Alcohol & Alcoholism Vol. 38, No. 1, pp. 2–10, 2003
doi:10.1093/alcalc/agg006, available online at www.alcalc.oupjournals.org

LIVER AND KIDNEY FUNCTION AND HISTOLOGY IN RATS EXPOSED TO CADMIUM AND ETHANOL

M. M. BRZÓSKA*, J. MONIUSZKO-JAKONIUK, B. PIŁAT-MARCINKIEWICZ[1] and B. SAWICKI[1]

Department of Toxicology, Medical Academy, Mickiewicza 2c str., 15-222 Bialystok and [1]Department of Histology and Embryology, Medical Academy, Kilińskiego 1 str., 15-230 Bialystok, Poland

(*Received* 21 *December* 2001; *first review notified* 27 *December* 2001; *in revised form* 9 *May* 2002; *accepted* 5 *July* 2002)

Abstract — Aims: The present study was performed to assess the function and histology of the liver and kidney in rats exposed to 50 mg Cd/l (as cadmium chloride) and/or 10% (w/v) ethanol (EtOH) for 12 weeks. **Methods:** The activities of alanine aminotransferase (ALAT) and asparate aminotransferase (AspAT) in serum were measured as indicators of the liver function. As parameters of the kidney function, creatinine, total protein and urea concentrations in serum and urine, as well as urinary alkaline phosphatase (ALP) activity were determined, and creatinine clearance was calculated. Both organs were subjected to histopathological analysis. **Results:** Daily Cd intake ranged from 3.17 to 4.28 mg/kg body weight and from 2.41 to 3.17 mg/kg body weight in the Cd and Cd + EtOH groups, respectively. The daily intake of 10% EtOH ranged from 47.5 to 86.9 g/kg body weight in the EtOH and from 47.3 to 63.4 g/kg body weight in the Cd + EtOH-exposed rats. Cd and EtOH, independently of separate or combined application, changed liver and kidney function and histology. Rats treated with Cd alone and those co-exposed to both substances showed qualitatively similar, but different magnitudes of changes, in liver and kidney histology. Blurred trabecular structure, vacuolar degeneration and increased density of nuclear chromatin with very compact nuclear structure were found in hepatocytes of zones 2 and 3. Moreover, mononuclear cell infiltrations and necrosis of single cells were evident in zone 1. In the kidney tubules, degeneration and hypertrophy of epithelial cells and dilation in the glomeruli were also observed. Some functional (increased serum AspAT and urinary ALP, decreased urinary urea) and structural changes in the liver and kidney were more evident in the case of combined exposure, while others were more evident after single exposure. However, a decrease in creatinine clearance, noted only in the animals treated with Cd and EtOH, shows that functional changes indicating renal insufficiency are more serious in the co-exposed group. **Conclusions:** Due to lower Cd and EtOH intake (resulting from a stronger aversion to drinking water containing both substances) in the co-exposed rats, as compared to the Cd- and EtOH-treated groups, it is difficult to draw a definite conclusion from this study. The findings, however, seem to indicate that EtOH increases Cd nephrotoxicity in rats, and thus may suggest a higher risk of kidney damage in alcoholics exposed to Cd. Unfortunately, this study does not provide clear evidence if, and to what extent, EtOH influences Cd hepatotoxicity.

INTRODUCTION

It is well known that toxic effects of a xenobiotic can be modified by other substances (Skoczyńska and Smolik, 1994; Brus *et al*., 1999; Institoris *et al*., 1999; Gupta and Gill, 2000). As simultaneous exposure to two or more xenobiotics can take place in the environment and/or under occupational conditions, the investigation of interactions between toxic substances is an important problem in modern toxicology. The interaction between cadmium (Cd) and ethanol (EtOH) can be a good example. Exposure of certain human populations to Cd is often rather high (World Health Organization, 1992; Schrey *et al*., 2000) and EtOH consumption continues to rise worldwide (Samson and Harris, 1992; Meyer *et al*., 2000); so those persons who are exposed to Cd may be simultaneously alcohol misusers (Maranelli *et al*., 1990; Schioeler, 1991).

Some publications provide data on Cd–EtOH interactions (Sharma *et al*., 1991, 1992; Brus *et al*., 1995) but many aspects are still not fully recognized. According to our earlier results short- and long-term EtOH administration affects Cd turnover in rats, and also modifies changes in the metabolism of some essential elements by this heavy metal (Moniuszko-Jakoniuk *et al*., 1999, 2001; Brzóska *et al*., 2000, 2002).

Liver and kidney are important organs of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites, and are especially vulnerable to damage. As the liver is an important target organ of EtOH (Bunout, 1999; Thurman *et al*., 1999), and the kidney of Cd toxicity (Kjellström, 1986; World Health Organization, 1992; Nordberg *et al*., 1994) we have also assessed liver and kidney function and histology.

MATERIALS AND METHODS

Animals

For the experiments, 40 inbred 10-week-old male Wistar rats of 250 g initial body weight were used. The animals were housed in stainless-steel cages under conventional conditions (temperature 22 ± 1°C; relative humidity 50 ± 10%, natural light–dark cycle) and had free access to drinking fluid and a standard rodent laboratory chow (LSM; Fodder Manufactures, Motycz, Poland). (The diet was prepared from: corn, wheat, barley, wheat bran, soya-bruised grain, meat starch, skimmed powdered milk, phosphate, fodder-chalk, mineral and vitamin premix. Metabolizable energy of the LSM diet was 12.2 MJ/kg.) The Cd content of the diet was 0.211 mg/kg (assessed in our laboratory).

Chemicals

All reagents and chemicals were of analytical grade or higher purity. Trace-free nitric acid (Merck, Dormstadt, Germany) and Cd standard solution assigned for atomic absorption spectrometry (Sigma, St Louis, MO, USA) were used for Cd analysis.

Experimental design

The experiment was conducted for 12 weeks. The animals were randomly allocated to four experimental groups of 10 rats each: (1) a control group, which received redistilled water;

*Author to whom correspondence should be addressed.

(2) an EtOH group, which received 10% (w/v) EtOH (POLMOS, Poland); (3) a Cd group, which was exposed to $CdCl_2$ (POCh, Gliwice, Poland) at a concentration of 50 mg Cd/l; (4) a Cd + EtOH group, which received a redistilled water containing 50 mg Cd/l and 10% EtOH. Fluid consumption was measured daily during the whole experiment.

After 12 weeks of treatment all rats were placed separately in glass metabolic cages for 24-h urine collection. After overnight starvation, blood was taken by cardiac puncture, the liver and kidney were removed under ether anaesthesia, washed thoroughly in ice-cold physiological saline [0.9% (w/v) NaCl], and weighed. Whole blood was centrifuged after clotting, and the serum was separated and stored frozen until further analysis.

The study was approved by the Local Ethics Committee for animal experiments in Bialystok, Poland.

