Food and Chemical Toxicology 65 (2014) 260–268

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Toxicological assessment of combined lead and cadmium: Acute and sub-chronic toxicity study in rats

Guiping Yuan [a,1], Shujun Dai [a,b,1], Zhongqiong Yin [b,*,1], Hongke Lu [b], Renyong Jia [b], Jiao Xu [b], Xu Song [b], Li Li [b], Yang Shu [b], Xinghong Zhao [b]

[a] Analytical & Testing Center, Sichuan University, Chengdu 610065, China
[b] College of Veterinary Medicine, Sichuan Agricultural University, Ya'an 625014, China

ARTICLE INFO

Article history:
Received 19 September 2013
Accepted 13 December 2013
Available online 3 January 2014

Keywords:
Pb and Cd
Acute toxicity test
Sub-chronic toxicity test

ABSTRACT

The exposure to chemical mixtures is a common and important determinant of toxicity and receives concern for their introduction by inhalation and ingestion. However, few in vivo mixture studies have been conducted to understand the health effects of chemical mixtures compared with single chemicals. In this study, the acute and 90 day sub-chronic toxicity tests of combined Pb and Cd were conducted. In the acute toxicity test, the LD_{50} value of $Pb(NO_3)_2$ and $CdCl_2$ mixture by the oral route was 2696.54 mg/kg by Bliss method. The sub-chronic treatment revealed that the low-dose combination of Pb and Cd exposures can significantly change the physiological and biochemical parameters of the blood of Sprague–Dawley (SD) rats with dose–response relationship and causes microcytic hypochromic anemia and the damages of liver and kidney of the SD rats to various degrees. Histopathological exams showed that the target organs of Pb and Cd were testicle, liver, and kidneys. These observations suggest that Pb and Cd are practically additive-toxic for the SD rats in oral acute toxicity studies. The lowest observed adverse-effect level in rats may be lower than a dose of 29.96 mg/(kg bw day) when administered orally for 90 consecutive days.

1. Introduction

Pb and Cd are widespread occupational and environmental toxicants and are recognized to be the most hazardous pollutants to various ecosystems and human health (Mol, 2011). Inhalation and ingestion are the two main routes of exposure to Pb and Cd (Johri et al., 2010), of which ingestion is the primary route of environmentally exposed people. After absorption, most Pb binds to proteins in erythrocytes and is distributed to soft tissues and bone; the latter is the main depot for this metal (Hambach et al., 2013b). Pb can cause mitochondrial damage, reactive oxygen species (ROS) production, gluthatione intracellular depletion, and apoptosis (Sabath and Robles-Osorio, 2012). Cd is transported in blood by albumin (ALB) to liver, where it bounds to metallothionein (MT). The Cd–MT complex is then released back into circulation (Hambach et al., 2013b). Johri et al. (2010)) found that Pb and Cd can interact with each other in a complex way. The co-exposure to Pb and Cd may induce additive or synergistic interactions or new effects that are not observed for single element exposure (Wang and Fowler, 2008). The similarities in the major target areas and mechanisms of toxicity (e.g., the inhibition of sulfhydryl group containing enzymes and the increased production of ROS) raise concerns regarding the possible toxicity of the combined exposure to Pb and Cd (Dai et al., 2013; Hambach et al., 2013b). They have been implicated as the cause of renal disturbances, lung insufficiency, bone lesions, cancer, and hypertension (Åkesson, 2011; Goyer, 1997; Joseph, 2009; Karavoltsos et al., 2008). Moreover, they can be carcinogenic, embryotoxic, teratogenic, and mutagenic (Järup, 2003).

Most current knowledge (e.g., neurotoxicity, nephrotoxicity, hepatotoxicity) induced by Pb and/or Cd has exposed human subjects and various experimental models (Baba et al., 2013; Hambach et al., 2013a; Kang et al., 2013; Schnaas et al., 2006; Sinha et al., 2008). In the majority of animal studies, single metal is used in high concentration; however in environment, population receives simultaneous multiple exposures, indicating the need for experimental work with combinations of substances.

Humans are typically exposed to low doses of combined chemicals rather than to one chemical at a time, yet most of the available toxicity data provide information on a single chemical rather than on two or more mixtures (Teuschler and Hertzberg, 1995). For example, the Pb and Cd concentrations in goat, cow, sheep, and buffalo milks from two large industrial cities were

* Corresponding author. Tel./fax: +86 835 2885614.
E-mail address: yinzhongq@163.com (Z. Yin).
[1] These authors contributed equally to this work and should be considered as first author.

0278-6915/$ - see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2013.12.041

higher than those in other regions of Iran (Rahimi, 2013). Some of the highest Cd and Pb concentrations were detected in the examined samples of non-certified organically produced foodstuffs from the Greek market (Karavoltsos et al., 2008). The environmental mixtures of chemicals constitute a prevalent issue in ecotoxicology, and reducing the uncertainties associated with their ecological risk assessment is a critical research need. A number of models have been explored to predict the potential combined effects of chemicals on species. These models, especially concentration addition and independent action, have been applied to a number of mixtures (Spurgeon et al., 2010).

In this study, $Pb(NO_3)_2$ and $CdCl_2{\cdot}2.5H_2O$ were used in a equitoxic mixture ratio design. The fixed mixture ratio was based on single toxicant LD_{50} value (median lethal dose value) for an oral acute study. The LD_{50} for the orally administered $Pb(NO_3)_2$ and $CdCl_2$ were 3163 mg/kg and 88 mg/kg in rats, respectively (Lu et al., 2012). The sub-chronic experimental model of rats treated with Pb and Cd at relatively low levels was based on the mixture toxicant LD_{50} value of our oral acute study for a 90-day period as a model of Pb/Cd-induced sub-chronic toxicological evaluation. The results of this study will provide an important reference of Pb and Cd co-exposure for possible disease diagnosis and health assessment in humans.

2. Materials and methods

2.1. Reagents

$Pb(NO_3)_2$ and $CdCl_2{\cdot}2.5H_2O$, which were analytical grade (AR > 99.0%), were obtained from Chengdu Kelong Chemical Co., Ltd. (Chengdu, China).

2.2. Experimental animals

Four week-old male and female SPF Sprague–Dawley (SD) rats weighing 100 ± 5 g were purchased from Chengdu Dossy Experimental Animals Co., Ltd. [License No. SCXK (Sichuan) 2008-24] and kept in animal houses at the Sichuan Agriculture University (Ya'an, China). The rats were placed separately in laboratory animal houses at 20–25 °C with 50–60% humidity and 12 h light/dark cycle with the lights off at 7 PM based on the Guidelines of the International Committee on Laboratory Animals. The rats were fed with a standard diet from Nuvital Nutrients (Colombo/PR, Brazil), allowed to access to distilled water ad libitum, and acclimated to laboratory conditions for 7 days.

