Influence of elevated content of cadmium and arsenic in diet containing feeding yeast on organisms of rats

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ABSTRACT: The influence of elevated cadmium content in diet on the content of this element in liver, kidney and testes of 68 male rats was studied in dependence on the chemical form of applied cadmium (as inorganic salt – $CdCl_2$ and organically bound in yeast cells); the influence of elevated arsenic content (as $NaAsO_2$) in diet on its content in the same organs was also investigated. The interactions between arsenic and cadmium in the above-mentioned organs were studied. The addition of cadmium to the diet of rats significantly ($P < 0.05$) increased cadmium content in several organs. The addition of yeast containing the natural level of Cd increased the content of cadmium in liver and kidney of experimental animals significantly ($P < 0.05$). A significantly ($P < 0.05$) increased cadmium accumulation in organs was observed after the addition of Cd as $CdCl_2$, compared with the addition of Cd as organically bound Cd in yeast cells. At the same time, the addition of yeasts containing the natural level of Cd decreased the Cd accumulation applied as $CdCl_2$ in the examined organs. The addition of sodium arsenite to the diet of rats led to a significantly ($P < 0.05$) increased arsenic content in all the analyzed organs. The addition of yeasts to the diet increased arsenic content in liver and at the same time suppressed its content in kidneys of experimental animals. The interaction between arsenic and cadmium applied simultaneously was evident. The addition of As to the diet decreased the accumulation of Cd in kidney and increased its accumulation in testes. The addition of Cd to the diet increased arsenic content in liver and kidney and decreased its content in testes.

Keywords: risk elements; interaction; accumulation; liver; kidney; testes

Contaminated soil can be a source of potentially toxic elements (As, Cd, Cr, Cu, Ni, Pb, Zn) for fodder plants resulting in a possible input of these elements into the food chain. Among the elements, Alloway (1990) showed that Cd, Mn, Mo and Zn were readily transported to the aboveground biomass of plants. Cadmium inputs to soil in fertilizers, biosolids, soil amendments and atmospheric deposition often exceed Cd outputs in crops and drainage water resulting in increasing Cd concentrations in many agricultural soils (BurgatSacaze et al., 1996; McLaughlin et al., 1999). Arsenic and its compounds undoubtedly enter the environment mostly from brown coal that contains high levels of arsenic and is burned in coal-fired power plants (Yudovich and Ketris, 2005) and various locations

Supported by Ministry of Education, Youth and Sports of the Czech Republic, GACR (Projects Nos. 523/06/0439 and MSM 6046070901).

characterized by Au- and Ag-bearing sulphide ore deposits accompanied by elevated contents of arsenic belong to the main sources of the food chain contamination including man on its top.

In biological systems, inorganic As exists as either arsenite or arsenate and can be metabolized in many cells via enzymatic methylation to various organometallic compounds such as methylarsonic or dimethylarsinic acid. Methylated arsenic compounds are less reactive compared to the inorganic ones and therefore less toxic in biological systems. Arsenite is directly toxic through its interaction with sulphhydryl groups in proteins (Romach et al., 2000). Vahter and Norin (1980) compared the effect of different arsenite and arsenate rates (0.4 and 4 mg per kg of body weight) in mice tissues. In the animals exposed to arsenite higher values of arsenic were observed in most tissues, especially in liver and bile where differences between individual forms of arsenic increased with increasing rate of administered arsenic. The application of lower arsenic rates (from 0.5 to 50 ug per animal) to rabbits did not lead to any significant differences in the distribution of either arsenite or arsenate to individual tissues 16 hours after intraperitoneal intoxication (Bertolero et al., 1980). Toxicity of cadmium and its accumulation in various tissues of organisms using model experiments with laboratory rats and mice were studied in detail by many authors. The results showed that low cadmium rates (from 1 to 30 mg/kg of the diet supplied for 3 months) did not affect either growth intensity of the experimental animals or feed consumption. No significant histopathological changes in liver, kidney and blood were observed either (Loeser and Lorke, 1977). The affinity of rat kidney to cadmium was confirmed by Sabbioni et al. (1978).