Analytical procedures

Cd and EtOH concentrations. Cd concentration in the blood, liver and kidney was determined by atomic absorption spectrometry (Zeiss Jena AAS 30) as described (Brzóska *et al*., 2000, 2002). Blood-EtOH concentration was analysed by head-space gas chromatography (Hewlett-Packard, model 5890, series II) according to the manufacturer's recommendations.

Alanine aminotransferase (ALAT) and asparate aminotransferase (AspAT) activities in serum. The activities of ALAT (EC 2.6.1.2.) and AspAT (EC 2.6.1.1.) were determined colorimetrically (SEMCO S/E-uv spectrometer) according to standard procedures using commercially available diagnostic laboratory tests (Lachema, Brno, Czech Republic).

Biochemical indicators of renal function. Total protein in serum and urine was determined according to Lowry *et al*. (1951). Concentrations of creatinine and urea in serum and urine, as well as urinary alkaline phosphatase (ALP, EC 3.1.3.1) activity, were assessed spectrophotometrically (SEMCO S/E-uv spectrometer) using diagnostic laboratory tests (POCh). Creatinine clearance was calculated.

Histopathological studies. Slices of the left liver lobe and left kidney (from seven animals of each group) were fixed in 10% formalin for 24 h, and were embedded in paraffin; 5–6 μm sections were routinely stained with haematoxylin and eosin (H&E) and assessed in a light microscope (Nikon Eclipse E400). All alterations from the normal structure were registered. The following criteria were used for scoring liver and kidney histology: ++++, a change was very often found in all animals of a group; +++, a change was relatively common in all animals of a group; ++, a change was rare in all animals of a group; +, a change was found in a few animals of a group; ±, a change was sporadic in a group.

Statistical analysis

Statistical analysis of results was performed using the Mann–Whitney non-parametric *U*-test. The level of significance was $P < 0.05$. In order to discern the possible interactions between Cd and EtOH, two-way analysis of variance (ANOVA/MANOVA) was used. *F* values having $P < 0.05$ were considered significant. A linear Pearson correlation was performed for testing relationships between certain parameters. All statistical calculations were done with the STATISTICA 5.0 computer program.

RESULTS

Fluid consumption and Cd and EtOH intakes

Cd or EtOH administered alone depressed the drinking fluid consumption, which was further reduced by their co-administration (Fig. 1). This effect was observed during the whole experiment. In the Cd, EtOH and Cd + EtOH groups, the mean consumption of drinking fluid was reduced by 37, 52 and 60%, respectively ($P < 0.001$ vs control). The daily Cd intake ranged from 3.17 to 4.28 mg/kg body weight in the Cd and from 2.41 to 3.17 mg/kg body weight in the Cd + EtOH groups, while the EtOH intake from 47.5 to 86.9 g/kg body weight (EtOH group) and from 47.3 to 63.4 g/kg body weight (Cd + EtOH group). The average Cd and EtOH intake in the Cd + EtOH groups were lower by 38 ($P < 0.001$) and 18% ($P < 0.01$), respectively, compared to their separate dosages.

Body weight gain, liver and kidney weight

The body weight gain of rats exposed to Cd or EtOH alone was similar to that of controls (Fig. 2), while combined administration of the two substances resulted in a significant retardation already during the first 4 weeks (Fig. 2). The final body weight of the co-exposed rats was lower by 39, 34 and 37% versus control, Cd and EtOH groups ($P < 0.001$), respectively. Two-way analysis of variance has shown that both Cd ($F = 25.7$, $P = 0.000$) and EtOH ($F = 15.9$, $P = 0.000$) had independent effects on the decrease in body weight gain and an interaction between the two substances ($F = 12.2$, $P = 0.001$) was also noted.

Cd and EtOH alone had no effect on kidney weight (Table 1), but the liver weight was reduced by 8% ($P < 0.05$) following Cd administration (Table 1). The co-exposure to Cd and EtOH decreased the absolute weight of both organs by 17 and 13% ($P < 0.01$), but the relative liver and kidney weights did not change (Table 1). The decrease in liver ($F = 30.0$,

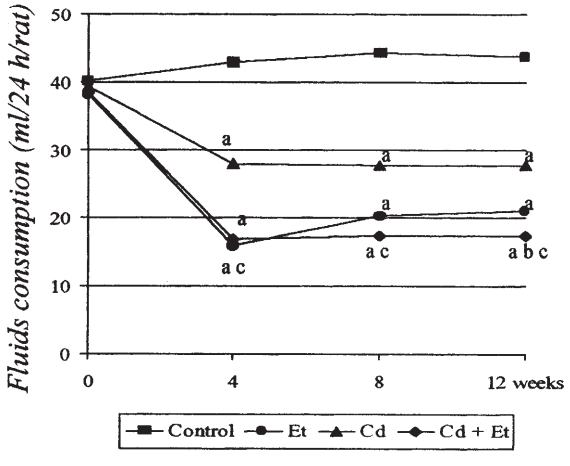

Fig. 1. Effect of Cd, EtOH (Et), and their co-administration on fluid consumption.

Each point represents the mean value of 10 rats. The animals were exposed to 10% EtOH or 50 mg Cd/l separately (EtOH and Cd groups) and to their combination (Cd + EtOH group) for 12 weeks. [a,b,c]Values were significantly different ($P < 0.05$; Mann–Whitney U-test) compared to the control, EtOH and Cd + EtOH groups, respectively.

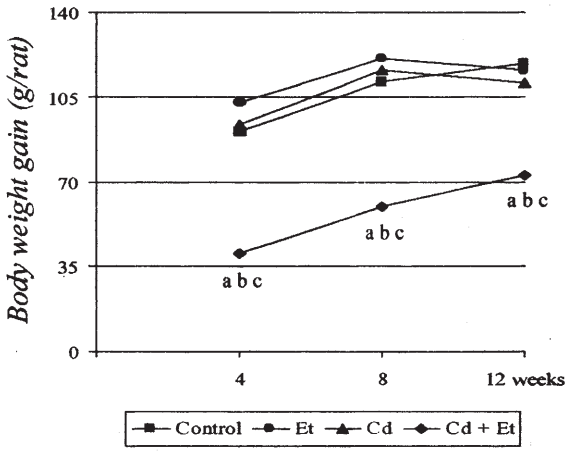

Fig. 2. Effect of Cd, EtOH (Et) and their co-administration on body weight gain.
[a,b,c]Values were significantly different ($P < 0.05$; Mann–Whitney *U*-test) compared to the control, EtOH and Cd + EtOH groups, respectively.

$P = 0.000$), and also in kidney weight ($F = 25.7$, $P = 0.000$) was mainly dependent on Cd administration, and an interactive effect between Cd and EtOH was also observed ($F = 9.2$, $P = 0.004$).