2.3. Oral acute toxicity

An oral acute study for calculating LD_{50} was performed according to the Organization for Economic Co-operation and Development (OECD) Guideline 425 "Up and Down procedure" (Jung and Choi, 1994; OECD, 2008; Rispin et al., 2002). In this method, animals are dosed once at a time. If the animal survived, the dose for the next animal is increased; if the animal died, the dose for the next animal is decreased. $Pb(NO_3)_2$ and $CdCl_2{\cdot}2.5H_2O$ were mixed according to the method of equitoxic ratio mixing (Smyth et al., 1970). Five experimental groups, with six rats each and an equal number of male and female, were formed. The five groups were fed with a mixture of $Pb(NO_3)_2$ and $CdCl_2{\cdot}2.5H_2O$ at doses of 1436(1402 + 34), 1968(1921 + 47), 2697(2633 + 64), 3694(3607 + 87), and 5062(4942 + 120) mg/kg. In each case, the product volume administered by gavage was 1 mL/100 g body weight (b.w.).

The animals were observed for gross behavioral, neurologic, autonomic, and toxic effects for 24 h and then daily for 14 days. The toxicological effect was assessed on the basis of mortality, which was expressed as an LD_{50} value. The LD_{50} of combinative toxicity was tested by Bliss method (Bliss, 1939). The results were evaluated through Keplinger evaluation system, in which the expected LD_{50} of a "mixture" was compared with the actual LD_{50} and expressed as a ratio (Keplinger and Deichmann, 1967).

2.4. Ninety-day sub-chronic oral toxicity

2.4.1. Study design

The study was performed in accordance with (A) FDA Redbook (FDA, 2000): Chapter IV.C.4.a Sub-chronic Toxicity Studies with Rodents, (B) OECD Guidelines for the Testing of Chemicals, Section 4, Health Effects (Part 408) (OECD, 1998): Repeated Dose 90-day Oral Toxicity Study in Rodents, and (C) Chinese Center for Disease Control and Prevention (CDC, 2008): chemical test method of repeated dose oral toxicity study in rodents. The study was conducted in accordance with the OECD principles of Good Laboratory Practice.

2.4.2. Treatments

Three groups of 20 rats, each containing 10 females and 10 males, consumed a daily dose of 29.96 (Group II, 29.25 + 0.71), 89.88 (Group III, 87.74 + 2.14), 269.65 (Group IV, 263.23 + 6.42) mg/kg b.w. with a mixture of $Pb(NO_3)_2$ and $CdCl_2{\cdot}2.5H_2O$ for at least 90 consecutive days. The doses of our study aimed to chronically expose rats to relatively low and environmentally realistic concentrations of Pb/Cd (Antonio Garcia and Corredor, 2004; Chen et al., 2013; Thijssen et al., 2007). In each case, the product volume administered by gavage was 10 mL/kg b.w. A vehicle-control group (Group I) formed by 20 rats consumed distilled water as drinking water during a 90-day period.

2.4.3. Clinical examination, b.w., and feed consumption

All animals were observed once daily for the clinical signs of toxicity and twice daily for mortality. The changes in gait, posture, and response to handling and the presence of clonic or tonic movements, stereotypes (e.g., excessive grooming, repetitive circling), or bizarre behavior (e.g., self-mutilation, walking backward) were also recorded. The ophthalmological examination was performed on all animals before the start and on day 90 of the study. Individual b.w.s were recorded at least two times prior to randomization. The test animals were weighed on day 1 (prior to study) and approximately weekly thereafter (with intervals of 7 ± 1 days). The feed efficiency was calculated and reported. All animals were fasted overnight prior to blood collection.

2.4.4. Clinical pathology

Clinical pathology was performed on all animals for the blood chemistry and hematology of the terminal sacrifice animals once toward the end of the in-life phase of the study. The blood samples for hematology (except coagulation samples) and clinical chemistry were collected via sublingual bleeding under isoflurane anesthesia on Day 86 of the test period. Approximately 0.5 mL was collected in a pre-calibrated tube containing heparin sodium for hematology assessments. The whole blood samples were stored under refrigeration and shipped on cold packs. Approximately 1 mL was collected into a tube containing no preservative for clinical chemistry assessments. These samples were centrifuged in a refrigerated centrifuge, and the serum was transferred to a labeled tube. On the day before the sample collection for the clinical pathology evaluation, the animals were placed in metabolism cages. The animals were fasted after 3 PM (at least 15 h prior to), and urine was collected from each animal.

2.4.4.1. Hematology. The hematological parameters included white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), platelet (PLT) count, and leukocyte differential count (lymphocytes, neutrophils, and monocytes).

2.4.4.2. Clinical chemistry. The clinical chemistry parameters measured included serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea nitrogen (BU), blood creatinine (CRE), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, fasting glucose (GLU), total serum protein (TP), ALB, globulin (GLO), calcium (CALC), and inorganic phosphorus (IPHS).

2.4.4.3. Urinalysis. The urinalysis parameters included quality, pH, ketone, color, glucose, bilirubin, clarity, specific gravity, blood volume, protein, urobilinogen, and microscopic urine sediment examination.

2.4.5. Pathology

2.4.5.1. Terminal necropsy and tissue collection. At termination, all animals were euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia. All animals in this study were subjected to a full necropsy. Kidneys, spleen, brain, liver, ovaries, uterus with oviducts, heart, and testes were weighed wet immediately after dissection to avoid drying. The tissues and organs were procured, preserved in 10% neutral buffered formalin, and processed for histopathological assessment.

2.4.5.2. Histopathological examination. The preserved organs and tissues of the animals from the control and high-dose groups (Groups 1 and 4, respectively) were subjected to histological examination. They were preserved in a fixation medium of 10% solution of buffered formalin (pH 7.4) and enclosed in paraffin-intended subsequent histopathological examination. A 5 μm section of each organ tissue was stained with hematoxylin and eosin. Each section was examined under an optical microscope.

2.4.6. Statistical analysis

Means and standard deviations were calculated for measurement data in each group, which included b.w., food consumption, clinical pathological data, and organ weights. Levene's test of the homogeneity of variance was performed; if the variances were homogeneous, the single factor analysis of variance was performed for inter-group comparison; when ANOVA showed significant differences, Dunnett t test would be performed; when the variances were not homogeneous, non-parameter test would be performed for inter-group comparisons.

3. Results

3.1. Acute toxicity

After treating for 30 min, the rats in the high-dose group moved slowly, chilled, showed extreme sensitivity to noise, and experienced convulsions. The rest of the dose group of poisoning decreased with decreasing amounts and ease. Death necropsy showed substantial liquid filling and swelling in the gastrointestinal tract of rats, including hepatomegaly and pulmonary venous plenum. The particulars of death of mice by the end of the study (Day 14) are shown in Table 1. The LD_{50} value of $Pb(NO_3)_2$ and $CdCl_2$ mixture by the oral route was 2696.54 mg/kg by Bliss method, and the confidence level of 95% was 2162.00 mg/kg to 3362.89 mg/kg. The results of mixture were evaluated through Keplinger evaluation system. The expected LD_{50} was 1849.280 mg/kg, obtained by calculation using the equation of Finney (Finney, 1971); the observed LD_{50} was 2696.54 mg/kg. The ratio of the expected to the observed LD_{50} was 0.68. Keplinger (Keplinger and Deichmann, 1967) reported that the ratios between 0.57 and 1.75 were indicative of a definite additive effect; thus, the acute toxicity oral of Pb with Cd had an additive effect.

3.2. day sub-chronic oral toxicity

3.2.1. Survival, clinical observations, and ophthalmological examination

Neither treatment-related mortality nor obvious clinical signs, including hair loss, scabbing, soft or mucoid feces, decreased defecation or feces smaller than normal, wet yellow material in the urogenital area, or vocalization upon handling, were found in any of the treated groups throughout the experimental period. The animals from all treatment groups did not appear serious diseases at the conclusion of the study period.