Margoshes and Vallee (1957) revealed metallothionein as a low-molecular-weight protein containing from 5 to 6% of cadmium and 2% of zinc. Later Kaegi and Vallee (1961) identified that 30% of amino acids contained cysteine represented in the protein as supported by a high content of sulphur in this protein (8.5%). Metallothioneins play an important role in the detoxification of heavy metals, in the homeostasis of essential metals, and in the scavenging of free radicals. Evidently, cadmium toxicity is given by the cadmium portion not bound in metallothionein (Liu et al., 2007). In the case of arsenic, the application of arsenic-enriched diet to hens led to the increased metallothionein-like proteins in liver but As bound to it was only in a trace amount (Falnoga et al., 2000).

Organisms are frequently exposed to the mixture of toxic metals and metalloids and the possible interactions among these elements should be evaluated to assess complex effects of these elements (Lopez Alonso et al., 2002; Fowler et al., 2004). Dietary cadmium, lead and arsenic in the mixture affected the parameters of oxidative stress in rats to a larger extent compared to single metals (Fowler et al., 2004; Jimi et al., 2004). The neurotoxicological effects provoked by an arsenic/lead mixture compared to single elements in the mice brain were also described (Mejia et al., 1997).

Yeast cells belong to the organisms with high ability to concentrate heavy metals and metalloids present in a culture medium where cadmium resorption is affected by pH of the medium (Gwenner et al., 1986) and by the presence of glucose in the medium resulting in an increase in cadmium content (Roesick et al., 1986). High contents of cadmium in feeding yeasts produced in the Czech Republic were documented by Cibulka et al. (1992). Tomsett (1988) and Inouhe et al. (1991) observed possible cadmium and copper detoxification in *Saccharomyces cerevisiae* yeasts via binding to the specific peptides, metallothioneins similar to animal metallothioneins. In comparison with cadmium arsenic showed to be less toxic to *Candida* spp. and *S. cerevisiae* yeasts (Berdicevsky et al., 1993). However, microorganisms including yeasts have evolved mechanisms for arsenic resistance and enzymes that oxidize As(III) to As(V) or reduce As(V) to As(III). The formation and degradation of organoarsenicals in yeast cells were also described (Mukhopadhyay et al., 2002).

In our experiment the influence of elevated dietary cadmium content on the content of this element in liver, kidney and testes of male Wistar rats was studied in dependence on the chemical form of applied cadmium (as inorganic salt – $CdCl_2$ and organically bound in yeast cells); the influence of elevated dietary arsenic content (as $NaAsO_2$) on its content in the same organs was also investigated. The main objective of the investigation was to assess the possibility of cadmium penetration from yeasts containing high levels of this element to the organisms of rats. Interactions between arsenic and cadmium were studied in the organisms of experimental animals at the same time. Hypothesis: (i) the form of cadmium in rat diet will affect cadmium bioavailability and (ii) the exposition of rat organisms to the mixture of Cd and As will be different from a single dose of the elements due to possible interactions of these elements.

Table 1. Experimental design

Group No.	Treatment – diet
0	control 1 – before the start of the experiment
1	control 2 – semisynthetic diet
2	semisynthetic diet + yeast with 3 mg Cd per kg
3	semisynthetic diet + yeast with 3 mg Cd per kg + $CdCl_2$
4	semisynthetic diet + $NaAsO_2$
5	semisynthetic diet + $CdCl_2$ + $NaAsO_2$
6	semisynthetic diet + yeast with 90 mg Cd per kg + $NaAsO_2$
7	semisynthetic diet + yeast with 90 mg Cd per kg
8	semisynthetic diet + $CdCl_2$

MATERIAL AND METHODS

Experimental design

Seventy-five male Wistar rats (average body weight 52.7 ± 5.3 g) were obtained from the breeder (Velaz, Prague). Animals at 21 days of age were housed in cages (1 animal per cage) in a room with controlled temperature (varying from 23 to 25°C) under natural light conditions. After 4 days of the adaptation period when the animals were fed a commercially available diet *ad libitum* 11 animals were randomly selected as group 0 (Table 1). The remaining animals were randomly divided into 8 groups per 8 animals and fed a treated semi-synthetic diet according to the experimental design (Tables 1 and 2) for 28 days. Feed and water were supplied to the animals *ad libitum*, feed consumption and body weight of animals were monitored weekly.

