MTL of dietary Hg for domestic animals (i.e. rabbit, cattle, sheep, swine, poultry and horse) is 2 mg/kg (NRC, 1980c). **The Hg content in root and tuber samples ranging from 3.44 to 30.0 mg/kg DM exceeded the MTL.**

The MTL of dietary Ni widely varies from 50 mg/kg for rabbit, cattle, sheep and horse to 100 and 300 mg/kg for swine and poultry (NRC, 1980d). **The dietary Ni concentrations were 50 and 500 mg/kg. The 500 mg/kg Ni in the diet exceeded the MTL for both broilers and rabbits.**

Body weight, feed intake. In young animals (e.g. calf, swine, rabbit, Japanese quail, chicken, rat), the different Cd salts can reduce feed intake and growth rate, but as $CdSO_4$ have no adverse effect on these parameters (PRITZL *et al*., 1974; NRC, 1980a; BOKORI *et al*., 1995; TURECKI *et al*., 1997). Decreased gain by $Pb(NO_3)_2$ of 500 mg/kg and $HgCl_2$ of 250 mg/kg was found in adult Japanese quails and in young chickens (NRC, 1980b, c). **The smaller body weight of rabbits fed carrot, potato or beetroot samples is probably due to the reduced dry matter and, consequently, the lower energy intake (<9.3 MJ DE/kg DM) from the carrots, potatoes and beetroots** (BERSÉNYI *et al*., 1999). Since the voluntary feed intake capacity of rabbits is a limiting factor (LEBAS and LAPLACE, 1980; DEHALLE, 1981; MAERTENS, 1995), the dilution of digestible energy has common effects on the overall body growth rate as well as on the relative growth of organs and on the body composition (OUHUYOUN, 1998, FODOR *et al*., 2001).

Moreover, the results of these experiments suggest that the adverse effect exerted by high concentrations of trace elements studied can hardly, if at all, be monitored by determining the classical zootechnical parameters (i.e. feed intake, body weight gain).

Nickel concentration of unsupplemented basal diet for rabbits (5.8 mg/kg) proved to be approximately 2-times higher than the corn-soybean type diet used by OSCAR and MITCHELL (1995). Chicks and rabbits fed more than 250-300 mg/kg Ni in diet exhibited depressed growth and reduced feed intake, partially caused by reduced palatability (SZILÁGYI *et al*., 1982; REGIUSNÉ, 1991a). **These findings are confirmed by the author, because by adding 500 mg/kg of Ni to the diet, reduced weight gain (by 10%), feed intake (by 4%) and worse FCE (by 5%) were observed in growing broiler cockerels.**

Digestibility of nutrients. **The trace element content of root and tuber samples, generally, did not decrease the digestibility of major nutrients (e.g. CP) in comparison to uncontaminated samples.** A crude fibre content of 13-16% with a digestibility of 10-30% is the optimum in feeds for rabbits (GIPPERT, 1984). Besides the lower content of crude fibre (7-10%) the digestibility of crude protein may also decrease. **In these studies the crude fibre intake was very small (<7%).** The lower digestibility of nutrients could be explained both by an impaired enzyme production of the pancreas and/or gut wall (NRC, 1980a; KÓSA *et al*., 2000) and by the disadvantageous dietetic effect of low-fibre diet having no "bulky" ("ballast") character (COLIN *et al*., 1976). The improved digestibility of CP and CF may be attributed to the influence of metals on the gut microflora (SCHNEIDER and FLATT, 1975).

On the other hand, the daily crude fibre intake of rabbits fed with commercial diet and pelleted alfalfa tended to maximum level of optimum (16%), which could significantly decrease the digestibility of nutrients (FEKETE *et al*., 2001).

The lower growth rate caused by Ni burden could be the consequence of decreased feed intake rather than its influence on the digestibility of nutrients. Nickel even of 500 mg/kg failed to reduce significantly the digestibility of nutrients (e.g. CP) in rabbits. The decreased digestibility of CF to approximately 20% is assumed to be within physiological ranges (CHEEKE, 1991).

The effect of heavy metal on the digestibility of nutrients depends not only on the character of the toxic elements, but also on the matrix of the feedstuff.