The ophthalmoscopy prior to the study initiation and near the experimental completion (Day 90) did not reveal any abnormalities. The confirmed blepharospasm was not associated with any conjunctival hyperemia or chemosis, and no intraocular abnormalities were noted. The blephrarospasm was attributed to the environmental irritation, a common cause of surface ocular irritation in laboratory animal species. These findings were not attributed to the exposure of the test substance; thus, the test article was not considered as an ocular toxicant.

3.2.2. B.w.s and feed consumption

In male rats, the mean weekly b.w.s (Fig. 1) for the treated groups at 29.96, 89.88, and 269.65 mg/(kg bw day) (Groups II to IV, respectively) were comparable with the control group values throughout the study. Compared with the control group, the male rats of Group IV had significantly lower weight gain from Weeks 7 to 14 after the initial dose ($P < 0.05$ or $P < 0.01$). Meanwhile, the b.w.s were significantly lower in Group III ($P < 0.05$ only in weeks 9, 10, and 14) in a dose-dependent manner. No other significant differences were indicated between the treatment and control groups in male rats throughout the experimental period. Similarly, the mean b.w.s for the treated female rats were comparable with the control group values throughout the study (Fig. 2). No significant difference in b.w. gain in female rats was attributable to the administration of Pb and Cd.

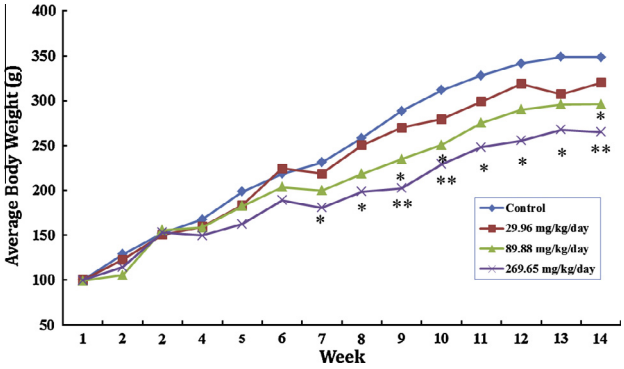

Fig. 1. Effect of lead and cadmium on body weights in male rats. Average body weights for male rats during a 90-day oral (gavage) toxicity study. The values are presented as means ± standard deviation (10 rats/sex/group). Compared with control group, $^{*}P < 0.05$; $^{**}P < 0.01$.

The mean daily feed consumption for the treated male and female rats at 29.96, 89.88, and 269.65 mg/(kg bw day) were comparable with the control group values throughout the study period (data not shown). No changes attributable to the administration of Pb and Cd were observed in the feed consumption or feed

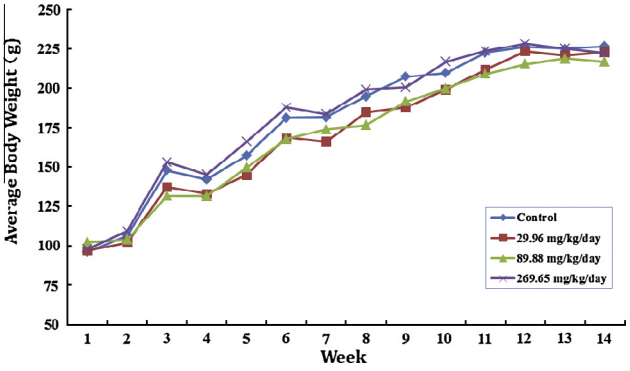

Fig. 2. Effect of lead and cadmium on body weights in female rats. Average body weights for female rats during a 90-day oral (gavage) toxicity study. The values are presented as means ± standard deviation (10 rats/sex/group).

Table 1
The result of combined acute toxicity test of lead and cadmium to rats.

Gruops	Combined dosages (mg/kg)	Dose logarithmic	Rat counts	Death counts	Mortality rate (%)
GruopI	1436(1402 + 34)	3.15 + 1.53	6	0	0
GruopII	1968(1921 + 47)	3.28 + 1.67	6	1	16
GruopIII	2697(2633 + 64)	3.42 + 1.80	6	3	50
GruopIV	3694(3607 + 87)	3.56 + 1.94	6	5	83
GruopV	5062(4942 + 120)	3.69 + 2.08	6	6	100

Combined dosages were equitoxic ratio mixing of lead nitrate and cadmium chloride.

efficiency in both genders. The sporadic statistically significant changes in food consumption and feed efficiency were considered spurious, unassociated with the test article administration.

3.2.3. Clinical pathology

3.2.3.1. Hematology. The hematological changes are shown in Tables 2 and 3. The same treatment-related biologically significant effects of the Pb and Cd treatments were found at dose levels of 29.96, 89.88, and 269.65 mg/(kg bw day) (Groups II to IV, respectively) in the hematological parameters in male and female rats. However, a more statistically significant trend was observed in female dose rats. Compared with the control group, WBC significantly decreased in both genders in Groups III and IV ($P < 0.05$) and showed an increasing trend in Group II but without significant difference. The RBC and PLT significantly decreased in male and female animals in Group IV, while HGB, MCV, MCH, and MCHC had the same changes in Groups III and IV ($P < 0.05$). Among the hematological parameters HCT (%) significantly decreased in all drug-dose group rats. The changes of other hematological parameters, including the absolute lymphocyte and neutrophil counts, were within the same as the WBC physiological range of rats. The results of the hematological parameter analyses suggest that the low-dose combination of Pb and Cd exposure can cause varying degrees of inflammatory injury in rats.

3.2.3.2. Clinical chemistry. The serum chemistry changes are shown in Tables 4 and 5. During the increasing dose levels of Pb and Cd in male rats, ALT, AST, BU, CRE, and TG showed a dose-related increasing trend in different dose groups. By contrast, a dose-related decrease in TP, ALB, GLO, ALB to GLO (A/G) ratio, ALP, GLU, TC, CALC, and IPHS were noted. Compared with the control group, a statistically significant increase in ALT and TG was noted in Group II, who received 29.96 mg/(kg bw day) of Pb and Cd ($P < 0.05$ or $P < 0.01$); a statistically significant increase in ALT, AST, and BU but a decrease in ALP and GLU levels were noted in Groups III and IV, who received 89.88 and 269.65 mg/(kg bw day) of the combined metals, respectively ($P < 0.05$ or $P < 0.01$);a decrease in TC, CALC, and IPHS levels were also observed at Group IV in male rats ($P < 0.05$). For female rats, similar treatment-related increased or decreased trends of Pb and Cd to male rats were found at dose levels of 92.6, 462.9, and 926.0 mg/(kg bw day) (Groups II, III, and IV, respectively) in the serum chemistry parameters. However, compared with the control group, TG significantly increased in Group II ($P < 0.05$); BU significantly increased but TP and IPHS significantly decreased in Groups III and IV ($P < 0.05$ or $P < 0.01$); ALT and AST significantly increased but ALP and CALC significantly decreased in Group IV ($P < 0.05$ or $P < 0.01$). The serum chemistry analysis results show that dose-related effects occurred in the serum chemistry parameters. Statistically significant changes in some biochemical parameters were also found, which might indicate that the hepatic function and renal function of rats had been damaged.