The yeasts *Candida utilis* used in the experiment were produced in a pilot equipment of

Table 2. Individual components and nutrient contents in experimental diets (%)

Component	Group No. 1, 4, 5, 8	Group No. 2, 3	Group No. 6, 7
Casein	19.3	14.1	14.1
Yeast – high Cd	–	–	10.0
Yeast – low Cd	–	10.0	–
DL-methionine	0.2	0.2	0.2
Mix. of minerals UPS XVIII[a]	5	5	5
Mix. of vitamins UPS XVII[a]	1	1	1
Polyethylene	4	4	4
Sucrose	6	6	6
Sunflower oil	5	5	5
Wheat starch ad	100.0	100.0	100.0
Nutrient			
Dry matter	90.6	91.6	91.3
Nitrogen substances	16.3	16.6	16.7
Fat	1.4	1.5	1.8
Fibre	3.7	4.6	3.6
Ash	4.5	5.0	4.9

Anonymous (1970)

Microbiological Institute, Czech Academy of Sciences, Prague. The yeasts with a high level of cadmium were cultivated in the presence of $CdCl_2$ and the final content of Cd in yeast biomass was 90 ± 3 mg/kg. The yeasts with a low level of Cd were cultivated without addition of Cd and the final Cd content was 3.1 ± 0.1 mg/kg. Arsenic content in both yeast samples was 0.28 ± 0.08 mg/kg. According to the design of the experiment (Table 1) $CdCl_2$ as $CdCl_2$. 2.5 H_2O, ACS (Merck, Germany) was added to the diets for group No. 3, 5, and 8 to obtain the final cadmium concentration 9 mg/kg diet like in the case of group 6 and 7 where 10% of contaminated yeasts was added. $NaAsO_2$ (Merck, Germany) was added to the diets for group 4, 5, and 6 to obtain the final As concentration 20 mg/kg.

The animals from group 0 were euthanized by exsanguination after anaesthetizing with ether immediately after the adaptation period, animals from groups 1–8 after the termination of the experiment, and liver, kidney and testes were sampled. The sampled tissues were kept at –18°C until analyses.

Analytical methods

To determine Cd and As contents the animal tissues were freeze-dried (Lyovac GT-2, Germany) and decomposed by a modified dry ashing procedure as follows: an aliquot (~ 0.8 g) of the freeze-dried liver tissue (whole kidney and testes, 0.5 g of yeast sample, 1 g of the experimental diet) was weighed to 1 mg into a borosilicate glass test-tube and decomposed in a mixture of oxidizing gases (O_2 + O_3 + NO_x) at 400°C for 10 hours in a dry mode mineralizer Apion (Tessek, Czech Republic). The ash was dissolved in 20 ml of 1.5% HNO_3 (electronic grade purity, Analytika Ltd., Czech Republic) and kept in glass tubes until analysis (Miholová et al., 1993). Blank samples represented 15% of the total number of digests, detection limit was calculated as mean + triple standard deviation of blanks. The detection limits were 0.001 mg/kg As and 0.002 mg/kg Cd for animal tissues, and 0.001 mg/kg As and 0.001 mg/kg Cd for yeast and diet samples. Cadmium concentrations in the digests were determined by atomic absorption spectrometry (AAS, VARIAN SpectrAA-400, Varian, Australia) in flame (groups 3, 5, 6, 7, 8, and corresponding diets) and flameless (groups 0, 1, 4, and corresponding diets) measurement modes. Arsenic was determined by a continuous hydride generation technique using a Varian SpectrAA-300 (Australia) atomic absorption spectrometer equipped with VGA-76 continuous hydride generator. For the quality assurance of analytical data certified reference materials NIST 1577a Bovine Liver, NIST 1568 Rice Flour, RM 12-02-01 Bovine Liver, and RM 12-02-03 Lucerne were used.

Statistics

The ANOVA followed by Scheffe's multiple range test was used for statistical evaluation (Statgraphics 5.1 plus for Windows; Anonymous, 1997) at the significance level $\alpha = 0.05$. Data were log-transformed where needed to obtain homogeneity of variance. If the log-transformation did not correct the inhomogeneity of variance, the non-parametric Kruskal-Wallis test was applied. Extreme values were tested by Grubbs' test for the identification of outliers.