Cd concentration in the whole blood, liver and kidney

The administration of EtOH alone had no influence on Cd concentration in the blood (Fig. 3), liver or kidney (Fig. 4). Cd concentration in the blood and liver of rats simultaneously treated with Cd and EtOH was in the range of values noted in the group exposed to Cd alone, while the kidney concentration was lower by 28% ($P < 0.01$) in the combined group compared to Cd alone.

The effect of EtOH on Cd metabolism in this experimental model has already been described (Brzóska *et al*., 2002).

Blood-EtOH concentration

The concentration of EtOH in the blood of rats which were not treated with EtOH (the control and Cd groups) was within the low physiological range (Fig. 5). In the animals drinking 10% EtOH alone, its concentration was significantly higher ($P < 0.001$), but the joint presence of Cd suppressed this increase (Fig. 5).

ALAT and AspAT activities in serum

In the serum of rats exposed to Cd, EtOH and to their combination, increased activity of ALAT and AspAT was measured versus control ($P < 0.001$), but no differences — except in one case — were observed between the enzyme activities of the treated groups (Fig. 6).

The changes in serum AspAT activity were independent of Cd ($F = 39.2$, $P = 0.000$) and EtOH ($F = 17.8$, $P = 0.000$), but an interaction between the two substances ($F = 5.9$, $P = 0.020$) was observed. On the other hand, serum activity of ALAT was mainly influenced by Cd ($F = 14.8$, $P = 0.001$), and an interactive effect of the substances ($F = 7.2$, $P = 0.011$) was also noted.

Biochemical indicators of renal function

Both Cd and EtOH exposure affected some biochemical markers of kidney function. As shown in Table 2, the intensity of these changes were dependent on whether Cd and EtOH were administered separately or in combination. The creatinine clearance was unaffected by Cd or EtOH alone, but their co-administration decreased it by 29% ($P < 0.05$) versus control and by 25% ($P < 0.05$) versus the Cd-treated group. The total protein concentration in urine was not influenced by either treatment alone. However, urinary protein excretion in the co-exposed rats was higher ($P < 0.05$) than in those receiving EtOH or Cd separately (by 11 and 14%, respectively). An increase in serum urea (by 23%, $P < 0.01$), and a decrease in serum total protein (by 27%, $P < 0.05$) accompanied by a decrease in urinary urea (by 32%, $P < 0.001$), and an increase in urinary ALP activity (2.6-fold, $P < 0.001$) were observed followed EtOH administration. Exposure to Cd alone decreased the urinary urea level (by 36%, $P < 0.001$), increased the urinary ALP activity (4.5-fold, $P < 0.001$) and the serum urea concentration (by 16%, $P < 0.001$), but had no effect on the total protein concentration in serum and urine. In co-exposed animals, serum protein concentration was unchanged, whereas serum urea was increased (by 24%, $P < 0.05$) vs controls. Furthermore, urinary excretion of urea was markedly reduced (2.1-fold, $P < 0.001$) while ALP activity was increased (2.4-fold, $P < 0.001$). In this group, the changes in urinary urea were more, while those in ALP were less pronounced than in the Cd-exposed group.

As revealed by two-way analysis of variance, depending on the parameter studied, the alterations in the indicators of kidney function were either significantly related to the intake of Cd or EtOH, or were a result of an interaction effect between the two substances (Table 2). An interactive effect was observed in serum total protein, in urinary urea, and in ALP activity. The changes of serum urea and creatinine clearance were mainly influenced by EtOH. In addition to the less or more pronounced interactive effect, total serum protein and urinary ALP were also influenced by Cd, while the urinary urea level was strongly independent from the effect of Cd and EtOH.

Table 1. Effects of Cd, EtOH and their co-administration on liver and kidney weight

Group	Liver weight (g)	Relative liver weight (g/100 g body weight)	Kidney weight (g)	Relative kidney weight (g/100 g body weight)
Control	8.7154 ± 0.1969	2.067 ± 0.054	0.9865 ± 0.0261	0.233 ± 0.003
EtOH	8.7551 ± 0.1840	2.068 ± 0.038	0.9912 ± 0.0254	0.234 ± 0.005
Cd	7.9980 ± 0.217[a,b]	1.974 ± 0.042	0.9350 ± 0.0188[b]	0.231 ± 0.006
Cd + EtOH	7.1976 ± 0.2297[a,b,c]	2.035 ± 0.059	0.8688 ± 0.0278[a]	0.245 ± 0.012

The animals were exposed to 10% EtOH and/or 50 mg Cd/l for 12 weeks. Values are means ± SEM of 10 animals.
[a,b,c]Values are significantly different ($P < 0.05$; Mann–Whitney *U*-test) compared to the control, EtOH and Cd groups, respectively.

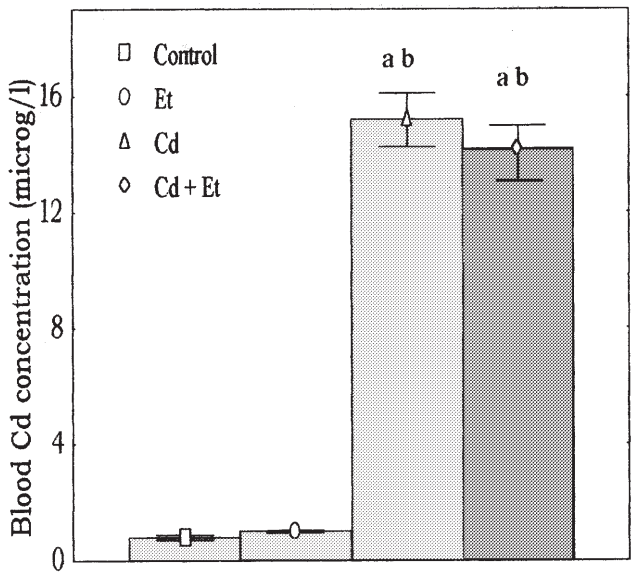

Fig. 3. Cd concentration in whole blood.
Each point represents the mean value ± SEM for 10 rats. [a,b]Values were significantly different ($P < 0.05$; Mann–Whitney *U*-test) compared to the control and EtOH groups, respectively.