Haematology. **Serum lead concentration of the treated rabbits increased approximately 2-fold after 4 weeks as a consequence of the mild Pb burden from 22 µg/L to 46 µg/L.** Mean blood Pb concentrations in clinically healthy laboratory rabbits were reported to be between 20 µg/L and 270 µg/L (GERKEN and SWARTOUT, 1986). **Although, our results were within the reference range, the increase of Pb concentration may be related to the elevated Pb intake.**

While Pb concentration in serum doubled, the ZPP concentration during the same period remained unchanged (114.57±37.80 µmol/mol hem), which is in agreement with previously reported tendencies (PETER and STRUNC, 1983). In the mentioned study, elevated Pb concentrations associated with normal ZPP values were found. This situation corresponds to the acute Pb poisoning in humans, in which high Pb concentration appears almost immediately after Pb loading, while the ZPP value is still well within normal range. The same phenomenon occurs in Pb-poisoned rabbits. **Such findings can be explained by assuming a dual effect in acute toxicity, namely interference with iron utilization, combined with inhibition of ferrochelatase enzyme activity.** These experiences prove obviously the limitation of the ZPP test. **From the data presented it can be suggested that both blood lead as well as ZPP analysis should be performed when Pb intoxication is suspected** and the increase of the ZPP concentration can increase as a consequence of the chronic Pb exposure (LABBÉ and RETTMER, 1989; LEUNG *et al*., 1993). KARAČIĆ *et al*. (1984), however, reported the biochemical indicators (i.e. ALAD, EP and Pb-B) had demonstrated increased absorption of Pb in cows grazing in Pb-contaminated pastures through lowered ALAD activity and increased both Pb-B (blood lead) and EP.

RBC, HGB and HCT are significantly decreased (by 13, 20 and 11%, respectively) while MCH and MCV are increased (by 9 and 4%) by Pb burden. These haematological data of the present study indicate that macrocytic hyperchromic anaemia has developed in rabbits treated with Pb (BERSÉNYI *et al*., 2003). Acute and chronic Pb intoxications cause anaemia associated with reticulocytosis and basophilic stippling of the erythroblast cells (REICHLMAYR-LAIS and KIRCHGESSNER, 1984) and a mild to moderate microcytic hypochromic or normocytic normochromic anaemia is usually seen only in chronic lead toxicities (NRC, 1980b; KANEKO, 1989).

The Cd and Hg burden did not alter the haematological parameters; all of them were within reference ranges for rabbits (WOLFORD *et al*., 1986; HILLYER and QUESENBERRY, 1997). These are in contrast with the significantly decreased values of WBC and RBC in chicken observed by SZILÁGYI *et al*. (1994), following a longer (6 weeks) load of higher (100 mg/kg) Cd concentration. In ruminants (cattle and sheep) exposed to Cd, anaemia with decreased RBC and HGB was observed (VAN BRUWAENE *et al*., 1984).

Serum biochemistry. **Cadmium and Hg loading via carrots did not change the activity of both AST and ALT. The increased activities of AST and ALT by oral administration of toxic elements** are supported by NOMIYAMA *et al*. (1998), who observed markedly elevated plasma AST and ALT in Cd-treated rabbits. BRAUN *et al*. (1997) also found a markedly increased ALT activity and a normal GGT level in fatal Pb-poisoned calves. **The significantly reduced activities of GGT caused by toxic metal burden (1-4 U/L), however, is below the normal range (6-17 U/L).** The altered activities of AST and ALT are confirmed in grazing cows by ROGA-FRANC *et al*. (1997).

The significantly higher CREA concentration (116 µmol/L) in Cd-treated rabbits is supported by SZILÁGYI *et al*. (1994), who described a significantly increased CREA level in Cd-treated chickens. **Plasma urea and the ratio of plasma urea to CREA remained at the normal level** which were also cited by NOMIYAMA *et al*. (1998).

Activities of ALP were significantly increased in rabbits by Cd, Pb and Hg treatment. These are in contrast with the inhibited ALP activity by administration of Cd in kidneys of guinea-pigs (RIBAS-OZONAS *et al*., 1970) and of Hg in rats, following the alterations in the kidney tubular cells (CARMIGNANI *et al*., 1991).