Table 3. Arsenic and cadmium contents in diets according to experimental groups (mg/kg)

Group No.	Treatment	As	Cd
1	control 2	0.033 ± 0.014	0.002 ± 0.001
2	yeast with low Cd	0.119 ± 0.022	0.335 ± 0.008
3	yeast with low Cd + $CdCl_2$	0.105 ± 0.033	9.3 ± 1.4
4	$NaAsO_2$	24.2 ± 2.9	0.002 ± 0.001
5	$CdCl_2$ + $NaAsO_2$	24.4 ± 2.3	9.3 ± 1.2
6	yeast with high Cd + $NaAsO_2$	23.9 ± 2.4	9.1 ± 0.2
7	yeast with high Cd	0.185 ± 0.028	9.1 ± 0.2
8	$CdCl_2$	0.183 ± 0.021	9.4 ± 1.2

n = 5; data are presented as mean ± standard deviation

Table 4. Body weight of animals monitored weekly during the experiment (g)

Group No.	Treatment	Age (days)				
		25	32	39	46	53
1	control 2	73 ± 7[a]	126 ± 6[a]	182 ± 7[a]	237 ± 12[a]	281 ± 18[a]
2	yeast with low Cd	74 ± 6[a]	123 ± 8[a]	177 ± 9[a]	234 ± 12[a]	280 ± 21[a]
3	yeast with low Cd + $CdCl_2$	74 ± 6[a]	126 ± 8[a]	184 ± 10[a]	232 ± 11[a]	281 ± 16[a]
4	$NaAsO_2$	74 ± 6[a]	126 ± 10[a]	182 ± 13[a]	235 ± 18[a]	275 ± 21[a]
5	$CdCl_2$ + $NaAsO_2$	74 ± 6[a]	124 ± 9[a]	178 ± 12[a]	226 ± 12[a]	271 ± 19[a]
6	yeast with high Cd + $NaAsO_2$	75 ± 6[a]	125 ± 9[a]	179 ±12[a]	229 ± 18[a]	272 ± 22[a]
7	yeast with high Cd	75 ± 6[a]	126 ± 12[a]	185 ± 18[a]	237 ± 18[a]	288 ± 19[a]
8	$CdCl_2$	74 ± 7[a]	126 ± 8[a]	181 ± 10[a]	233 ± 14[a]	278 ± 16[a]

The means marked by the same letter did not significantly differ at $P < 0.05$ within individual columns, $n = 8$; data are presented as mean ± standard deviation

RESULTS

The arsenic and cadmium contents in diets prepared for individual treatments are summarized in Table 3. The relatively high content of Cd in yeasts cultivated in the medium without Cd addition (3.1 ± 0.1 mg/kg) was expectable (Cibulka et al., 1992) and resulted in slightly increased Cd content in the low-Cd yeast supplemented diet. Arsenic content in the yeasts was low (0.28 ± 0.08 mg/kg) as well as arsenic content in whole diets without addition of sodium arsenite.

Body weight of the animals was weekly monitored (Table 4) as well as body weight gain per week and feed consumption. For all the three parameters the one-way ANOVA did not demonstrate any significant differences among individual treatments if monitored from week to week. However, the results in groups 5 and 6, where both As and Cd were supplied, tended to decrease (the differences were not significant). Lower total weight gain and feed consumption (data not tabulated) were reported at the end of the monitored period (after 28 days of the experiment) for groups 5 and 6, and in the case of group 5 the difference was significant ($P < 0.05$). The total body weight gain varied between 195 g (group 5) and 213 g (group 7) whereas total feed consumption increased from 464 g (group 5) and 504 g (group 7). The cadmium supplementation did not affect either body weight gain or feed consumption of the experimental animals as observed also by Loesser and Lorke (1977).

Arsenic content in the liver of group 0 rats (sampled before the start of the experiment) varied between 0.043 and 0.075 mg/kg and did not substantially differ from control group 1 sampled at the end of the experiment (Table 5). Dietary arsenic led to a significantly higher ($P < 0.05$) content of arsenic in the liver of rats of groups 4, 5, and 6 compared to control group 1. Moreover, the presence of cadmium in the diet, regardless of the form of dietary cadmium, increased the arsenic accumulation in rat liver. Therefore, arsenic content in the liver of group 4 rats fed the diet supplemented with $NaAsO_2$ was significantly lower compared to groups 5 and 6 where cadmium was supplemented either in the form of $CdCl_2$ or incorporated in the feeding yeasts. Arsenic content in the kidney of group 0 was very low compared to control group 1 sampled at the end of the experiment. As expected, the addition of sodium arsenite to the diet increased significantly ($P < 0.05$) the arsenic levels in the kidney of groups 4, 5, and 6. The total arsenic content in liver and kidney was comparable confirming the results presented by Bertolero et al. (1980) and Vahter and Norrin (1980). Differently to the liver samples, the arsenic level was not significantly affected by the presence of Cd in the diet and the addition of yeasts resulted in the lower accumulation of arsenic in kidney. Whereas the arsenic content in rat liver increased if As + Cd were supplemented, a decreased arsenic content was found in the testes (Table 5) and the effect of yeasts was not significant in this case.