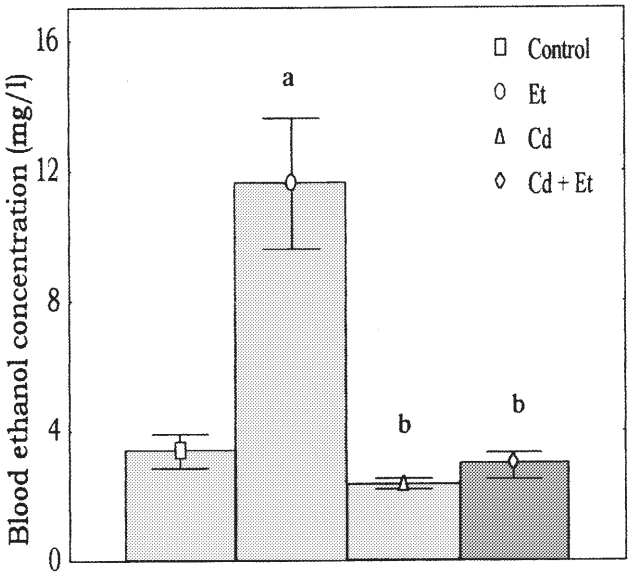

Fig. 5. EtOH (Et) concentration in whole blood.
Each point represents the mean value ± SEM for 10 rats. [a,b]Values were significantly different ($P < 0.05$; Mann–Whitney *U*-test) compared to the control and EtOH groups, respectively.

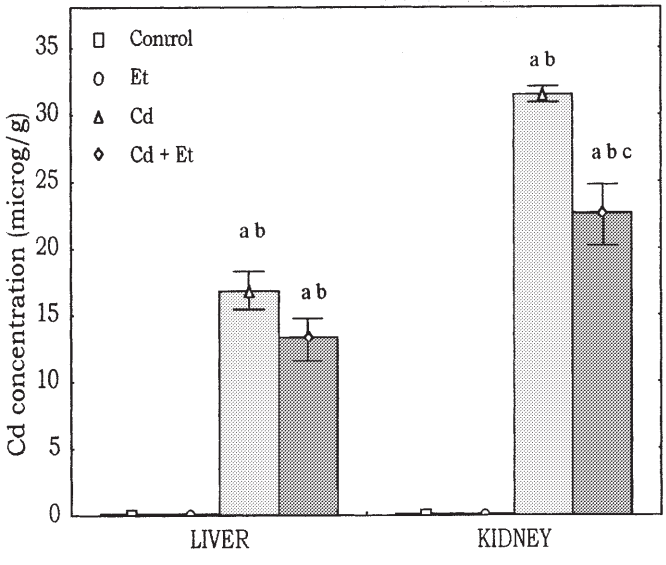

Fig. 4. Cd concentration in liver and kidney.
Each point represents the mean value ± SEM for 10 rats. [a,b,c]Values were significantly different ($P < 0.05$; Mann–Whitney *U*-test) compared to the control, EtOH and Cd + EtOH groups, respectively.

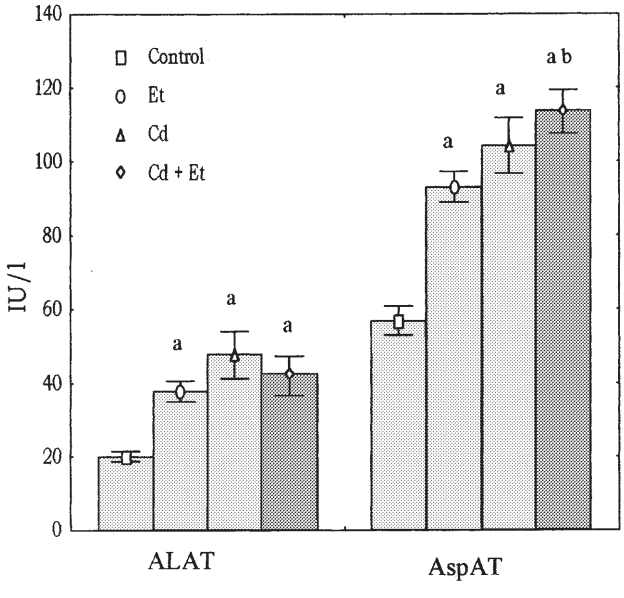

Fig. 6. Effects of Cd, EtOH (Et) and their co-administration on serum ALAT and AspAT activities in serum.
Each point represents the mean value ± SEM for 10 rats. [a,b]Values were significantly different ($P < 0.05$; Mann–Whitney *U*-test) compared to the control and EtOH groups, respectively.

Liver and kidney histopathology

The liver of control rats showed a normal structure (Fig. 7), which was influenced by the administration of Cd and/or EtOH (Table 3 and Figs 8–10). Following exposure to EtOH alone (Fig. 8), the trabecular structure of the lobules was slightly or distinctly blurred. The cytoplasm of hepatocytes of zone 2 and 3, contained empty vacuole-like spaces, and were enlarged. Some sinusoids were overfilled with erythrocytes and the walls of most sinusoids showed numerous Kupffer cells. Locally, mononuclear cell infiltrates were observed, most frequently in the hepatocytes of zone 1. In a few animals of this group, an increased density of nuclear chromatin and a very compact nuclear structure were noted (zones 2 and 3). Sporadically, single necrotic cells were evident in zone 1. After exposure to Cd alone (Fig. 9), the trabecular liver structure

Table 2. Effects of Cd, EtOH and their co-administration on biochemical indicators of renal function

Group	Creatinine clearance (ml/min)	Total protein in serum (g/100 ml)	Urea in serum (mg/100 ml)	Total protein in urine (mg/mg creatinine)	Urea in urine (mg/24 h)	ALP in urine (IU/l)
Control	1.18 ± 0.06	3.07 ± 0.22	34.11 ± 0.76	3.02 ± 0.12	602.4 ± 23.7	1.64 ± 0.23
EtOH	1.07 ± 0.07	2.25 ± 0.14[a]	41.98 ± 1.71[a]	2.76 ± 0.09	409.6 ± 19.10[a]	4.26 ± 0.26[a]
Cd	1.11 ± 0.05	2.85 ± 0.16[b]	39.61 ± 0.87[a]	2.67 ± 0.11	385.9 ± 16.3[a]	7.43 ± 0.53[a,b]
Cd + EtOH	0.84 ± 0.11[a,c]	3.22 ± 0.10[b,c]	42.22 ± 2.72[a]	3.05 ± 0.10[b,c]	289.7 ± 9.5[a,b,c]	4.07 ± 0.19[a,c]
Main effect of						
EtOH	$F = 6.2$, $P = 0.018$	NS	$F = 9.4$, $P = 0.004$	NS	$F = 65.2$, $P = 0.000$	NS
Cd	NS	$F = 5.7$, $P = 0.022$	NS	NS	$F = 88.3$, $P = 0.000$	$F = 70.1$, $P = 0.000$
Interactive effect[d]	NS	$F = 14.0$, $P = 0.001$	NS	$F = 8.8$, $P = 0.005$	$F = 7.3$, $P = 0.011$	$F = 85.9$, $P = 0.000$

The rats were exposed to 10% EtOH and/or 50 mg Cd/l for 12 weeks. Values are means ± SEM of 10 animals.
[a,b,c]Values are significantly different ($P < 0.05$; Mann–Whitney *U*-test) compared to control, EtOH and Cd groups, respectively.
[d]Two-way analysis of variance (ANOVA/MANOVA). NS, non-significant.