3.2.3.3. Urinalysis. No treatment-related adverse effects were observed in the urinalysis parameters in male and female rats (data not shown) following the administration of Pb and Cd at dose levels of 29.96, 89.88, and 269.65 mg/(kg bw day) (Groups II, III, and IV, respectively). The urine volume, pH, and specific gravity were almost within normal limits. Only in the high-dose group, the incidences of positive urine occult blood and urine pH $\geqslant$ 9.0 increased. Some statistically significant differences from the control

Table 2
Effect of lead and cadmium on hematological parameters in male rats.

Parameter	Units	mg/kg/day			
		(G-I) 0	(G-II) 29.96	(G-III) 89.88	(G-IV) 269.65
WBC	10^{-9}/L	5.98 ± 0.49	7.23 ± 0.39	12.30 ± 1.01*	15.55 ± 1.72*
RBC	10^{-12}/L	8.61 ± 0.38	8.10 ± 0.16	7.91 ± 0.21	7.13 ± 0.83*
HGB	g/L	157.67 ± 7.23	151.67 ± 3.51	146.25 ± 2.51*	135.46 ± 6.03*
HCT	%	48.33 ± 0.71	46.13 ± 1.01*	45.60 ± 0.89*	43.83 ± 1.00*
MCV	fL	64.27 ± 0.84	60.20 ± 1.54	57.27 ± 1.40*	52.87 ± 4.48*
MCH	pg	22.13 ± 1.65	20.40 ± 1.20	19.20 ± 0.78*	17.70 ± 1.11*
MCHC	g/L	344.42 ± 9.50	337.67 ± 8.50	325.32 ± 11.50*	313.26 ± 7.77*
PLT	10^{-9}/L	984.27 ± 91.80	933.36 ± 70.76	871.56 ± 35.64	775.32 ± 59.63*

The values are presented as means ± standard deviation (10 rats/sex/group). WBC = white blood cell count; RBC = red blood cell count; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelet count. Compared with control group, *$P < 0.05$. Parameters that were not influenced by lead and cadmium during dosing are not listed.

Table 3
Effect of lead and cadmium on hematological parameters in female rats.

Parameter	Units	mg/kg/day			
		(G-I) 0	(G-II) 29.96	(G-III) 89.88	(G-IV) 269.65
WBC	10^{-9}/L	5.80 ± 0.78	7.93 ± 0.54	13.21 ± 1.32*	16.55 ± 1.88*
RBC	10^{-12}/L	8.34 ± 0.19	8.01 ± 0.26	7.88 ± 0.24	7.01 ± 0.44*
HGB	g/L	155.67 ± 6.31	148.33 ± 4.51	143.75 ± 3.41*	132.46 ± 6.22*
HCT	%	45.33 ± 0.71	42.13 ± 2.01*	41.60 ± 1.89*	39.42 ± 1.30*
MCV	fL	63.27 ± 1.84	58.60 ± 2.54	55.27 ± 2.48*	50.87 ± 4.47*
MCH	pg	18.25 ± 2.65	15.48 ± 1.28	14.20 ± 0.78*	11.78 ± 0.61*
MCHC	g/L	350.42 ± 9.88	341.67 ± 8.61	329.72 ± 10.50*	317.29 ± 7.65*
PLT	10^{-9}/L	864.27 ± 81.80	843.38 ± 65.76	751.56 ± 44.64	673.32 ± 79.63*

The values are presented as means ± standard deviation (10 rats/sex/group). WBC = white blood cell count; RBC = red blood cell count; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelet count. Compared with control group, *$P < 0.05$. Parameters that were not influenced by lead and cadmium during dosing are not listed.

Table 4
Effect of lead and cadmium on clinical chemistry parameters in male rats.

Parameter	Units	mg/kg/day			
		(G-I) 0	(G-II) 29.96	(G-III) 89.88	(G-IV) 269.65
TP	g/L	70.37 ± 5.35	69.17 ± 6.38	68.40 ± 5.51	67.07 ± 4.41
ALB	g/L	43.13 ± 3.61	42.50 ± 4.98	41.30 ± 5.29	41.30 ± 2.30
GLO	g/L	27.87 ± 2.89	27.86 ± 4.12	27.10 ± 0.70	23.94 ± 1.25
A/G		1.80 ± 0.12	1.54 ± 0.26	1.52 ± 0.18	1.49 ± 0.15
ALT	U/L	40.17 ± 3.27	50.43 ± 2.54*	63.87 ± 3.67**	101.67 ± 7.64**
AST	U/L	122.00 ± 12.17	153.33 ± 25.17	182.67 ± 14.50**	221.33 ± 15.63**
ALP	U/L	188.66 ± 7.76	169.00 ± 18.52	140.33 ± 2.51**	121.33 ± 9.07**
BU	mmol/L	4.82 ± 0.45	4.98 ± 0.17	5.26 ± 0.61*	6.17 ± 1.64*
CRE	μmol/L	31.33 ± 2.52	33.67 ± 2.08	34.00 ± 1.00	33.33 ± 6.81
GLU	mmol/L	7.06 ± 0.63	6.68 ± 0.27	6.08 ± 0.42*	6.03 ± 0.63*
TC	mmol/L	1.70 ± 0.43	1.34 ± 0.20	1.35 ± 0.08	1.19 ± 0.08*
TG	mmol/L	0.44 ± 0.07	1.45 ± 0.27**	0.74 ± 0.08	0.52 ± 0.10
CALC	μg/mL	26.65 ± 1.13	24.85 ± 1.53	22.90 ± 1.04	21.85 ± 1.18*
IPHS	μg/mL	430.50 ± 10.10	416.09 ± 11.55	411.34 ± 12.70	385.98 ± 9.24*

The values are presented as means ± standard deviation (10 rats/sex/group). Total serum protein (TP), albumin (ALB), globulin (GLO), albumin/globulin ratio(A/G), serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea nitrogen (BU), blood creatinine (CRE), fasting glucose (GLU), total cholesterol (TC), triglycerides (TG), calcium (CALC), inorganic phosphorus (IPHS). Compared with control group, $^{*}P < 0.05$, $^{**}P < 0.01$. Parameters that were not influenced by lead and cadmium during dosing.

Table 5
Effect of lead and cadmium on clinical chemistry parameters in female rats.