Cadmium content in the liver of group 0 rats (sampled before the start of the application of experimental diets) varied between < 0.002 and 0.010 mg/kg and did not markedly differ from con-

Table 5. Arsenic contents in the liver, kidney and testes of animals (mg/kg of wet matter)

Group No.	Treatment	Liver	Kidney	Testes
0	control 1	0.058 ± 0.010	0.016 ± 0.001	0.017 ± 0.000
1	control 2	0.062 ± 0.011^{a,b}	0.084 ± 0.009^{a}	0.064 ± 0.014^{b}
2	yeast with low Cd	0.059 ± 0.014^{a}	0.077 ± 0.019^{a}	0.067 ± 0.015^{b}
3	yeast with low Cd + $CdCl_2$	0.077 ± 0.021^{a,b}	0.048 ± 0.020^{a}	0.035 ± 0.019^{a}
4	$NaAsO_2$	7.0 ± 1.3^{d}	6.2 ± 1.7^{c}	2.33 ± 0.81^{c}
5	$CdCl_2$ + $NaAsO_2$	10.2 ± 1.6^{e}	9.6 ± 2.5^{c}	1.73 ± 0.49^{c}
6	yeast with high Cd + $NaAsO_2$	10.6 ± 1.7^{e}	4.8 ± 2.0^{c}	1.93 ± 0.46^{c}
7	yeast with high Cd	0.163 ± 0.033^{c}	0.197 ± 0.027^{b}	0.051 ± 0.022^{b}
8	$CdCl_2$	0.083 ± 0.019^{b}	0.093 ± 0.017^{a}	0.058 ± 0.027^{b}

The means marked by the same letter did not significantly differ at $P < 0.05$ within individual columns, $n = 11$ for control 1, $n = 8$ for all the remaining treatments; data are presented as mean ± standard deviation

trol group 1 sampled at the end of the experiment (Table 6). Expectably, the addition of cadmium to the diet increased its content in rat liver ($P < 0.05$) even in group 2, where yeasts with natural content of Cd were supplemented to the diet. The addition of yeasts resulted in a lower cadmium content in rat liver compared to the animals fed the diet supplemented with $CaCl_2$. A significant decrease in cadmium content in liver was observed in group 3 (yeasts with low Cd + $CaCl_2$) as compared to groups 5 and 8, where $CdCl_2$ and no yeasts were applied to the diet. However, group 3 showed a significantly higher Cd content in liver than groups 6 and 7 fed the diet containing yeasts with a high Cd level. The cadmium content in the liver of groups 4, 5, and 6 did not reflect significantly the addition of arsenic to the diet. Therefore, we did not confirm a decreasing Cd level in rat liver exposed to the mixture of As and Cd (Yanez et al., 1991). In the case of kidney, similar results like in the case of rat liver can be derived. Differently, the cadmium level in the kidney of groups 5 and 6, where both Cd and As were added to the diet, tended to be lower compared to groups 7 and 8, where the corresponding level of Cd and no As were supplemented. These results are different from the findings of Schmolke et al. (1992) when the accumulation of Cd in rat kidney was independent of the As content in the diet. However, more pronounced renal toxicity of the Cd and As mixture than the exposure to single metals was described by many authors in mice (Liu et al., 2000) and humans (Buchet et al., 2003; Nordberg et al., 2005). The increasing Cd content in the rat diet reflected an increasing content of this

Table 6. Cadmium contents in the liver, kidney, and testes of animals (mg/kg of wet matter)

