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Risk assessment on renal dysfunction caused by co-exposure to arsenic and cadmium using benchmark dose calculation in a Chinese population

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Abstract

Arsenic and cadmium are important inorganic toxicants in the environment. Humans certainly have the potential to be exposed to the mixtures of arsenic and cadmium, but the toxicological interactions of these inorganic mixtures are poorly defined. A general population co-exposed to arsenic and cadmium, was selected in China. The total number of participants was 245, made up of 122 in the arsenic-cadmium polluted area, 123 in the non polluted area. Urinary arsenic (UAs) and cadmium (UCd) were determined by atomic absorption spectrometry as exposure biomarkers and β_2-microglobulin (Uβ_2MG), albumin (UALB), N-acetyl-β_2-glucosaminidase (UNAG) in urine were determined as effect biomarkers. The benchmark dose (BMD) and the lower confidence limit on the benchmark dose (LBMD) were calculated to estimate the critical concentration of UAs and UCd. UAs and UCd concentrations in the polluted area were significantly higher than those in the non polluted area ($P < 0.01$). The levels of Uβ_2MG, UALB and UNAG in the polluted area were significantly higher than those in the non polluted area ($P < 0.01$). The BMD/LBMD of UAs and UCd for a 10% level of risk above the background level were estimated as 121.91/102.11 μg/g creatinine and 1.05/0.88 μg/g creatinine. It was suggested that the lower confidence limit of population critical concentration of UAs and UCd for renal dysfunction for 10% excess risk level above the background, which is obtained from LBMD, may need to be kept below 102 and 0.88 μg/g creatinine in order to prevent renal damage in general population co-exposed to arsenic and cadmium. It is indicated that combined effect of arsenic and cadmium were additive effect and/or synergistic effect, and cadmium may potentiate arsenic nephrotoxicity during the long-term and co-exposure to arsenic and cadmium in humans.

Introduction

Toxic consequences following the exposure of inorganic elements such as arsenic or cadmium have been extensively studied both in experimental animals and humans. Arsenic and cadmium are the top two metals in site frequency counted by the ATSDR Completed Exposure Pathway Site Count Report (ATSDR 2000). In addition, as confirmed by ATSDR using the HazDat database, these metals most often occur together; they are present in 5 of 10 of the top 10 Binary Combinations of Contaminants in soil and water, respectively (Fay & Mumtaz 1996). The kidney is a well known target organ of cadmium in occupationally or environmentally exposed populations and in animals. Evidence of renal toxicity of arsenic (kidney cancer excepted) is limited to animal models using relatively high doses. It has recently been observed in mice that, while chronic exposure to cadmium produces more renal toxicity than arsenic, the combination of cadmium and arsenic produces even more renal injury than caused by either of the chemicals given alone (Liu *et al.* 2000).

Nearly 2 million Chinese are in the risk of arsenism. Only in Guizhou province, China, where the arsenism by burning coal containing arsenic, arsenic concentrations reach about 100–8300 mg/kg in coal, 0.22 mg/m^3 in air, 1096.60 mg/kg in capsicum and 11.30 mg/kg in corn because of burning coal contain

arsenic. But arsenic concentration in drinking water is less than 50 μg/l (Zhang *et al.* 2000). The peoples lived in these areas took fishes and shrimps in very few occasion.

The protection of human health from environmental toxicants typically relies on setting criteria for the exposure to chemicals as single agents. However, people are exposed to a myriad of chemicals, including both organic and inorganic agents. Examination of potential toxicant interactions is an important, but often difficult, aspect of toxicology. It is clear that with inorganics, exposure to multiple agents is the rule rather than the exception (Snow 1992). Arsenic and cadmium co-exposure, for instance, frequently occurs in a variety of settings (IARC 1993). These inorganics are frequently found as co-pollutants in our environment (Diaz-Barriga *et al.* 1993). Arsenic and cadmium are also by-products obtained from processing other metals, leading to common exposure in industrial settings (IARC 1993). Thus, there is a clear potential for simultaneous or sequential exposure to arsenic and cadmium in general populations, either in the environment or from the workplace. Interactions between arsenic and cadmium in acute liver injury have been reported (Hochadel & Waalkes 1997), but little is known about their potential interaction in renal dysfunction in human populations, especially during chronic exposure. Therefore, the primary goal of the present study was to characterise the interaction of arsenic and cadmium in producing renal dysfunction and to assess the critical concentration of UAs and UCd for renal dysfunction in general population coexposure to arsenic and cadmium in China.