Parameter	Units	mg/kg/day			
		(G-I) 0	(G-II) 29.96	(G-III) 89.88	(G-IV) 269.65
TP	g/L	73.33 ± 4.87	70.70 ± 1.83	68.13 ± 1.76*	63.90 ± 3.99*
ALB	g/L	42.53 ± 1.59	42.26 ± 4.27	38.77 ± 5.53	38.03 ± 5.78
GLO	g/L	32.67 ± 5.35	31.07 ± 3.55	25.93 ± 6.64	25.60 ± 1.15
A/G		1.67 ± 0.11	1.63 ± 0.55	1.38 ± 0.22	1.20 ± 0.34
ALT	U/L	43.30 ± 9.11	45.50 ± 8.43	63.77 ± 4.29	116.07 ± 25.06**
AST	U/L	137.00 ± 12.00	163.00 ± 14.93	193.67 ± 31.66	571.33 ± 72.29**
ALP	U/L	236.33 ± 20.79	181.33 ± 45.35	156.67 ± 24.85	140.33 ± 15.14**
BU	mmol/L	4.63 ± 0.74	4.97 ± 0.23	6.33 ± 0.58**	7.36 ± 0.64**
CRE	μmol/L	27.33 ± 2.89	28.00 ± 4.00	30.00 ± 3.61	32.00 ± 1.73
GLU	mmol/L	6.52 ± 0.88	6.88 ± 0.50	5.04 ± 2.55	2.45 ± 0.67**
TC	mmol/L	1.92 ± 0.21	1.74 ± 0.16	1.67 ± 0.21	1.43 ± 0.90**
TG	mmol/L	0.69 ± 0.21	1.48 ± 0.63*	1.03 ± 0.17	0.95 ± 0.12
CALC	μg/mL	27.85 ± 1.13	25.85 ± 1.70	24.10 ± 1.10	22.90 ± 1.15*
IPHS	μg/mL	379.00 ± 9.81	361.50 ± 7.79	320.00 ± 8.08*	227.50 ± 9.76**

The values are presented as means ± standard deviation (10 rats/sex/group). Total serum protein (TP), albumin (ALB), globulin (GLO), albumin/globulin ratio(A/G), serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea nitrogen (BU), blood creatinine (CRE), fasting glucose (GLU), total cholesterol (TC), triglycerides (TG), calcium (CALC), inorganic phosphorus (IPHS). Compared with control group, $^{*}P < 0.05$, $^{**}P < 0.01$. Parameters that were not influenced by lead and cadmium during dosing are not listed.

were not in a dose-related manner and were considered to be incidental and unrelated to the treatment.

3.2.4. Organic coefficient

The changes noted in the organic coefficient (g/100 g) between the groups following the treatment with Pb and Cd at dose levels of 29.96, 89.88, and 269.65 mg/(kg bw day) for 90 days are summarized in Table 6. No toxicologically significant changes in organ weights were observed in the groups compared with those of the vehicle control group. However, Group IV female relative kidney-to-b.w. ratio statistically increased. This increase in kidney weight was considered as incidental and toxicologically insignificant because of the lack of dose-dependency and correlating changes in the clinical chemistry and histopathology.

3.2.5. Histopathological analysis

The incidence and severity of the histopathological findings following the treatment with Pb and Cd at dose levels of 29.96, 89.88, and 269.65 mg/(kg bw day) for 90 days had varying degrees of damage on liver, kidneys, and testicle. The consistent treatment-related histopathological changes were found in both sexes.

In the liver of the control group (Group I), the cross-section showed the normal appearance of liver, central vein, sinusoids, and hepatocytes in a clearly conserved form (Fig. 3A). Group II showed the rat hepatic lobule central venous, interlobular vein and liver blood sinus congestion, and neutrophilic granulocyte infiltration in hepatocytes. Granular degeneration was also observed in hepatocytes, and a part of the cell nucleus appeared nuclear enrichment, i.e., serious karyolysis, only remaining nuclear membrane (Fig. 3B). Group III showed hepatic lobule losed funicular structures and appeared focal necrosis (Fig. 3C). The most serious tissue lesions a large number of vesicular degeneration of liver cells, cell death, nuclei concentration, fracture, and dissolved were all discovered in Group IV.

In Group I, the cross section showed that the appearances of kidney, glomerulus, and renal tubule structure were normal (Fig. 4A). Group II showed capillary of glomerulus and interstitial angiectasis hyperemia, renal tubular epithelial cell (RTEC) swelling, and granular degeneration, and some of them were separated from

Table 6
Effect of lead and cadmium on terminal body weight and organic coefficient (g/100 g) in grams of male and female rats.

Organ	sex	(G-I) 0 mg/kg/day	(G-II) 29.96 mg/kg/day	(G-III) 89.88 mg/kg/day	(G-IV) 269.65 mg/kg/day
		Body weight in grams, organic coefficient in g/100 g			
Body weight	M	304.1 ± 33.9	290.8 ± 22.6	299.4 ± 26.4	288.6 ± 21.8
Brain	M	0.60 ± 0.05	0.63 ± 0.04	0.62 ± 0.08	0.61 ± 0.08
Heart	M	0.29 ± 0.01	0.31 ± 0.02	0.31 ± 0.02	0.28 ± 0.05
Liver	M	2.75 ± 0.21	2.90 ± 0.02	2.81 ± 0.28	2.85 ± 0.49
Spleen	M	0.18 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.14 ± 0.02
Lung	M	0.55 ± 0.07	0.59 ± 0.07	0.61 ± 0.13	0.53 ± 0.09
Kidney	M	0.61 ± 0.07	0.68 ± 0.06	0.63 ± 0.03	0.69 ± 0.07
Testes	M	1.14 ± 0.36	1.09 ± 0.12	1.02 ± 0.11	1.3 ± 0.28
Body weight	F	236.3 ± 18.4	248.5 ± 20.5	242.3 ± 15.6	244.5 ± 16.3
Brain	F	0.78 ± 0.03	0.71 ± 0.08	0.75 ± 0.01	0.73 ± 0.04
Heart	F	0.31 ± 0.04	0.32 ± 0.07	0.30 ± 0.03	0.29 ± 0.01
Liver	F	3.35 ± 0.21	2.90 ± 0.28	2.85 ± 0.35	3.05 ± 0.07
Spleen	F	0.22 ± 0.06	0.19 ± 0.02	0.19 ± 0.04	0.22 ± 0.03
Lung	F	0.73 ± 0.10	0.73 ± 0.05	0.74 ± 0.13	0.76 ± 0.01
Kidney	F	0.59 ± 0.01	0.57 ± 0.08	0.60 ± 0.04	**0.73 ± 0.05***
Uterus and oviducts	F	0.65 ± 0.03	0.64 ± 0.17	0.59 ± 0.04	0.61 ± 0.03

The values are presented as means ± standard deviation (10 rats/sex/group). Compared with control group, $^{*}P < 0.05$.

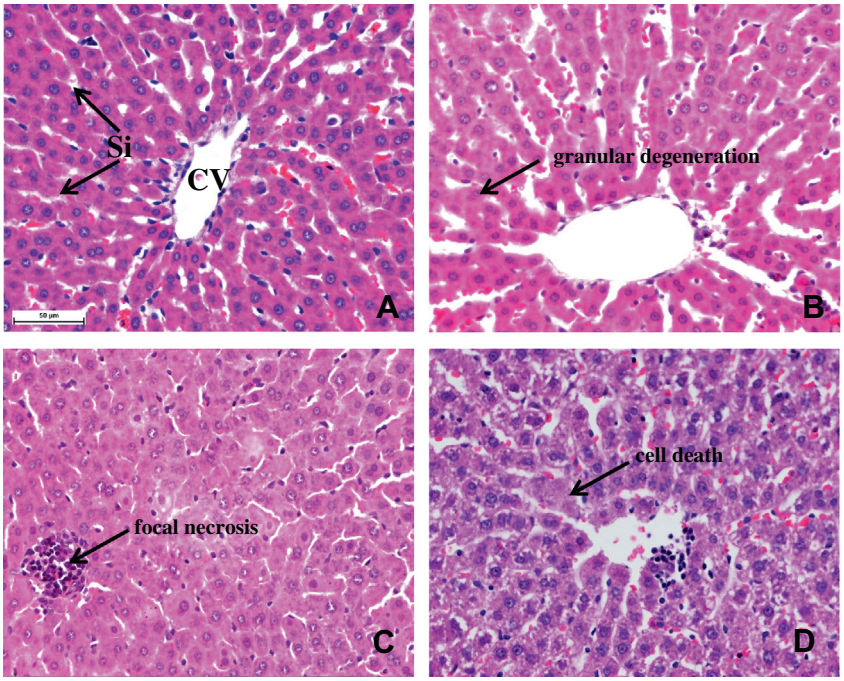

Fig. 3. Effect of lead and cadmium on the microstructures of liver of rats after administration for 90 days. Panel A: Group I (0 mg/kg, HE 400×); Panel B: Group II (29.96 mg/kg, HE 400×); Panel C: Group III (89.88 mg/kg, HE 400×); Panel D: Group IV (269.65 mg/kg, HE 400×). CV: central vein; Si: sinusoids.