Environmental heavy metal contaminants may cause an insufficiency in different organisms. These influences may be resulted in oxidative stress syndrome. Cadmium can generate free radical processes or reactive intermediates, resulting in decreased activity of GSHPx and increased CK (SZILÁGYI *et al*., 1997). **All toxic metals (i.e. Cd, Pb and Hg) decreased the activity of GSHPx and increased CK in rabbits. The findings may cause only insignificant alteration in the relative weight of different organs.**

Nickel supplementation did not have any significant effect on enzyme activities in broiler chickens and rabbits. The significant increase in activity of GGT (from 7 U/L to 12 U/L) in rabbits by 500 mg/kg Ni is within the normal range. The findings are in agreement with the results of REGIUSNÉ *et al*. (1984b).

Based on our results the blood haematology and serum biochemistry can be considered as a good but unspecific indicator of the toxic metal (i.e. Cd, Hg, Pb) loading in mammals, including humans and companion animals.

Histopathology. **The increased activity of both AST and ALT and reduced activity of CHE (50-60% of the control) are referring to the damage of the liver parenchyma. The results of serum biochemistry have been confirmed by pathological focal fatty infiltration.**

It is well established that Cd causes renal dysfunction with morphological and functional disorders in human and animals. Experiences associated with Cd intoxication in rabbits suggest that ALP and urinary GGT (besides acid phosphatase and urinary trehalase) could be a good index of renal tubular injury (NISHIMURA *et al*., 1986). **Both the reduced activity of GGT and the increased activity of ALP indicate toxicity of trace elements to the kidneys and/or liver.**

Due to the increased concentration of CREA as well, Cd burden of body could cause toxic effect to kidneys correspondig with KANEKO (1989) and SZILÁGYI *et al*. (1994). **Although, slight tubulonephrosis developed, the toxic metal burden did not seriously affect the renal function because of the CREA and UREA results**, in contrast to SMITH *et al*. (1991), who noticed proteinuria is one of the most consistent findings in animals and humans with early failure caused by Cd toxicity. In the parenchyma organs (i.e. liver, kidneys, myocardium, pancreas) of Japanese quails degenerative lesions could be seen as a result of the Cd exposure (BOKORI *et al*., 1994). NOMIYAMA *et al*. (1998) reported that hepatic dysfunction appears at the same time or little earlier than renal dysfunction in animals exposed to Cd. Nevertheless, RIBAS-OZONAS *et al*. (1970) did not observe the specific necrotic effects of Cd on the kidney of guinea pig possibly because the exposure time was very short.

Altered activities of AST, CHE and TRIG caused by Ni exposure are referring to the damage of the liver parenchyma. The results of serum biochemistry have been confirmed by mild or moderate form of pathological focal fatty infiltration in boilers.

Although mortality was only exhibited by chicks fed 1100 mg/kg diet (NIELSEN, 1987), **supplemental 500 mg/kg Ni reduced the immune state in broilers and signs of epicarditis, pneumonia and air-sacculitis were found.**

The markedly increased relative weight of lungs by toxic metal burden is, however, assumed to be an attempt to compensation.

The toxic metals failed to damage the function of the bone marrow. This finding corrensponds with HUMPHREYS (1991), who noticed that the orally administered Pb entered the bones and its level in bone marrow were similar to control animals.

Pancreatic enzymes. **The reduced activity of amylase, trypsin and total protease, and lipase by trace elements (especially in case of Pb and Hg) suggested their toxicity to the pancreas**, with a potential decrease in exocrine and endocrine pancreatic functions as it was stated in other studies (BANERJEE and BHATTACHARYA, 1997; CHOWDHURY *et al*., 1993).

Reproductive organs. Successful reproduction requires a well developed genital tract, including a good cooperation between hypothalamus, pituitary gland, ovaries and uterus on the female side and good sperm quality and motility on the male side. Toxic metals may cause adverse effects to the development and function of the female reproductive system. On the male side, mainly the production and differentiation of sperm cells that can be affected is of importance (PEEREBOOM-STEGEMAN, 1987). Continuous exposure to Pb may cause significant suppression of serum sex steroid concentrations in both male and female rats (RONIS *et al*., 1998).

Metals which are bound to metallothionein are not toxic, but unbound Cd causes effects on follicular growth (ovarian atrophy) in ovarian tissue and necrosis in testis is produced (KOSTIAL, 1986; MASSÁNYI and UHRÍN, 1996). The degeneration of the seminiferous epithelium and the other changes occurring in testes after Cd exposure are secondary and mainly due to vascular disturbances. The effects occurring in the testis (focal oedema, haemorrhage and vascular thrombosis) can be likewise induced in the ovaries, in the placenta and in the uterus (PEEREBOOM-STEGEMAN, 1987).