Materials and methods

Study population

The population was selected from people living in arsenic-polluted dominating areas where burning coal contain arsenic, arsenic concentrations reach about 100–8300 mg/kg in coal, 15.70 mg/kg in soil, Guizhou province in China. As a result, it was shown that the average arsenic content of ambient air was 0.022 mg/m^3, 1.82 mg/kg in rice, 11.30 mg/kg in corn and 1096.60 mg/kg in capsicum because of burning coal contain arsenic. Also, cadmium concentrations were 0.02, 0.06, 0.11, 0.17 and 2.11 mg/kg in corn, capsicum, soil, rice and coal, respectively. And arsenic and cadmium level in the tobacco is 0.39 mg/kg and 2.00 mg/kg, respectively. A non arsenic-cadmium polluted area also selected. All participants were randomly selected from people living in both area matched with age and sex. The total number of participants was 245, made up of 122 in the polluted area, 123 in the control area. No statistically significant differences were observed in the two areas in the mode of life, and social and economic status. Subjects were asked to answer a detailed questionnaire by trained and supervised interviewers, and to provide urine for biological measurements.

The study was carried out with the permission of the local authority and the ethics committee of Shanghai Medical University or Fudan University and with the informed consent of each participating individual. The participation was on a completely voluntary basis.

Urine collection and analytical method

Urine samples were collected from all participants, and were kept frozen at -20 °C until analysis. Each urine sample was divided into three parts immediately after collection. Of these, a part, which was used for arsenic and cadmium measurement, was acidified with concentrated nitric acid. A part was treated with 0.1 M NaOH and was used for the measurement of β_2MG. The third part was assayed for ALB, NAG and creatinine without pretreatment. UAs and UCd concentrations were measured by graphite-furnace atomic absorption spectrometry (David *et al.* 1991; Jin *et al.* 2002). For analytical quality assurance, both calibration standards and one run of reference materials (Seronorm Trace Elements Urine, USA) for every analytical run were used. Uβ_2MG and UALB were measured by ELISA (Neuman & Cohen 1989) and UNAG was measured as described by Price *et al.* (1992). β_2MG, ALB and NAG kits were purchased from the Debo Bioengineering Ltd., China. Creatinine was measured by the Jaffe reaction method (Hare 1951). All urinary parameters were adjusted for creatinine in urine.

Benchmark dose estimation

The benchmark dose (BMD) was defined by Crump (1984) as a statistical lower confidence limit to the dose producing some predetermined increase in response rate such as 1–10%. It has been suggested that the BMD dose could be used in risk assessment to replace the NOAEL (No Observed Adverse Effect Level) or LOAEL (Lowest Observed Adverse Effect Level) in setting acceptable daily intakes (ADI) for

Table 1. Results of UAs, UCd and renal markers(geometric means).

Group	N (μg/g creatinine)	UAs (μg/g creatinine)	UCd (μg/g creatinine)	Uβ_2MG (mg/g creatinine)	UALB (U/g creatinine)	UNAG
Control	123	56.23 (12.41-476.54)	0.86 (0.07-8.09)	114.81 (21.26-624.05)	4.49 (0.07-30.75)	3.68 (0.03-62.16)
Exposed	122	288.40□□ (33.34-1973.90)	2.16□□ (0.06-17.57)	213.80□□ (5.17-1250.57)	13.12□□ (2.39-118.29)	11.88□□ (1.06–157.21)

□□ $P < 0.01$,vs control group; the data in ( ) is the range.

Table 2. Results of UB2M, UALB, UNAG in each combination-dose groups (geometric means).

As or Cd μg/g creatinine	Group N	Uβ_2MG μg/g creatinine	UALB mg/g creatinine	UNAG U/g creatinine
As 0 and Cd 0	56	87.10 (21.26-208.62)	3.47 (0.07-12.54)	2.98 (0.03-43.57)
As 50 and Cd 0	78	131.83□□ (32.35-526.50)	6.94□□ (1.05-31.72)	4.13 (0.04-24.89)
As 200 and Cd 0	29	218.78□□▲▲ (88.70-612.75)	12.80□□▲▲ (5.16-36.73)	10.22□□▲▲ (3.64-42.77)
As 50 and Cd 2	37	138.04□ (5.17-456.83)	5.02■■ (0.44-27.68)	5.03■ (0.03-23.40)
As 200 and Cd 2	26	323.59□□▲▲▼▼ (101.98-1224.36)	19.89□□▲▲▼▼ (5.60-52.73)	22.44□□▲▲■■▼▼ (5.98-119.21)
As 200 and Cd 5	19	478.63□□▲▲■■▼▼ (128.71-1250.57)	29.20□□▲▲■■▼▼ (7.91-118.29)	56.54□□▲▲■■▼▼□□ (13.43-157.21)