the basement membrane (Fig. 4B). Group III showed RTECs in obvious hydatid degeneration, with nuclei concentration, fracture, and dissolved. Some pathologic nuclear fission also appeared in RTEC (Fig. 4C). Group IV showed massive inflammatory cells, especially neutrophilic granulocyte infiltrated in the glomeruli and nephric tubules, glomeruli swelling, nephric tubules obstruction, RTEC degeneration, and necrosis, segregated with the basilar membrane. The renal tubule revealed protein cast (Fig. 4D).

The cross section of Group I also showed a normal appearance of testicle; the seminiferous tubule (ST) structure was intact, and the spermatogenic cells (SCs) at all levels were arranged in order and spermatozoon (Fig. 5A). Group II showed that the number of spermatid cells was reduced, and the SCs of STs had owned part of vesicular degeneration (Fig. 5B). In Groups III and IV, the basic structure of ST was damaged, SCs were seriously dissolved and disappeared, and the sperms within the seminiferous lumen almost completely disappeared (Fig. 5C and D).

4. Discussion

Considering the wide distribution in potential health risk and the lack of co-exposure toxicological properties, the toxicological profiles of combined Pb and Cd must be clarified. In this study, we present a comprehensive toxicological evaluation on Pb and Cd co-exposure by performing acute and 90 day sub-chronic oral toxicity studies in SD rats.

In the acute toxicity study, the exposure of rats in Pb and Cd had an additive effect based on Keplinger evaluation system. The acute combined toxicity (LD_{50}) test provided information on the range of

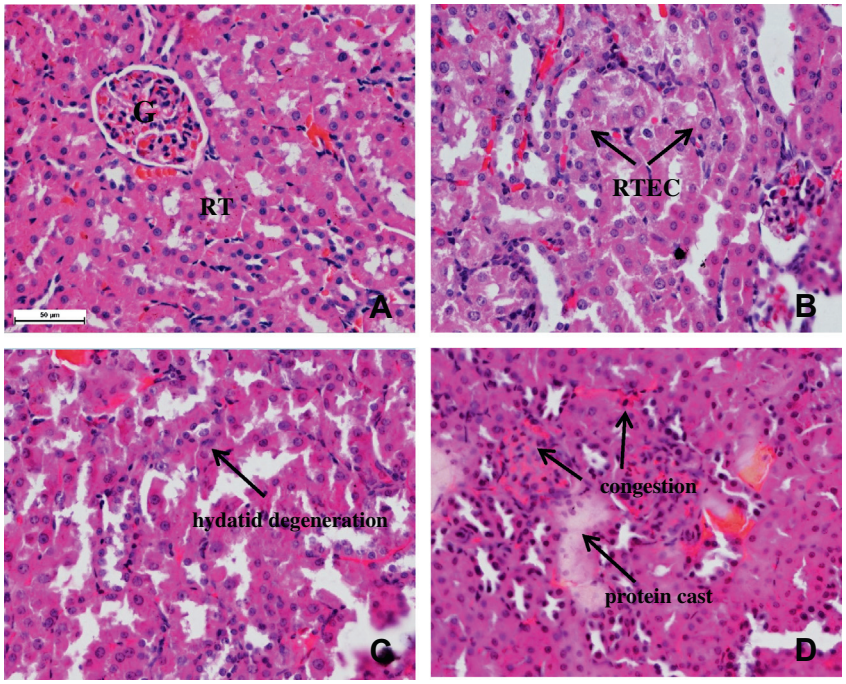

Fig. 4. Effect of lead and cadmium on the microstructures of kidney of rats after administration for 90 days. Panel A: Group I (0 mg/kg, HE 400×); Panel B: Group II (29.96 mg/kg, HE 400×); Panel C: Group III (89.88 mg/kg, HE 400×); Panel D: Group IV (269.65 mg/kg, HE 400×). G: glomerulus; RT: renal tubule; RTEC: renal tubular epithelial cells.

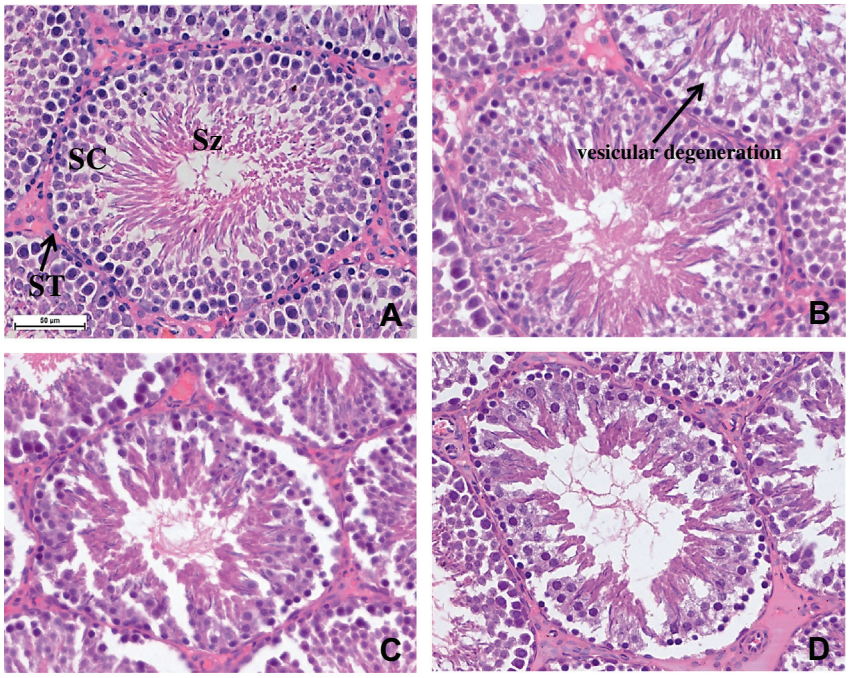

Fig. 5. Effect of lead and cadmium on the microstructures of testicle of rats after administration for 90 days. Panel A: Group I (0 mg/kg, HE 400×); Panel B: Group II (29.96 mg/kg, HE 400×); Panel C: Group III (89.88 mg/kg, HE 400×); Panel D: Group IV (269.65 mg/kg, HE 400×). ST: seminiferous tubules; SC: spermatogenic cells; Sz: spermatozoon.

doses that could be used in subsequent toxicity testing and estimating the therapeutic index of xenobiotics (Aniagu et al., 2005).