Table 3. Histopathological findings in liver of rats treated with Cd or/and EtOH[a]

Finding	Treatment		
	EtOH	Cd	Cd + EtOH
Blurred trabecular structure of the lobules	++	++++	+++
Vacuolar degeneration-type changes, enlarged cell sizes	+++	++++	+++
Increased density of nuclear chromatin and very compact nuclear structure	+	++++	++
Necrosis of single cells — pycnosis of nuclei, strongly acidophilic cytoplasm	±	++	+
Increased number of Kupffer cells	++	+++	+++
Sinuses overfilled with blood with mononuclear cell infiltrations	++++	+++	++++

Animals were exposed to 10% EtOH and 50 mg Cd/l separately (EtOH and Cd groups) and in combination (Cd + EtOH group) for 12 weeks.
++++, the change was very often found in all animals; +++, the change was relatively common in all animals; ++, the change was rare in all animals; +, the change was found in a few animals; ±, the change was sporadic.
[a]The study was done for seven rats of each group.

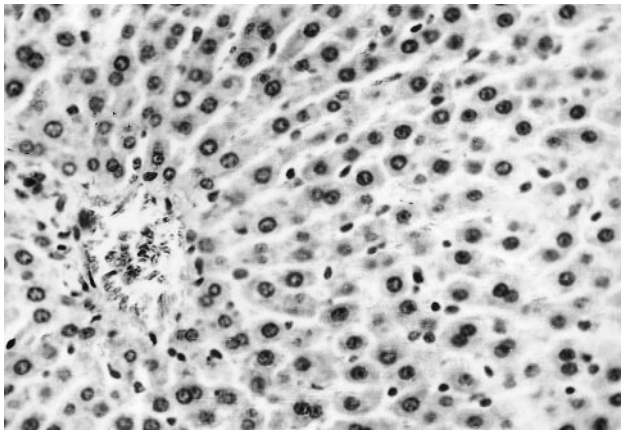

Fig. 7. Liver of a control rat.
It is composed of hexagonadal or pentagonadal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective tissue. Hepatocytes are arranged in trabecules running radiantly from the central vein and are separated by sinusoids containing Kupffer cells. They are regular and contain a large spheroidal nucleus with a distinctly marked nucleolus and peripheral chromatin distribution. Some cells have two nuclei each. H&E, ×300.

was more seriously affected than after EtOH administration (Fig. 8). Cd-induced degenerative changes were evident in numerous hepatocytes of zones 2 and 3; the cells were enlarged and had light and foamy cytoplasm filled with vacuoles. The walls of the sinusoids in both zones showed numerous Kupffer cells. In a few zone 1 hepatocytes, necrotic changes were evident; a small, pycnotic cellular nucleus with condensed chromatin, lack of nucleolus and strongly acidophilic cytoplasm were observed. Mononuclear cell infiltrates were also noted in zone 1 hepatocytes. In rats co-exposed to Cd and EtOH (Fig. 10), the trabecular structure of the lobules was blurred. The cytoplasm of some hepatocytes was light, enlarged and contained vacuoles (less numerous than after Cd alone). Numerous Kupffer cells were found in the sinusoid walls. These changes were observed mainly in the hepatocytes of zone 3. Mononuclear cell infiltrates were evident in zone 1. Moreover, increased density of nuclear chromatin and a very compact nuclear structure (zones 2 and 3) were rarely noted in all rats of this group. In a few animals, necrosis of single cells was evident (zone 1).

Normal structure of the cortex and medulla was observed in the kidney of control rats (Figs 11 and 13). The animals exposed to Cd and EtOH separately as well as in combination showed similar changes, but of different intensity, in the renal tubules and glomeruli (Table 4, Figs 12 and 14). Hypertrophy of epithelial cells and degeneration of epithelia of renal tubules with infiltration of mononuclear cells, dilation of glomeruli as well as hyperaemia of medullary and cortical parts with mononuclear cell infiltrates were evident in all animals treated with Cd and/or EtOH. The most advanced change after EtOH exposure was the dilatation of capillaries filled with erythrocytes both in the cortical and medullary parts of the kidney. In the rats treated with Cd alone or in combination, an enlargement

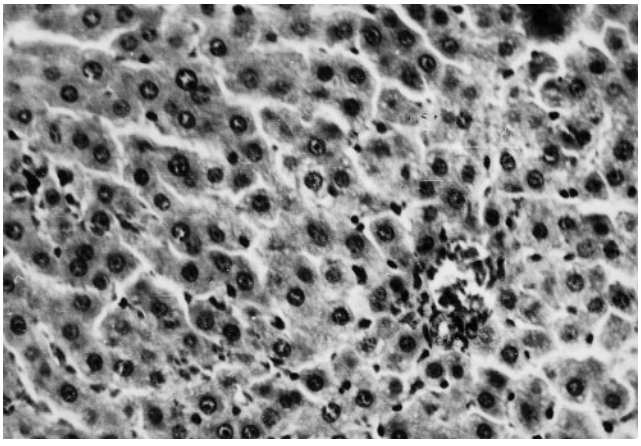

Fig. 8. Liver of a rat exposed to 10% EtOH for 12 weeks.
In most hepatic lobules, the trabecular structure is lightly blurred and, in the remaining lobules, distinctly blurred. The cytoplasm of some cells shows rare empty vacuole-type spaces. A considerable number of Kupffer cells are observed in the sinusoid walls. The sinuses exhibit erythrocytes and mononuclear cell infiltration in the vicinity of sinusoids. H&E, ×300.

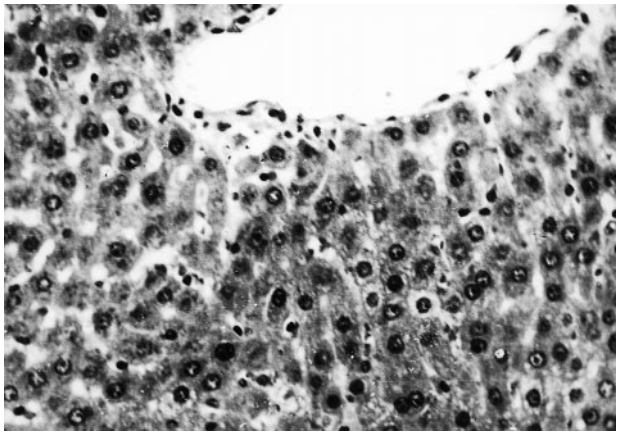

Fig. 10. Liver a of rat exposed simultaneously to 50 mg Cd/l and 10% EtOH for 12 weeks.
The trabecular structure of the lobules is blurred in places. The cytoplasm of some hepatocytes is enlarged, light, with vacuoles. In most hepatocytes, the structure of nuclei is normal. Moreover, an increased number of erythrocytes are observed in the lobular sinusoid lumen. H&E, ×300.