Group No.	Treatment	Liver	Kidney	Testes
0	control 1	0.003 ± 0.002	0.003 ± 0.000	< 0.002
1	control 2	0.001 ± 0.000^{a}	0.008 ± 0.001^{a}	< 0.002^{a}
2	yeast with low Cd	0.009 ± 0.001^{c}	0.018 ± 0.005^{b}	< 0.002^{a}
3	yeast with low Cd + $CdCl_2$	0.791 ± 0.137^{e}	1.19 ± 0.32^{d}	0.009 ± 0.003^{d}
4	$NaAsO_2$	0.002 ± 0.001^{b}	0.003 ± 0.001^{a}	0.003 ± 0.001^{a,b}
5	$CdCl_2$ + $NaAsO_2$	1.84 ± 0.73^{f}	2.02 ± 0.43^{e}	0.021 ± 0.008^{e}
6	yeast with high Cd + $NaAsO_2$	0.367 ± 0.056^{d}	0.490 ± 0.07^{c}	0.005 ± 0.002^{c}
7	yeast with high Cd	0.428 ± 0.027^{d}	0.715 ± 0.16^{c}	0.003 ± 0.001^{b,c}
8	$CdCl_2$	1.58 ± 0.43^{f}	2.94 ± 0.93^{e}	0.016 ± 0.005^{e}

The means marked by the same letter did not significantly differ at $P < 0.05$ within individual columns, $n = 11$ for control 1, $n = 8$ for all the remaining treatments; data are presented as mean ± standard deviation

element in rat testes to a lesser extent compared to liver and kidney (Table 6) regardless of the form of cadmium supplemented to the diet. In group 2, where low Cd yeasts were applied, no significant increase of Cd in testes was observed. Contrary to kidney samples, the addition of arsenic to the diet resulted in a slightly increased Cd content in testes.

DISCUSSION

Evidently, the form of dietary cadmium is the factor determining the extent of cadmium accumulation in rat tissues where cadmium bound in the yeast cells as type II metallothionein (Tomsett, 1988; Inouhe et al., 1991) demonstrated lower availability for rats compared to $CdCl_2$. Moreover, the presence of yeasts also affected the cadmium availability. Similarly, Fuciková et al. (1995) observed a combined effect of dietary Cd and yeasts at the levels comparable to this experiment on selected haematological and immunological parameters (haemoglobin values, counts of monocytes). The availability of dietary Cd in the form of $CdCl_2$ and incorporated in the feeding yeasts was investigated by Turecki et al. (1998). They determined a significantly higher cadmium content in the duodenum of rats supplemented with $CdCl_2$ without yeasts compared to cadmium incorporated in the feeding yeasts suggesting higher availability of inorganic Cd and supporting our findings.

Humans and animals are frequently exposed to combinations of various risk elements especially in the vicinity of metallurgical works (Fischer et al., 2002; Koreneková et al., 2002). The effects of long-term exposition to low levels of these elements and their possible interactions resulting in possible symptoms of oxidative stress should be taken into account in this context (Fowler et al., 2004). Jadhav et al. (2007a,b) investigated the effect of subchronic exposure of rats to drinking water containing arsenic, cadmium, lead, mercury, chromium, nickel, manganese, and iron. The results showed that the long-term (90 days) exposure of rats to the mixture of elements can lead to substantial changes in humoral and cell-mediated immune responses, biochemical parameters and even in vascular, degenerative, and necrotic changes in brain, liver and kidney of rats. Yanez et al. (1991) stated that the toxicity of a mixture of As + Cd cannot be predicted from the toxic mechanisms of single components as also suggested by our results. They also demonstrated differences between the mechanisms of toxic behaviour of both elements. In their experiment, the mixture of As + Cd behaved as arsenic in the induction of lipid peroxidation and glutathione and as cadmium in the metallothionein induction. As already mentioned above, the addition of arsenic can increase the level of metallothionein-like proteins but As is bound to this protein only in trace amounts (Falnoga et al., 2000). Arsenic pre-treatment can lead to a decrease in cadmium hepatotoxicity because of an increase in liver levels of metallothioneins in which cadmium will be blocked. Cadmium pre-treatment did not affect the arsenic toxicity although it increases the liver metallothionein levels even to a larger extent (Hochadel and Waalkes, 1997). In our experiment, the presence of cadmium significantly increased the arsenic content in rat liver. Generally, the results suggested possible interactions of dietary Cd and As in rat organs. However, more detailed investigations including the determination of metallothionein level as well as biochemical parameters should be conducted in further research for better elucidation of these relations.

Acknowledgements

The authors thank Dr. Petr Kujan, Czech Academy of Sciences, Prague, for the preparation of yeasts used in this experiment and Assoc. Prof. Jiří Cibulka, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences in Prague, for substantial help with management of the experiment.