To predict the hazard of long term, low-dose exposure to a particular mixture compound, sub-chronic toxicity studies are almost always invaluable in evaluating the safety profile of xenobiotics (Aniagu et al., 2005). The 90 day sub-chronic co-exposure to Pb and Cd test showed that the target organ toxicity and the pathological damages of rats were liver, kidney, and testis.

In our study, no obvious influences on the general condition and appearance of the animals, feed consumption, clinical observations, and ophthalmoscopy were noted, which suggested that low-dose Pb and Cd had no apparent toxic effects on the vision and function of most organs. All examined organ coefficients had also no statistical difference compared with those of the control group. However, a significant effect of retardations of b.w. gain was found in the mid and later stages of male dosing (Groups III and IV).

Hematopoietic system is one of the most sensitive parameters to assess the toxicity of drugs in humans and animals (Liju et al., 2013). This study indicated that the low-dose combination of Pb and Cd exposure can cause varying degrees of inflammatory injury in rats (Tables 2 and 3). The decrease in MCV, MCH, and MCHC indicates that the low-dose combination of Pb and Cd exposure can cause microcytic hypochromic anemia of rats (Lodia and Kansala, 2012; Sharma et al., 2011). The heavy metal accumulation in kidney, spleen, and liver might suppress the activity of these hematopoietic tissues; this idea was supported by the study of Gill and Epple (Gill and Epple, 1993). This condition might lead to anemia that impairs erythropoiesis caused by a direct effect of metals on hematopoietic centers (kidney/spleen), accelerated erythroclasia because of the altered membrane permeability and/or increased mechanical fragility, and defective Fe metabolism or failure of intestinal uptake of Fe because of mucosal lesions.

Liver is the main site of the synthesis of plasma proteins, especially ALB. Changes in serum total proteins or the ratio of A/G may indicate hepatic dysfunction. These changes in plasma proteins usually do not appear, except in chronic or severe liver dysfunction that may be evidenced by a decrease in ALB and an increase in GLO concentration. Changes were observed in TP, ALB, GLO levels, and A/G ratio, which suggest that the combination of Pb and Cd may induce liver damage, and the synthetic function of liver may be more adversely affected with prolonged exposure to rats.

Any damage to liver results in the elevations of both ALT and AST in blood. The liver enzymes AST, ALT, and ALP are cellular enzymes that are present in low concentrations of serum under normal conditions. ALT found in serum is considered as the first sign of cell and liver damages (Liju et al., 2013; Mukinda and Eagles, 2010). CRE is known as a good indicator of renal function, i.e., rises in CRE means an obvious damage to functional nephrons (Liju et al., 2013). Significant differences in ALT, AST, ALP, CRE, and BU were found in treated animals compared with the control group. These results suggest that Pb and Cd could alter the renal and hepatic function. The significant decrease of ALP activity indicates that high-dose Cd can reinforce competitive inhibition with Zn, and ALP has the strong dependence of enzyme to Zn. Hence, the low-dose combination of Pb and Cd exposure can cause more hepatotoxicity. Moreover, positive urine occult blood and higher incidence of urine pH $\geqslant$ 9.0 indicate a portentous injury in kidney, which is correlated with the oral administration of Pb and Cd.

Our data show a trend of decrease in TC in all test groups compared with the control rats. By contrast, all test groups showed increase trend in TG compared with the control rats. Previous studies have shown that single heavy metal poison has a decrease trend in TG for animal bodies (Robinson and Tuovinen, 1984; Vallee and Ulmer, 1972). On one hand, reduced TC concentrations cause acute liver necrosis and hardening, severe anemia, malnutrition, and other diseases. On the other hand, elevated TG concentrations cause hyperlipidemia, diabetes, kidney disease syndrome, and liver dirty disease. The decrease in TC and the increase in TG are in agreement with the findings of the above hematopoietic and biochemical parameters, in which blood, hepatotoxicity, and nephrotoxicity have been proved. However, the mechanisms by which Pb and Cd supplementation increases blood TG need further investigations. The decrease of CALC and IPHS was affected by bone injury. As an important target tissue, bone is the largest repository of Pb and Cd in body. Previous studies have shown that the low level of Pb or Cd exposure can affect Ca and P metabolism and decreases blood Ca and P (Dongre et al., 2013; Kazantzis, 2004).

The characteristic histopathological findings in the present study included mainly granular and vacuolar degeneration in the liver, kidney, and testicle of rats. Necrocytosis and pathological mitotic figure were observed in the liver and kidney of Groups III and IV, which indicates that low-dose Pb and Cd can lead to body carcinogenic (mutagenesis, carcinogenesis, teratogenesis). These findings are considered to be treatment-related adverse effects, because they are well consistent with the hematology and biochemical findings.

5. Conclusion

The LD_{50} value of $Pb(NO_3)_2$ and $CdCl_2$ mixture by the oral route was 2696.54 mg/kg. Pb and Cd co-exposure had an additive effect in the rats in the acute toxicity study. The sub-chronic oral toxicity study, in which a combined experimental animal model was created, showed that the main target organs of the toxic effects were blood, liver, kidneys, and testicle. Meanwhile, the changes of hematological and blood biochemical indicators were more sensitive in female animals. The lowest observed adverse-effect level of Pb and Cd co-exposure is proposed to be <29.96 mg/(kg bw day) following daily oral administrations to SD rats for 90 days. This animal model may help in detecting the early events of chronic Pb/Cd intoxication with relatively low and environmentally realistic concentrations.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency document associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fct.2013.12.041.

Acknowledgements

This study was supported by the National Nature Science Foundation of China (NO. 31101860) and the Sichuan Youth Science and Technology Innovation Research Team for waterfowl disease prevention and control (NO. 2013TD0015).

References

Åkesson, A., 2011. Cadmium Exposure in the Environment: Renal Effects and the Benchmark Dose, In: Jerome, O.N. Editor-in-Chief (Ed.), Encyclopedia of Environmental Health. Elsevier, Burlington, pp. 465–473.

Aniagu, S.O., Nwinyi, F.C., Akumka, D.D., Ajoku, G.A., Dzarma, S., Izebe, K.S., Ditse, M., Nwaneri, P., Wambebe, C., Gamaniel, K., 2005. Toxicity studies in rats fed nature cure bitters. Afr. J. Biotechnol. 4, 72–78.

Antonio Garcia, T., Corredor, L., 2004. Biochemical changes in the kidneys after perinatal intoxication with lead and/or cadmium and their antagonistic effects when coadministered. Ecotox. Environ. Safe. 57, 184–189.

Baba, H., Tsuneyama, K., Yazaki, M., Nagata, K., Minamisaka, T., Tsuda, T., Nomoto, K., Hayashi, S., Miwa, S., Nakajima, T., Nakanishi, Y., Aoshima, K., Imura, J., 2013. The liver in itai–itai disease (chronic cadmium poisoning): pathological features and metallothionein expression. Mod. Pathol. 26, 1228–1234.

Bliss, C., 1939. The toxicity of poisons applied jointly. Ann. Appl. Biol. 26, 585–615.

CDC, 2008. Chemicals—Test Method of Repeated Dose Oral Toxicity Study in Rodents. General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (AQSIQ).

Chen, X., Wang, G., Li, X., Gan, C., Zhu, G., Jin, T., Wang, Z., 2013. Environmental level of cadmium exposure stimulates osteoclasts formation in male rats. Food. Chem. Toxicol. 60, 530–535.