In the reproductive organs of bulls the Cd content was lower than in cows, and in all samples of testes the concentration was under the detection limit of 0.01 mg/kg (MASSÁNYI *et al*., 1995b).

Significant correlations were observed between Cd-B (blood cadmium) levels and the volume of semen (negative correlation), midpiece defects and immature forms of spermatozoa in men. High Cd-B levels may, therefore, have an effect on spermatogenesis (CHIA *et al*., 1992). Cadmium (2.0 mg/ml as $CdCl_2$) *in vitro* expressively affected all motility parameters (i.e. motility, progressive movement, path velocity, straightness, mean pixel count, mean intensity) of bovine spermatozoa (MASSÁNYI *et al*., 1996).

In Pb burden men, a deterioration of sperm counts, sperm motility and morphology was observed (PEEREBOOM-STEGEMAN, 1987; CHIA *et al*., 1992). There was no significant Pb-related change in the testicular weight of male rats after Pb administration (RONIS *et al*., 1998). Rats treated with Pb (8 mg/kg as Pb acetate) did not show marked degenerative changes in the tubules, the histopathological examination of testis revealed disturbance in spermatogenesis and degenerating *Leydig* cells (SAXENA *et al*., 1987).

No significant differences were noted between Hg-B levels and human semen parameters (CHIA *et al*., 1992). On the other hand, spermatogenic inhibition and degeneration of the seminipherous tubules were observed in different animal species treated with high doses of $HgCl_2$ (CARMIGNANI *et al*., 1991).

The Cd content in ovaries of cattle ranged from <0.01 to 0.02 mg/kg (w/w). The highest levels were observed in ovaries (0.02-0.07 mg/kg, w/w), while the concentrations of Cd in the uteri were up to 0.05 mg/kg, w/w (MASSÁNYI *et al*., 1995b).

$CdCl_2$ for female rats, even at 1.0 mg/kg/week, fails to alter the ovarian cycle, progesterone production, and oestradiol-17 *ß* production of the ovary (VARGA *et al*., 1991). There was an atrophy observed in the ovaries and oviducts of Japanese quails (BOKORI *et al*., 1994).

After daily oral application of Cd (1 mg/kg of $CdCl_2$) for female rabbits, during 5 months, the highest concentration was found in the kidneys (54 mg/kg), then in the liver (13 mg/kg), ovaries, and uterus. With higher Cd content, there is a lower amount of primary follicles and stroma in the ovaries. Cadmium does not affect the diameter of follicles. The number of atretic follicles was significantly higher in case of administration of Cd. The lower glandular epithelium in the endometrium and the more stroma are signs of oedematization of uterus as a toxic cellular effect of Cd administration. It is very similar to that in ovaries or oviducts, and is caused by a damage in the wall of blood vessels (MASSÁNYI and UHRÍN, 1996, 1997; MASSÁNYI *et al*., 1997). Although relatively high concentrations of Cd accumulated in the ovaries and uterus (0.5 and 0.2 mg/kg, respectively), does had a normal number of offspring and no malformation (MASSÁNYI *et al*., 1995a).

In our studies, no significant effects were observed on testis weight when expressed as a percentage of body weight. In testes of rabbits, both Cd and Pb content were, generally, below the detection limit, while markedly elevated their concentrations could be detected in ovaries of the rabbits. On the other hand, considerably higher Hg levels were measured in ovaries (2.9 mg/kg) than in testes (<0.5 mg/kg).

Histological examinations revealed a reduced rate of spermatogenesis in the testes by Cd and Pb loading. The alterations caused by Hg could be found only at its highest (30 mg/kg DM) concentration. Due to the toxic metal exposure the 4-5 layers of germ cells in the seminipherous tubules and the number of mature spermatocytes are reduced. A large number of syncytial giant cells and degenerated cells indicating abnormal meiosis were observed among the spermatogenic cells.

In case of heavy metal-exposed does, ovarian follicles containing healthy ova of different developmental stages were found, but signs of actual ovulation (*corpus luteum*) were not observed.

The increased Ni concentration of ovaries together with the histopathological findings (inhibited the ovarial activity) suggest a possible ovarial dysfunction in Ni-exposed rabbits.