Note:□ $P < 0.05$ Compared to group As 0 and Cd 0; ▲ $P < 0.05$ ▲▲ $P < 0.01$, vs group As50 Cd 0; ■ $P < 0.05$ ■■ $P < 0.01$,vs group As200 Cd 0; ▼▼ $P < 0.01$,vs group As50 Cd2; □□ $P < 0.01$, vs groupAs200 Cd2.
The data in ( ) is the range.

human exposure to potentially toxic substances. Recently, Gaylor *et al.* (1998) have redefined the BMD as the point estimate of the dose corresponding to a specified low level of risk, and suggested that LBMD (lower confidence limit on the benchmark dose) to be used as replacement for the NOAEL or LOAEL. The LBMD is identical with the original concept of benchmark dose defined by Crump (1984). Generally, a suitable LBMD is often defined as the lower 95% confidence limit estimate of dose corresponding to a 1 to 10% level of risk above background (Gaylor *et al.* 1998). In the present study, we use 10% level of risk above background in BMD and LBMD procedure to estimate low confidence limit of population critical concentration of urinary arsenic and cadmium. Benchmark Dose Software (BMDS) Version 1.3.2 (U.S.EPA) has been used for calculation of the BMD and LBMD.

Statistical analysis

Procedures of the SPSS version 11.0 software were used for frequency, correlation, variance, regression analyses. The cut-off points (abnormal values) for the criterion variables were defined as the 95% upper limit values, which were calculated from the control group. For comparisons between more than two groups, a one-way analysis of variance (ANOVA) was used. Distributions of the biological measurements were normalized by logarithmic transformation. The data were expressed in terms of geometric means.

Results

The concentrations of arsenic, cadmium, β_2MG, ALB and NAG in the urine are shown in Table 1. It was clearly shown that UAs and UCd concentrations in the polluted areas were significantly higher than those in

Table 3. Partial correlation analysis between variables.

Variable 1	Variable 2	Correlation coefficient	Sig.
UAs	UCd	0.4822	0.000
UAs	β_2MG	0.4669	0.000
	ALB	0.6323	0.000
	NAG	0.5101	0.000
UCd	β_2MG	0.2844	0.000
	ALB	0.1527	0.017
	NAG	0.3899	0.000

the control areas. In particular, in the polluted area, the geometric mean of UAs and UCd were 288.40 μg/g creatinine and 2.16 μg/g creatinine, respectively. The levels of Uβ_2MG, UALB and UNAG in the polluted area were also significantly higher than those in the control areas. There were no significant differences in UAs, UCd and urinary parameters of renal dysfunction between males and females in the exposed or control area.

To identify the factors that affected the renal dysfunction (β_2MG, ALB, NAG), multiple regression analyses and logistic regression analyses were carried out separately. For this study the following independent variables were considered: UAs, UCd, age, sex, smoking and drinking habits. For each dependent variable, the two regression methods were examined with consideration of all subjects (The data have not been shown). An increased urinary excretion of β_2MG, ALB and NAG was associated with parameters of arsenic and cadmium co-exposure while taking possible confounders (age, sex, smoking, drinking) into account.

The cut-off point was defined based on the upper 5% limit value in the non polluted area. The cut-off values of Uβ_2MG, UALB and UNAG were 0.30 mg/g creatinine, 15.00 mg/g creatinine, 23.00 U/g creatinine, respectively. The geometric mean values of β_2MG, ALB and NAG in all subjects at each combination-dose groups of UAs and UCd were calculated, the results are shown in Table 2. It was clearly shown that there was a significantly increased urinary level of β_2MG, ALB and NAG with increasing arsenic and cadmium excretion in urine. The increases were statistically significant in the ANOVA test for means (Table 2). By analysis with Chi-square test for trend a statistically significant, relationship between combination-dose groups of UAs and UCd and the prevalence of renal dysfunction was demonstrated (data not shown).

It was suggested that co-exposures to arsenic and cadmium may be partly related based on the good and significant partial correlation coefficients between UAs and UCd (Table 3). It was indicated that combined effect of arsenic and cadmium were additive effect and/or synergistic effect in Table 2 (e.g., there is effect on ALB for As but not for Cd , There is an effect for Cd+As). And also more increased excretion of urinary β_2MG, NAG and ALB was found in the combined exposure groups compared to those with only As or Cd only exposure (Table 2).