REFERENCES

Alloway B.J. (1990): Heavy Metals in Soil. J. Wiley and Sons, New York, USA.

Anonymous (1970): Official Methods of Analysis. Association of Official Agricultural Chemists, Washington, USA, 800–801.

Anonymous (1997): Statgraphics Plus for Windows User Manual. Manugistics, Inc., Rockville, USA.

Berdicevsky I., Duek L., Merzbach D., Yannai S. (1993): Susceptibility of different yeast species to environmental toxic metals. Environmental Pollution, 80, 41–44.

Bertolero F., Marafante E., Rade J., Sabbioni E. (1980): Pharmacokinetics and intracellular-distribution of

inorganic arsenic in the rabbit. Toxicological Letters, 93, 1.

Buchet J.P., Heilier J.F., Bernard A., Lison D., Jin T., Wu X., Kong Q., Nordberg G.F. (2003): Urinary protein excretion in humans exposed to arsenic and cadmium. International Archives of Occupational and Environmental Health, 76, 111–120.

BurgatSacaze V., Craste L., Guerre P. (1996): Cadmium in the food chain: A review. Revue de Medecine Veterinaire, 147, 671–680. (in French)

Cibulka J., Turecki T., Miholová D., Máder P., Száková J., Brabec M. (1992): Cadmium, lead and mercury levels in feeding yeast produced in Czechoslovakia. Science of the Total Environment, 114, 73–86.

Falnoga I., Stilbilj V., Tusek-Znidaric M., Slejkovec Z., Mazej D., Jacimovic R., Scancar J. (2000): Effect of arsenic trioxide on metallothionein and its conversion to different arsenic metabolites in hen liver. Biological Trace Element Research, 78, 241–254.

Fischer A.B., Georgievá R., Nikolová V., Hálková J., Bainová A., Hristeva V., Penkov D., Alandjiisk D. (2002): Health risk for children from lead and cadmium near a non-ferrous smelter in Bulgaria. International Journal of Hygiene and Environmental Health, 205, 1–14.

Fowler B.A., Whittaker M.H., Lipsky M., Wang G.S., Chen X.Q. (2004): Oxidative stress induced by lead, cadmium and arsenic mixtures: 30-day, 90-day, and 180-day drinking water studies in rats: An overview. Biometals, 17, 567–568.

Fučiková A., Slamová A., Száková J., Cibulka J., Heger J. (1995): The influence of dietary cadmium on hematological parameters and phagocytic activity of leukocytes in rats. Živočišná Výroba, 40, 15–18.

Gwenner C. (1986): Enrichment of cadmium in biomasses. Acta Biotechnologica, 6, 339–343. (in German)

Hochadel J.F., Waalkes M.P. (1997): Sequence of exposure to cadmium and arsenic determines the extent of toxic effects in male Fisher rats. Toxicology, 116, 89–98.

Inouhe M., Inagawa A., Morita M., Tohoyama H. (1991): Native cadmium-metallothionein from the yeast *Saccharomyces cerevisiae*: Its primary structure and function in heavy-metal resistance. Plant and Cell Physiology, 32, 475–482.

Jadhav S.H., Sarkar S.N., Ram G.C., Tripathi H.C. (2007a): Immunosuppressive effect of subchronic exposure to a mixture of eight heavy metals, found as groundwater contaminants in different areas of India, through drinking water in male rats. Archives of Environmental Contamination and Toxicology, 53, 450–458.

Jadhav S.H., Sarkar S.N., Patil R.D., Tripathi H.C. (2007b): Effects of subchronic exposure *via* drinking water to a mixture of eight water-contaminating metals: A biochemical and histopathological study in male rats. Archives of Environmental Contamination and Toxicology, 53, 667–677.

Jimi S., Uchiyama M., Takaki A., Suzumiya J., Hara S. (2004): Mechanisms of cell death induced by cadmium and arsenic. Mitochondrial Pathogenesis: From Genes and Apoptosis to Aging and Disease, 1011, 325–331.

Kaegi J.H.R., Vallee B.L. (1961): Metallothionein: A cadmium and zinc containing protein from equine renal cortex. II. Physicochemical properties. Journal of Biological Chemistry, 236, 2435–2442.

Koreneková B., Skalická M., Nad P. (2002): Concentration of some heavy metals in cattle reared in the vicinity of a metallurgic industry. Veterinarski Arhiv, 72, 259–267.