In conclusion, it can be said that toxic metal exposure may result in disturbed steroidgenesis and an impairment of reproductive functions, and may consequently decrease fertility in rabbits.

Accumulation. Dietary Cd absorption from the gastrointestinal tract in animals is low. The Cd administered daily to the beagle dogs covered a wide range, up to 100 mg/day, and the absorbed amount was in the range of 6-150 µg. Cadmium is distributed into the body tissues, especially in the liver and kidneys of dogs (VAN BRUWAENE *et al*., 1984; MATSUNO *et al*., 1991).

Due to the very low level of absorption, total body retention in male goats was 0.3 to 0.4% of the 100 mg/kg dietary Cd (in form of $CdCl_2$) with about one-half of this in the liver,

one-fourth in the kidneys. Bone, muscle, hair, skin, blood contained very small amounts of it (MILLER *et al*., 1969). The Cd concentration increased most in kidneys of goats, then the liver, indicating Cd exposure most securely and it was followed by hair. Since it is changed yearly, it shows exogenous Cd emissions in the living area. Cardiac and skeleton muscles store little Cd and are not suitable as indicator organs. Meat only accumulates unimportant amount of Cd, therefore, the meat of animals from exposed areas can also be used as food (MASAOKA *et al*., 1986).

Cadmium burden of body is highly reflected by kidneys. Comparing the Cd content of the different organs to kidneys, the liver, hair and the remaining part of body have 30, 17%, and much less of capacity to accumulate Cd, respectively (REGIUSNÉ, 1991b). Cadmium supply and load can be detected by kidney, hair and eventually by faeces (REGIUSNÉ *et al*., 1984a).

The highest levels of Cd were found in the kidneys of all investigated animal species (i.e. fallow-deer /*Dama dama*/, sheep, brown hare /*Lepus europaeus*/, and rabbit /*Oryctolagus cuniculus*/). The lowest values were recorded in the reproductive organs and in the muscle. In comparison of wild and farm animals, higher levels of Cd were detected in the organs of wild animals. Low levels of Cd in the muscle (0.005-0.01 mg/kg, w/w) are important, because they are the main part of the animal body used for human consumption (TOMAN and MASSÁNYI, 1996).

The Cd status of horses is also best reflected by the kidneys and much worse by the liver and hair. Nevertheless, the Cd content of hair and blood serum might be of particular importance for practical purposes since these samples can always be taken (ANKE *et al*., 1989).

The highest mean Cd concentrations were found in the kidneys of hens and chickens. Cadmium concentrations in the liver were about 3-times lower, followed by spleen and gizzard (DOGANOC, 1996).

Although Cd accumulated in kidney, liver, and ovaries of Holstein heifers with increasing dietary concentration of Cd (0, 1, and 5 mg/kg), it did not create any functional abnormalities. Cadmium has not accumulated in muscles (either skeletal or heart), bone, or pancreas. Although dietary Cd has never been reported to accumulate in skeletal muscle of dairy cows, accumulation of Cd in these tissues of grazing beef cattle and steers was observed (SCHENKEL, 1988; SMITH *et al*., 1991).

KING *et al*. (1992) also noticed Cd levels in both the kidney and liver of pigs between 8 and 90 kg live weight increased in a linear manner with dietary Cd content increased, but this increase could not be detected in meat and fat.

There was no accumulation of Cd observed in milk and meat from either cows or swine, or eggs from poultry after exposure to Cd-supplemented ration (in form of $CdCl_2$ or $CdSO_4$). Increasing the concentration of Cd in animal feed up to or more than 10 mg/kg will cause no appreciable change in the human dietary intake of this metal through the consumption of these organs. Increasing levels of Cd were observed, however, in the liver and kidneys of cows, swine, and chicken and in poultry meat. Any additional Cd in human food certainly increases the dietary intake of this metal. Since the threshold level of Cd intake for adverse effects on humans ranges from 57 to 71 µg/day, a consideration of an upper limit of 1 mg/kg in any dietary component for animals or man might be useful. If these limits suggested by WHO/FAO are valid, the liver and kidney and possibly poultry meat should be avoided in case of a contamination of animal feed with Cd. Moreover, the increases in the background levels of Cd in feed grains and forages should be prevented (LEACH *et al*., 1979; VAN BRUWAENE *et al*., 1984; MÜLLER *et al*., 1994; BRAMLEY, 1990; ANTONIOU and ZANTOPOULOS, 1992). Cadmium concentration in chicken meat (0.3 mg/kg) is far less than that found in sea foods (0.8 mg/kg) and other meats (0.9 mg/kg). Some of these high values