Dai, S., Yin, Z., Yuan, G., Lu, H., Jia, R., Xu, J., Song, X., Li, L., Shu, Y., Liang, X., He, C., Lv, C., Zhang, W., 2013. Quantification of metallothionein on the liver and kidney of rats by subchronic lead and cadmium in combination. Environ. Toxicol. Pharm. 36, 1207–1216.

Dongre, N.N., Suryakar, A.N., Patil, A.J., Hundekari, I.A., Devarnavadagi, B.B., 2013. Biochemical effects of lead exposure on battery manufacture workers with reference to blood pressure, calcium metabolism and bone mineral density. Indian J. Clin. Biochem. 28, 65–70.

Fda, U., 2000. Chapter IV. C. 4. a. Subchronic Toxicity Studies with Rodents. Toxicological Principles for the Safety Assessment of Food Ingredients. Redbook 2000.

Finney, D.J., 1971. Probit Analysis, third ed. Cambridge University Press.

Gill, T.S., Epple, A., 1993. Stress-related changes in the hematological profile of the American Eel Anguilla rostrata. Ecotox. Environ. Safe. 25, 227–235.

Goyer, R.A., 1997. Toxic and essential metal interactions. Annu. Rev. Nutr. 17, 37–50.
Hambach, R., Lison, D., D'Haese, P., Weyler, J., François, G., De Schryver, A., Manuel-Y-Keenoy, B., Van Soom, U., Caeyers, T., van Sprundel, M., 2013a. Adverse effects of low occupational cadmium exposure on renal and oxidative stress biomarkers in solderers. Occup. Environ. Med. 70, 108–113.
Hambach, R., Lison, D., D'Haese, P.C., Weyler, J., De Graef, E., De Schryver, A., Lamberts, L.V., van Sprundel, M., 2013b. Co-exposure to lead increases the renal response to low levels of cadmium in metallurgy workers. Toxicol. Lett. 222, 233–238.
Järup, L., 2003. Hazards of heavy metal contamination. Brit. Med. Bull. 68, 167–182.
Johri, N., Jacquillet, G., Unwin, R., 2010. Heavy metal poisoning: the effects of cadmium on the kidney. Biometals 23, 783–792.
Joseph, P., 2009. Mechanisms of cadmium carcinogenesis. Toxicol. Appl. Pharm. 238, 272–279.
Jung, H., Choi, S.C., 1994. Sequential method of estimating the LD_{50} using a modified up-and-down rule. J. Biopharm. Stat. 4, 19–30.
Kang, M.-Y., Cho, S.-H., Lim, Y.-H., Seo, J.-C., Hong, Y.-C., 2013. Effects of environmental cadmium exposure on liver function in adults. Occup. Environ. Med. 70, 268–273.
Karavoltsos, S., Sakellari, A., Dassenakis, M., Scoullos, M., 2008. Cadmium and lead in organically produced foodstuffs from the Greek market. Food. Chem. 106, 843–851.
Kazantzis, G., 2004. Cadmium, osteoporosis and calcium metabolism. Biometals 17, 493–498.
Keplinger, M.L., Deichmann, W.B., 1967. Acute toxicity of combinations of pesticides. Toxicol. Appl. Pharm. 10, 586–595.
Liju, V.B., Jeena, K., Kuttan, R., 2013. Acute and subchronic toxicity as well as mutagenic evaluation of essential oil from turmeric (*Curcuma longa* L). Food. Chem. Toxicol. 53, 52–61.
Lodia, S., Kansala, L., 2012. Antioxidant activity of rubia cordifolia against lead toxicity. Int. J. Pharmacol. Sci. Res 3, 2224–2232.
Lu, H., Dai, S., Yin, Z., Yuan, G., Wang, C., 2012. Study on damage of bone in rat induced by experimental acute combined exposure to lead and cadmium. Chin. Vet. Sci., 1278–1282.
Mol, S., 2011. Levels of heavy metals in canned bonito, sardines, and mackerel produced in Turkey. Biol. Trace Elem. Res. 143, 974–982.
Mukinda, J.T., Eagles, P.F., 2010. Acute and sub-chronic oral toxicity profiles of the aqueous extract of Polygala fruticosa in female mice and rats. J. Ethnopharmacol. 128, 236–240.
OECD, 1998. Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents. OECD Publishing.
OECD, 2008. Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. OECD Publishing.
Rahimi, E., 2013. Lead and cadmium concentrations in goat, cow, sheep, and buffalo milks from different regions of Iran. Food. Chem. 136, 389–391.
Rispin, A., Farrar, D., Margosches, E., Gupta, K., Stitzel, K., Carr, G., Greene, M., Meyer, W., McCall, D., 2002. Alternative methods for the median lethal dose (LD50) test: The up-and-down procedure for acute oral toxicity. ILAR J. 43, 233–243.
Robinson, J.B., Tuovinen, O., 1984. Mechanisms of microbial resistance and detoxification of mercury and organomercury compounds: physiological, biochemical, and genetic analyses. Microbiol. Rev. 48, 95.
Sabath, E., Robles-Osorio, M.L., 2012. Renal health and the environment: heavy metal nephrotoxicity. Nefrologia 32, 279–286.
Schnaas, L., Rothenberg, S.J., Flores, M.-F., Martinez, S., Hernandez, C., Osorio, E., Velasco, S.R., Perroni, E., 2006. Reduced intellectual development in children with prenatal lead exposure. Environ. Health Persp. 114, 791.
Sharma, V., Kansal, L., Sharma, A., Lodi, S., Sharma, S., 2011. Ameliorating effect of Coriandrum sativum extracts on hematological and immunological variables in an animal model of lead intoxication. J. Pharm. Allied Health Sci. 1, 16–29.
Sinha, M., Manna, P., Sil, P.C., 2008. Cadmium-induced neurological disorders: prophylactic role of taurine. J. Appl. Toxicol. 28, 974–986.
Smyth Jr., H.F., Weil, C.S., West, J.S., Carpenter, C.P., 1970. An exploration of joint toxic action: II. Equitoxic versus equivolume mixtures. Toxicol. Appl. Pharm. 17, 498–503.
Spurgeon, D.J., Jones, O.A., Dorne, J.L., Svendsen, C., Swain, S., Sturzenbaum, S.R., 2010. Systems toxicology approaches for understanding the joint effects of environmental chemical mixtures. Sci. Total Environ. 408, 3725–3734.
Teuschler, L.K., Hertzberg, R.C., 1995. Current and future risk assessment guidelines, policy, and methods development for chemical mixtures. Toxicology 105, 137–144.
Thijssen, S., Maringwa, J., Faes, C., Lambrichts, I., Van Kerkhove, E., 2007. Chronic exposure of mice to environmentally relevant, low doses of cadmium leads to early renal damage, not predicted by blood or urine cadmium levels. Toxicology 229, 145–156.
Vallee, B.L., Ulmer, D.D., 1972. Biochemical effects of mercury, cadmium, and lead. Annu. Rev. Biochem. 41, 91–128.
Wang, G., Fowler, B.A., 2008. Roles of biomarkers in evaluating interactions among mixtures of lead, cadmium and arsenic. Toxicol. Appl. Pharm. 233, 92–99.