Arsenic could induce nephrotoxicity both glomerular and tubular dysfunction. Cadmium mainly affects on tubuli during the long-term and coexposure with arsenic and cadmium.

The mean UAs and UCd concentrations of different UAs strata (0, 50, 100, 200, 400 μg/g creatinine) and UCd strata (0, 0.50, 1.00, 2.00, 5.00 μg/g creatinine) and the related prevalence values for each urinary renal dysfunction marker were calculated (does-response curves for ALB shown in Figures 1 and 2). The goodness of fit of the model (Probit model) was better for UALB ($P = 0.5341$) than Uβ_2MG ($P = 0.0913$) and UNAG ($P = 0.0576$) in co-exposure to arsenic and cadmium. The dose-response data were used to estimate the BMD and LBMD for different urinary parameters of renal dysfunction. The estimated parameters and the corresponding BMD and LBMD are given in Table 5. It was show that, the estimated LBMD of UAs was 102.11 μg/g creatinine, 136.98 μg/g creatinine and 144.44 μg/g creatinine for UALB, Uβ_2MG and UNAG, respectively. And for UCd was 0.88 μg/g creatinine, 1.13 μg/g creatinine and 1.24 μg/g creatinine, respectively. Figures 1 and 2 show the does-response curves of Urinary As or Cd in relation to prevalence of ALB (above cut off level) as well as the 10% BMD and LBMD points of urinary As or Cd.

Discussion

Renal effects of co-exposure to arsenic and cadmium have received very limited study in humans. Recently, Buchet *et al.* (2003) reported renal effects a Chinese population co-exposed to cadmium and arsenic. The distributions of the biomarkers of exposure (UCd, UAs) showed definite differences in exposure intensity to both elements in Chinese populations.

Table 4. Tests of interaction between cadmium and arsenic.

Source	Uβ_2MG		UALB		UNAG	
	F	Sig.	*F*	Sig.	*F*	Sig.
As	23.006	0.000	39.890	0.000	18.626	0.000
Cd	3.032	0.045	1.445	0.238	13.207	0.000
As×Cd	0.518	0.597	5.660	0.004	4.448	0.036

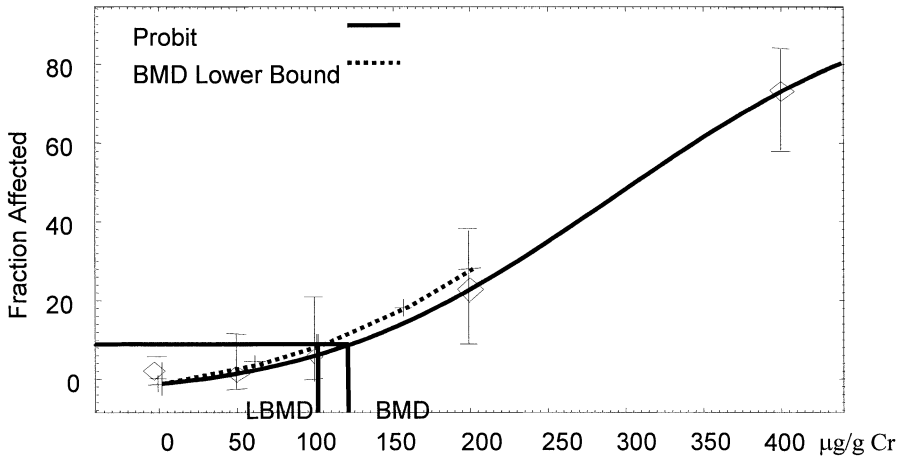

Fig. 1. Probit Model the dose response relationship between UAs and UALB with 0.95 confidence level and the BMD-10 and LBMD-10 are indicated.

Results show that the same exposure parameters and, in addition, UAs proved to be significant determinants of renal tubular and glomerular dysfunction parameters, while UCd was consistently (positive relationship) associated with renal parameters. Co-exposure to cadmium and arsenic did add their respective effect on protein excretion and a synergism was detected. The potentiation by arsenic of the cadmium nephrotoxicity observed in mice includes a statistically significant additivity and/or synergism between both elements (Liu *et al.* 2000) and a similar situation is observed in humans in our study.

It is generally assumed that the concept of additivity is operative on low-level exposures to chemical mixtures (Svendsgaard & Hertzberg 1994). If significant departure from additivity is found, then an interaction can be claimed at the mixture levels tested. In this study, 2 types of responses to the metal mixture were seen (i.e., additivity and synergism), but not seen antagonism, depending highly on both the renal parameters examined and the dose of the metal mixture. With some exceptions, the general trend of nephrotoxicity by the 2-metal mixture appears to be hormesis to additivity to synergism as dose level increases (Table 4).