probably occur from surface contamination of meat through processing. High Cd contents, thus, of sliced bacon, frankfurters, and canned food products were also above the Cd residues of most of the tissues and products of animals treated with Cd-supplemented feed (except for liver and kidneys). It is, therefore, likely that a major source of contamination of food with Cd may be processing and storage, rather than the dietary intake of metal by food producing animals. Consumption of organs like liver and kidney should be avoided if animal feed was contaminated with Cd (SHARMA *et al*., 1979; SCHENKEL, 1988).

Cadmium burden of rabbits is highly reflected by the kidneys, followed by the liver, similar to other farm and wild animals. Comparing the Cd content of the different organs to kidneys, the liver and hair as well as the remaining part of the body have 20-40% and much less of capacity to accumulate Cd, respectively. Cadmium content of rabbit muscle never exceeded the value found in normal rabbits. Cadmium content in the kidneys of rabbit could exceed the maximum permissible limit of 0.03-0.5 mg/kg (w/w) in some cases.

The highest mean Pb concentrations were presented in the bones of hens, with a 5-times lower concentration in the kidney followed by spleen, liver and skin. In the liver Pb was about two times less than in the kidneys (DOGANOC, 1996).

Compared to hens, the highest mean concentration of Pb was observed in the renal tissues of beefs since the kidney is one of the organs that mainly accumulates toxic heavy metals in mammalian organisms. Lead levels in liver were higher than in muscle tissues. Nevertheless all values measured were lower than the exposure limits for Pb in foods (ZANTOPOULOS *et al*., 1990).

Lead content in all kidney samples of cattle was lower than half of the limit. The maximum permissible load for Cd was exceeded in 5% of the cases (VON KÖFER and FUCHS, 1993). It is confirmed by VON SCHMIDT *et al*. (1988), who described in 26% of the kidneys the Cd concentrations were above the limit of 1.0 mg/kg (w/w) and in 4.6% of the dairy cows' kidneys the levels were even higher than double the acceptable limit and by ANTONIOU and ZANTOPOULOS (1992), who observed that 3% of goat livers and 22% of kidneys are over tolerance limits. Furthermore, in some cases both Pb and Cd content in meat (femoral and pectoral muscle), liver, kidney and egg of laying hens (but not chickens) from industrially contaminated areas exceeded the official tolerance levels (0.5 and 0.1, 1.0 and 0.5, 1.0 and 1.0, 0.25 and 0.005 mg/kg, respectively). These foods, therefore, are unsuitable for human consumption (DOGANOC, 1996).

In contrast to hens but similarly to beef cattles, the highest mean concentrations of Pb were observed in the kidneys of rabbits.

The highest mean Hg concentrations were presented in the kidneys of rabbits. Hair could also be a good indicator of the Hg burden but not for Cd or Pb in agreement with finding of DEMICHELE (1984) and DRASCH *et al*. (1997).

In some cases both Pb and Hg content in kidneys have exceeded the tolerance level of 0.5-2.0 mg/kg (w/w) and 0.01-0.05 mg/kg (w/w). Muscle, liver, lung, bone and spleen of rabbits can accumulate sufficient Pb and Hg.

Considerable toxic metals were observed in soft faeces. It could be uptaken by rabbits. As a consequence of re-absorption of toxic metals, rabbits may be more susceptible to toxic metals than other animal species. This is similar to the finding of

FEKETE *et al*. (1989) with T-2 toxin-loaded rabbits. **Therefore, rabbits could be excellent models to study the toxic metal burden of the body.**

Nickel accumulated in the kidneys, bones, heart and liver, which slightly differs from the order of rib bones, liver and kidneys cited by REGIUSNÉ *et al.* (1982) and REGIUSNÉ (1991a). **Moreover, our data of Ni concentration in organs are much higher than those presented by other studies** (REGIUSNÉ *et al*., 1990a; REGIUSNÉ, 1991a). **The very low Ni retention of hair makes it impossible to use this tissue as an indicator. On the other hand, the faeces Ni level is high and proportional to the intake, consequently, it is able to indicate the environmental Ni loading. Supplemental Ni failed to interact with Zn, Mn, Fe and Cu content of the different organs/tissues of rabbits.** Findings partly confirm the results found in young bulls by REGIUSNÉ *et al*. (1987), because the liver, spleen and ribs contained significantly less Cu.