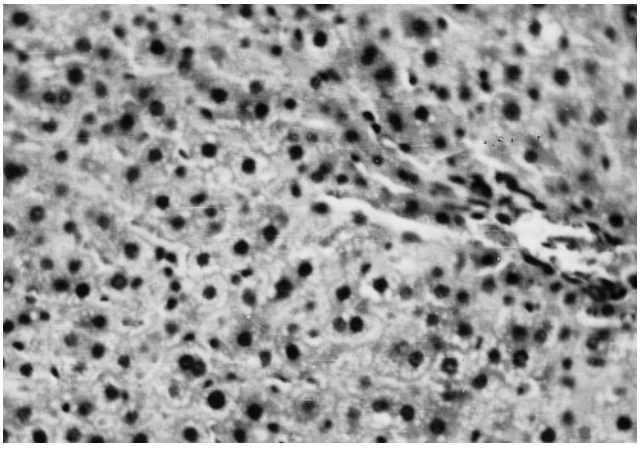

Fig. 9. Liver of a rat exposed to 50 mg Cd/l for 12 weeks.
The trabecular structure of the liver is blurred. The hepatocyte cytoplasm is light, foamy and filled with vacuoles; cell sizes are enlarged, nuclear chromatin is more compact, slightly smaller nucleoli are not conspicuous. Necrosis of single hepatocytes — nuclei are contracted, pycnotic with condensed chromatin, cytoplasm is strongly acidophilic. Accumulation of mononuclear cells in the vicinity of sinusoids. The sinusoid walls show numerous Kupffer cells. H&E, ×300.

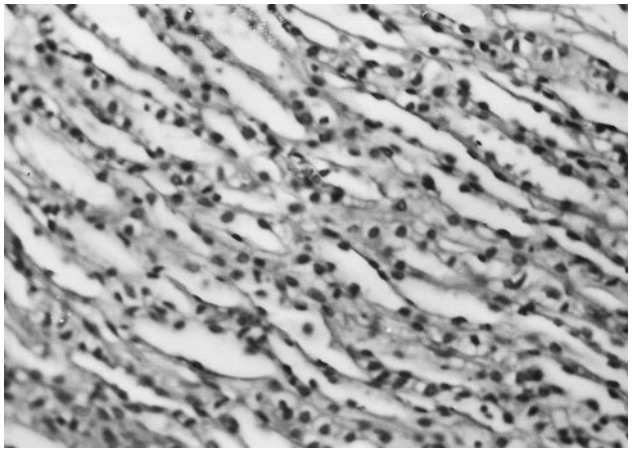

Fig. 11. Kidney (medullary part) of a control rat.
Collecting tubules are lined with the relatively low simple cubic epithelium. The thick descending and ascending parts of Henle's loops and collecting coils of small calibre, and a small amount of interstitial tissue can be seen in the cross-sections. H&E, ×300.

of renal glomeruli and epithelial cells of the I-row tubules in the cortical part of the kidney were found; a few renal tubules showed single epithelial cells desquamated to their lumen (Fig 12). Mononuclear cell infiltrates were observed in some places of the medullary part of the kidney, and at these sites the inflowing cells blurred the tubular structure (Fig. 12). Generally, the histological changes in kidney cortex and medulla of Cd and Cd + EtOH groups were more serious than those observed after EtOH alone (Table 4).

DISCUSSION

The present study was undertaken to evaluate the function and structure of the liver and kidney in conditions of co-exposure to EtOH and Cd. Both substances are hepato- and nephrotoxic, but they affect these organs in different ways (Kjellström, 1986; World Health Organization, 1992; Nordberg *et al*., 1994; Epstein, 1997; Sakurama, 1998; Bunout, 1999; Thurman *et al*., 1999). Long-term EtOH consumption damages mainly the liver (Bunout, 1999; Thurman *et al*., 1999), whereas chronic exposure to Cd results, first of all, in tubular

Table 4. Histopathological findings in kidney of rats treated with Cd or/and EtOH[a]

Finding	Treatment		
	EtOH	Cd	Cd + EtOH
Hypertrophy of epithelial cells of renal tubules	++	++++	+++
Degeneration of tubular epithelia with simultaneous infiltration of mononuclear cells	++	+++	+++
Hyperaemia of medullary and cortical part with mononuclear cell infiltration	++++	+++	++++
Dilation of renal glomeruli	++	++++	+++

Animals were exposed to 10% EtOH and 50 mg Cd/l separately (EtOH and Cd groups) and in combination (Cd + EtOH group) for 12 weeks.
++++, the change was very often found in all animals; +++, the change was relatively common in all animals; ++, the change was rare in all animals; +, the change was found in a few animals; ±, the change was sporadic.
[a]The study was done for seven rats of each group.

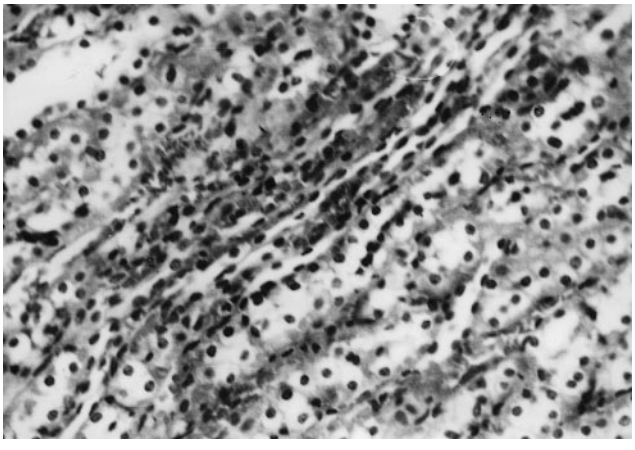

Fig. 12. Kidney (medullary part) of a rat exposed to 50 mg Cd/l for 12 weeks.
A transverse cross-section of thick Henle's loops shows hypertrophy of epithelial cells, frequently with signs of oedema. Distinct infiltration of mononuclear cells can be seen in places where tubular epithelium has undergone degenerative changes. H&E, ×300.

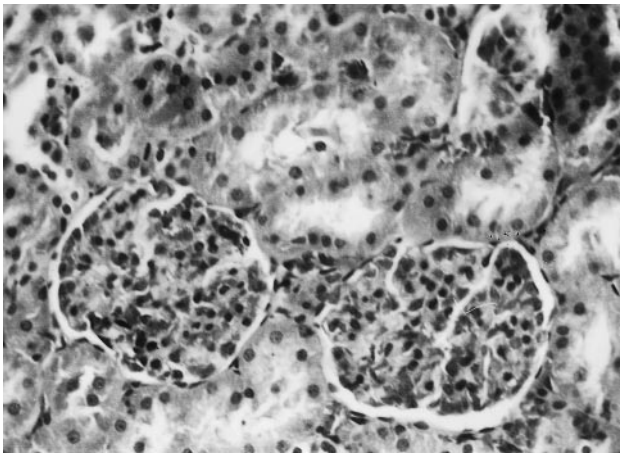

Fig. 14. Kidney (cortical part) of a rat exposed simultaneously to 50 mg Cd/l and 10% EtOH for 12 weeks.
Vascular glomeruli are enlarged, tightly filling the Bowmann's capsule. Some cells of the I-row tubular epithelium show features of oedema. Capillaries are filled with blood cells, some tubules contain single desquamated cells. H&E, ×300.