Liu J., Liu Y., Habeebu S.M., Waalkes M.P., Klaassen C.D. (2000): Chronic combined exposure to cadmium and arsenic exacerbates nephrotoxicity, particularly in metallothionein-I/II null mice. Toxicology, 147, 157–166.

Liu J., Cheng M.-L., Yang Q., Shan K.-R., Shen J., Zhou Y., Zhang X., Dill A.L., Waalkes M.P. (2007): Blood metallothionein transcript as a biomarker for metal sensitivity: Low blood metallothionein transcripts in arsenicosis patients from Guizhou, China. Environmental Health Perspectives, 115, 1101–1106.

Loeser E., Lorke D. (1977): Semichronic oral toxicity of cadmium. 2. Studies on rats. Toxicology, 7, 215–224.

Lopez Alonso M., Benedito J.L., Miranda M., Castillo C., Hernandez J., Shore R.F. (2002): Interactions between toxic and essential trace metals in cattle from a region with low levels of pollution. Archives of Environmental Contamination and Toxicology, 42, 165–172.

Margoshes M., Vallee B.L. (1957): A cadmium protein from equine kidney cortex. Journal of the American Chemical Society, 79, 4813–4814.

McLaughlin M.J., Parker D.R., Clarke J.M. (1999): Metals and micronutrients – food safety issues. Field Crops Research, 60, 143–163.

Mejia J.J., DiazBarriga F., Calderon J., Rios C., Jimenez-Capdeville M.E. (1997): Effects of lead-arsenic combined exposure on central monoaminergic systems. Neurotoxicology and Teratology, 19, 489–497.

Miholová D., Mader P., Száková J., Slamová A., Svatoš Z. (1993): Czechoslovakian biological certified reference materials and their use in the analytical quality assurance system in a trace element laboratory. Fresenius Journal of Analytical Chemistry, 345, 256–260.

Mukhopadhyay R., Rosen B.P., Pung L.T., Silver S. (2002): Microbial arsenic: from geocycles to genes and enzymes. FEMS Microbiology Reviews, 26, 311–325.

Nordberg G.F., Jin T., Hong F., Zhang A., Buchet J.P., Bernard A. (2005): Biomarkers of cadmium and arsenic interactions. Toxicology and Applied Pharmacology, 206, 191–197.

Roesick E., Mangir M., Lochman E.R. (1986): Different uptake of cadmium in *Saccharomyces* and *Rhodotorula* cells. Chemosphere, 15, 981–983. (in German)

Romach E.H., Zhao C.Q., Del Razo L.M., Cebrian M.E., Waalkes M.P. (2000): Studies on the mechanisms of arsenic-induced self tolerance developed in liver epithelial cells through continuous low-level arsenite exposure. Toxicological Sciences, 54, 500–508.

Sabbioni E., Marafante E., Amantiny L., Ubertalli L., Pietra L. (1978): Cadmium toxicity studies under long-term low level exposure /LLe/ conditions. I. Metabolic patterns in rats exposed to present environmental dietary levels of Cd for two years. Science of the Total Environment, 10, 135–161.

Schmolke G., Elsenhans B., Ehtechami C., Forth W. (1992): Arsenic-copper interaction in the kidney of the rat. Human and Experimental Toxicology, 11, 315–321.

Tomsett A.B. (1988): Molecular biology of metal to lerances of plants. Plant Cell and Environment, 11, 383–394.

Turecki T., Cibulka J., Slámová A., Barcalíková R. (1998): Effects of organic and inorganic forms of dietary cadmium on cadmium, zinc, copper, iron and manganese availability to rats. Journal of Animal Physiology and Animal Nutrition, 78, 119–128.

Vahter M., Norin H. (1980): Metabolism of 75As-labelled trivalent and pentavalent inorganic arsenic in mice. Environmental Research, 21, 132–151.

Yanez L., Carrizales L., Zanatta M.T., Mejia J.D., Batres L., DiazBarriga F. (1991): Arsenic-cadmium interaction in rats – toxic effects in the heart and tissue metal shifts. Toxicology, 67, 227–234.

Yudovich Y.E., Ketris M.P. (2005): Arsenic in coal: a review. International Journal of Coal Geology, 61, 141–196.

Received: 2008–05–30
Accepted after corrections: 2008–09–16

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