Assuming that the accumulation of toxic metals is organ-dependent, it may have characteristic pathological consequences with morphological and/or functional disorders in animals.

However, many of the results discussed above raise an important point in relation to the human consumption of potentially toxic metal-contaminated animal product, namely, that normal cuts of meat, excluding liver and kidney, will probably be quite safe for human consumption and will contain toxic metals at concentrations below the acceptable limits. In contrast, the offal (i.e. kidney, liver, lung, bone) from such animals may not be fit for human consumption or for consumption by other animals, e.g. companion pets.

Nevertheless, as a conclusion, with regard to PTWI (provisional Total Weekly Intake), UL (Tolerable Upper Intake Level) and NOAEL (No-observed-Adverse-Effect Level) for toxic metals, it can be said that a quantity of such an offal hazardous the health of human beings. The PTWI is as follows: 0.4-0.5 mg for Cd; 3 mg for Pb and 0.3-0.4 mg for Hg (RDA, 1989).

The UL (mg/day) for Ni is as follows (DRI, 2001):

Infants	0 – 12 months	No data
Children	1 – 8 years	0.2
Males/Females	9 – 13 years	0.6/0.6
	14 years ≤	1.0/1.0
Pregnancy	≤18 – 50 years	1.0
Lactation	≤18 – 50 years	1.0

The NOAEL for Ni is 5 mg/kg BW/day (DRI, 2001).
The estimated mean daily dietary intakes by French consumers were as follows: 3.6 µg for Cd; 34 µg for Pb, 9 µg for Hg and 74 µg for Ni. Values for individual toxic elements were below tolerable limits (NOEL *et al*., 2003).

6. NEW SCIENTIFIC RESULTS

1. The accumulation of toxic metals (i.e. Cd, Pb and Hg), and their effect on the digestibility of nutrients, biochemical and histopathological changes (in the liver, kidneys, testes) are treat-dependent, namely, it depends not only on the character of the pollutant, but also on the matrix of the feedstuff.
2. Orally administered Cd increases the serum ALT activity; Pb increases the AST activity and Hg increases both the ALT and AST activity in rabbits. The ivestigated toxic metals (i.e. Cd, Pb, Hg) reduce the activity of pancreatic enzymes.
3. Toxic metals (i.e. Cd, Pb, and Hg) reduce the rate of spermatogenesis in testes, resulting in reproductive impairment of male rabbits. Nevertheless, they fail to inhibit the ovarial activity of female rabbits.
4. Under the experimental condition of sub-acute Pb burden, the ZPP concentration remain practically unchanged in rabbits.
5. In case of Ni burden, approximately 98% of Ni is eliminated from the body of the rabbits via the faeces and 0.5-1.5% with the urine and approximately 1% is retained in the body. With increasing the Ni-load, the Ni content of muscle and ovaries increases. Dietary 500 mg/kg Ni inhibits the ovarial activity in female rabbits.
6. Supplemental 50 mg/kg of Ni improves the weight gain by 3% in broilers, while the 500 mg/kg of Ni reduces the weight gain by 10% and the feed conversion efficiency in broiler cockerels.
7. Even 50 mg/kg of Ni damages the liver parenchyma induces pathological focal fatty infiltration in broilers and rabbits.
8. Nickel burden of body failed to affect the Cu, Zn, Fe, and Mn concentration of the different organs in rabbits.

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7.1. LITERATURES based on this DISSERTATION

1. **BERSÉNYI, A.**, FEKETE, S., HULLÁR, I., KÁDÁR, I., SZILÁGYI, M., GLÁVITS, R., KULCSÁR, MARGIT, MÉZES, M., ZÖLDÁG, L. (1999): Study of the soil-plant (carrot)-animal cycle of nutritive and hazardous minerals in a rabbit model. Acta Vet. Hung., 47:(2):181-190.

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3. **BERSÉNYI, A.**, FEKETE, S., SZŐCS, Z., BERTA, ERZSÉBET (2003): Effect of ingested heavy metals (Cd, Pb and Hg) on haematology and serum biochemistry in rabbits. Acta Vet. Hung., 51:(3):297-304.

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