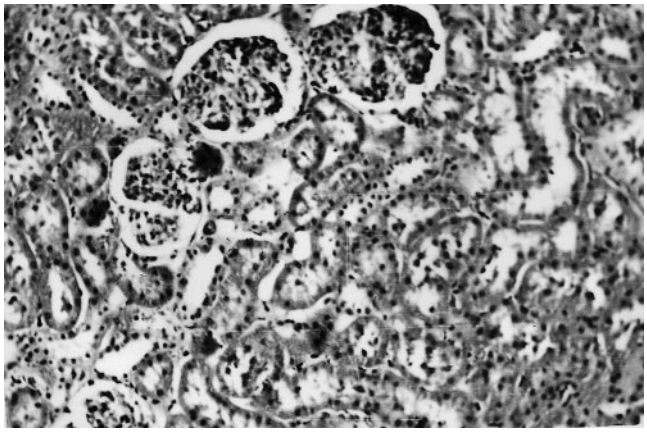

Fig. 13. Kidney (cortical part) of a control rat.
Renal glomeruli show normal structure. The I-row renal tubules are lined with typical thick cubic epithelium. The II-row tubules show a considerably lower cubic epithelium. The tubules have a relatively regular distinct lumen. Lobular organization of the glomerule and a flat epithelium lining the glomerular capsule can be seen. H&E, ×150.

dysfunction (Kjellström, 1986; World Health Organization, 1992; Nordberg *et al*., 1994). Unfortunately, no data are available on the function and structure of both organs in conditions of co-exposure to Cd and EtOH.

We evaluated liver function by measuring plasma ALAT and AspAT activities. As parameters of kidney function, creatinine, total protein and urea concentrations in serum and urine as well as urinary ALP activity, were determined, and creatinine clearance was also calculated. The structure of both organs was assessed on the basis of histopathological analyses.

The level of Cd treatment used in this study corresponds to human (especially smokers) occupational exposure to this heavy metal, or environmental exposure in heavily contaminated areas (World Health Organization, 1992). The level of intoxication with EtOH may be tantamount to its misuse in man (Wiśniewska-Knypl and Wrońska-Nofer, 1994).

Since the relative liver and kidney weights did not change in the co-exposed rats, the decrease in their weights reflects a retardation in body weight gain, which is a consequence of reduced food (Brzóska *et al*., 2002) and water intake, and

of Cd–EtOH interaction. Other authors also reported the unfavourable effect of co-exposure to Cd and EtOH on body weight gain (Tandon and Tewari, 1987; Gupta and Gill, 2000).

As animals receiving Cd and EtOH simultaneously develop a stronger aversion to drinking than those intoxicated separately, so they ingest less Cd and EtOH. The difference of intake is noteworthy and has to be taken into account in interpretation of the present results.

Cd accumulation in the liver and kidney of rats exposed to this metal alone as well as in combination with EtOH resulted in serious changes in the histology and function of these two organs. Similar or more advanced changes in liver and kidney histology and function under Cd influence, have been reported by others (Aughey *et al*., 1984; Kjellström, 1986; Mitsumori *et al*., 1998). Aughey *et al*. (1984) noted early pathological changes in rat kidney already after 6 weeks of administration of 50 mg Cd/l in drinking water. After 12 weeks, they revealed signs of tubular necrosis, interstitial fibrosis and glomerular epithelial cell hypertrophy in small areas of the kidney cortex. Pathological changes in kidney ultrastructure (injured brush-border microvilli and swollen mitochondria in the proximal convoluted tubular cells) were observed when Cd concentration in this organ exceeded 10 μg/g and they became more pronounced as concentration increased. At a Cd level of about 30 μg/g, necrotic changes were observed (Aughey *et al*., 1984). In our experiment, Cd concentration in kidney ranged from about 20 to 30 μg/g, depending on whether Cd was administered alone or in combination with EtOH. The results of this study and of other investigations (Aughey *et al*., 1984) show that the critical Cd concentration in the kidney cortex is lower than 200 μg/g (the kidney cortex/whole kidney ratio of Cd concentration is about 1.25). Such high Cd concentrations in the kidney cortex were measured in rats fed with diet containing 200 mg Cd/kg for 2–4 months (Mitsumori *et al*., 1998).

Increased serum transaminase activities were observed in our study following Cd and EtOH co-administration and similar changes have been reported by other authors (Tandon and Tewari, 1987; Thurman *et al*., 1999).

Morphological observations, together with functional tests, show that Cd and EtOH, administered separately and especially in combination, lead to liver and kidney injury, thus posing a serious risk for health. The changes observed in these organs of co-exposed rats can be a result of an independent effect of Cd and EtOH and also of their interaction. Since EtOH alone also had affected the liver and kidney, on the basis of this study it is difficult to make any definite assessment as to whether EtOH influenced Cd toxicity, and if so, to what extent. However, such an effect of EtOH is very likely, and can be linked to changes in Cd body burden. In this work, we measured the Cd concentrations only in the liver and kidney, but in a previous study a profound effect of EtOH on Cd turnover was reported in the same experimental model (Brzóska *et al*., 2002). We have noted that in the Cd + EtOH group the whole Cd pool in the internal organs was at the same level as in those receiving Cd alone, in spite of its lower intake. In the absence of the modifying effect of EtOH, the concentrations and content of Cd in the co-exposed animals should be lower, compared to the Cd-only exposed ones. Thus, our results clearly show that EtOH influences Cd turnover (increases gastrointestinal absorption and retention of absorbed metal), making the organism more susceptible to its accumulation.

Due to the different intakes of Cd and EtOH during their co-administration, than after their separate dosages, we cannot correctly interpret the interactive effects of the two substances on the liver and kidney. Nevertheless, our findings allow us to conclude that EtOH increases Cd nephrotoxicity, although the present results give no clear evidence of enhanced Cd hepatotoxicity. However, it seems likely that, if the consumption of Cd and EtOH were the same in co-exposed and separately exposed animals, the disturbances in liver and kidney function as well as histology, would be more serious in the co-exposed ones. On the basis of the present and previous studies (Brzóska *et al*., 2000, 2002), we hypothesize that subjects exposed simultaneously to Cd and EtOH are more vulnerable to Cd accumulation and thus its deleterious health effects, including kidney damage. Further studies are needed to explain Cd–EtOH interactions in conditions of long-term co-exposure and their consequences for health.

REFERENCES

Aughey, E., Fell, G. S., Scott, R. and Black, M. (1984) Histopathology of early effects of oral cadmium in the rat kidney. *Environmental and Health Perspectives* **54**, 153–161.

Brus, R., Kostrzewa, R. M., Felińska, W., Plech, A., Szkilnik, R. and Frydrych, J. (1995) Ethanol inhibits cadmium accumulation in brains of offspring of pregnant rats that consume cadmium. *Toxicology Letters* **76**, 57–62.

Brus, R., Szkilnik, R., Nowak, P., Oswiecimska, J., Kasperska, A., Sawczuk, K., Slota, P., Kwiecinski, A., Kunanski, N. and Shani, J. (1999) Effect of lead and ethanol, consumed by pregnant rats, on behavior of their grown offsprings. *Pharmacology Reviews and Communications* **10**, 175–186.

Brzóska, M. M., Moniuszko-Jakoniuk, J., Jurczuk, M., Gałażyn-Sidorczuk, M. and Rogalska J. (2000) Effect of short-term ethanol administration on cadmium retention and bioelements metabolism in rats continuously exposed to cadmium. *Alcohol and Alcoholism* **35**, 439–445.

Brzóska, M. M., Moniuszko-Jakoniuk, J., Jurczuk, M. and Gałażyn-Sidorczuk, M. (2002) Cadmium turnover and changes of zinc and copper body status of rats continuously exposed to cadmium and ethanol. *Alcohol and Alcoholism* **37**, 213–221.

Bunout, D. (1999) Nutritional and metabolic effects of alcoholism. Their relationship with alcoholic liver disease. *Nutrition* **7–8**, 583–589.

Epstein, M. (1997) Alcohol's impact on kidney function. *Alcohol Health and Research World* **21**, 84–92.

Gupta, V. and Gill, K. D. (2000) Influence of ethanol on lead distribution and biochemical changes in rats exposed to lead. *Alcohol* **20**, 9–17.

Institoris, L., Siroki, O., Desi, I. and Undeger, U. (1999) Immunotoxicological examination of repeated dose combined exposure by dimethoate and two heavy metals in rats. *Human and Experimental Toxicology* **18**, 88–94.

Kjellström, T. (1986) Renal effects. In *Cadmium and Health: A Toxicology and Epidemiological Appraisal*, Vol. 2, Friberg, L., Elinder, C. G., Kjellström, T. and Norgderg, G. F. eds, pp. 21–109. CRC Press, Boca Raton, FL.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.

Maranelli, G., Apostoli, P. and Ferrari, P. (1990) Influence of smoking, alcohol, and dietary habits on blood Pb and Cd levels. *Bulletin of Environmental Contamination and Toxicology* **45**, 804–810.

Meyer, C., Rumpf, H. J., Hapke, U., Dilling, H. and John, U. (2000) Prevalence of alcohol consumption, abuse and dependence in a country with higher per capita consumption: findings from the German TACOS study. Transitions in alcohol consumption and smoking. *Social Psychiatry and Psychiatric Epidemiology* **35**, 539–547.

Mitsumori, K., Shibutani, S., Sato, S., Onodera, H., Nakagawa, J., Hayashi, Y. and Ando, M. (1998) Relationship between the development of hepato-renal toxicity and cadmium accumulation in rats given minimum to large amounts of cadmium chloride in the long-term: preliminary study. *Archives of Toxicology* **72**, 545–552.

Moniuszko-Jakoniuk, J., Jurczuk, M., Gałażyn-Sidorczuk, M., Brzóska, M. M. and Świątek, E. (1999) The changes in chosen parameters of iron metabolism in rats after exposure to cadmium and ethanol. *Polish Journal of Environmental Studies* **8** (Suppl. 2), 158–162.

Moniuszko-Jakoniuk, J., Gałażyn-Sidorczuk, M., Brzóska, M. M., Jurczuk, M. and Kowalczyk, M. (2001) Effect of short-term ethanol administration on cadmium excretion in rats. *Bulletin of Environmental Contamination and Toxicology* **66**, 125–131.

Nordberg, G. F., Jin, T. and Nordberg, M. (1994) Subcellular targets of cadmium nephrotoxicity: cadmium binding to renal membrane proteins in animals with or without protective metallothionein synthesis. *Environmental Health Perspectives* **102** (Suppl. 3), 191–194.

Sakurama, K. (1998) Effect of long-term ethanol administration on the kidneys, bones and muscles of mice. *Nippon Arukoru Yakubutsu Igakkai Zasshi* **33**, 703–717.

Samson, H. H. and Harris, H. (1992) Neurobiology of alcohol abuse. *Trends in Pharmacological Sciences* **13**, 206–211.

Schioeler, P. (1991) Alcohol-related problems for primary health care workers. In *Development of National Training Seminar*. WHO Euro, Copenhagen.

Schrey, P., Wittsiepe, J., Budde, U., Heinzow, B., Idel, H. and Wilhelm, M. (2000) Dietary intake of lead, cadmium, copper and zinc by children from the German North Sea island Amrum. *International Journal of Hygiene and Environmental Health* **203**, 1–9.

Sharma, G., Sandhir, R., Nath, R. and Gill, K. (1991) Effect of ethanol on cadmium uptake and metabolism of zinc and copper in rats exposed to cadmium. *Journal of Nutrition* **121**, 87–91.

Sharma, G., Nath, R. and Gill, K. D. (1992) Effect of ethanol on the distribution of cadmium between the cadmium metallothionein- and non-metallothionein-bound cadmium pools in cadmium-exposed rats. *Toxicology* **72**, 251–263.

Skoczyńska, A. and Smolik, R. (1994) The effect of combined exposure to lead and cadmium on serum lipids and lipid peroxides level in rats. *International Journal of Occupational Medicine and Environmental Health* **7**, 263–271.

Tandon, S. K. and Tewari, P. C. (1987) Effect of co-exposure to ethanol and cadmium in rats. *Bulletin of Environmental Contamination and Toxicology* **39**, 633–640.

Thurman, R. G., Bradford, B. U., Iimuro, Y., Frankenberg, M. V., Knecht, K. T., Connor, H. D., Adachi, Y., Wall, C., Arteel, G. E., Raieigh, J. A., Forman, D. T. and Mason, R. P. (1999) Mechanisms of ethanol-induced hepatotoxicity: studies in rats. *Frontiers in Bioscience* **4**, 42–46.

Wiśniewska-Knypl, J. M. and Wrońska-Nofer, T. (1994) Biological markers of oxidative stress induced by ethanol and iron overload in rats. *International Journal of Occupational Medicine and Environmental Health* **7**, 355–363.

World Health Organization (1992) *Environmental Health Criteria, 134 Cadmium*. IPCS, Geneva.