Present exposure to toxic metals such as arsenic and cadmium has decreased to a small fraction, compared to the leve in the past The risk assessment of the health effect caused by low level exposure to metals are therefore of great importance. An increasing number of studies have shown that renal dysfunction, which is regarded as the critical effect of long-term cadmium exposure, may develop at lower levels than previously believed. It is well known that UAs and UCd is closely related to the body or kidney burden of arsenic and cadmium, so that UAs and UCd is two appropriate parameters to use in the risk assessment of the renal effects caused by arsenic and cadmium (Järup *et al.* 1998; Zhang *et al.* 2000).

The critical effects of cadmium are crucial for the preventive action (Nordberg 1992). American Conference of Governmental Industrial Hygienists (ACGIH 1996) commendation the threshold limit values of UCd concentration is 5.00 μg/g creatinine for cadmium exposure. An important and frequently cited study of the effects of cadmium on the general population indicated that a significant proportion (10%)

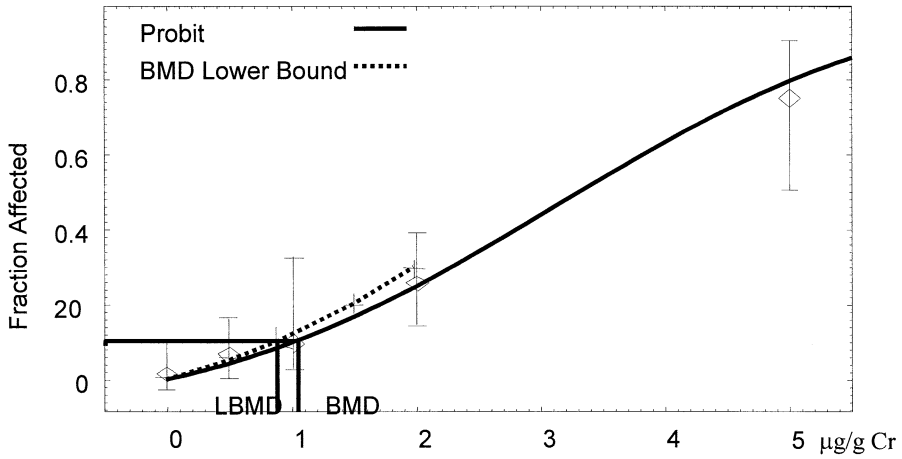

Fig. 2. Probit Model the dose response relationship between UCd and UALB with 0.95 confidence level and the BMD-10 and LBMD-10 are indicated.

of a population showed evidence of renal damage at UCd concentrations exceeding 2–4 μg/g creatinine (Buchet *et al.* 1990; Bernard *et al* 1992). A recent study from China also showed a significantly increased excretion of UNAG isoform B in subjects environmentally exposed to cadmium at UCd concentrations of 2–5 μg/g creatinine (Jin *et al.* 1999). As pointed out by Järup et al. (1998) in a review of the health effects of cadmium all recent findings suggest that the critical concentrations of cadmium in the urine and the kidney have both been overestimated. In generally, UAs concentration is low than 50.00 μg/l in general population not exposed to arsenic. When the UAs concentration over 100.00 μg/l its mean there exist arsenic exposure (Klaassen *et al.* 2002). ACGIH (1996) commendation the threshold limit values of UAs concentration is 50.00 μg/g creatinine. In the present study, the degree of renal injure (renal parameter) is gradually increased as the UAs and UCd concentration changes increased. The renal function significant changed when UAs and UCd concentration about 50.00 μg/g creatinine, 2.00 μg/g creatinine, respectively, include tubular and glomerular function. There is significant dose-effect relationship.

The BMD process is mainly used to estimate the ADI or RfD of toxic substances as a replacement for the NOAEL or LOAEL in the safety assessment process (WHO 2002). The benchmark dose is related to a specific change in the effect above the background, expressed as a percentage change in the effect relative to the background (Kalliomaa *et al.* 1998). In the present study, the BMDs have been used to calculate the BMD based on population data of Uβ_2MG, UALB and UNAG as parameters of renal dysfunction. The LBMD, is the lower confidence level of the estimated 10% level of renal dysfunction above the background according to the 'Bechmark dose' terminology. In the present study, the results showed that there were different BMD and LBMD values depending on the urinary parameter of renal tubular and glomerular dysfunction used. The LBMD for UALB gave the lowest value for UAs (about 102.11 μg/g creatinine) and UCd (about 0.88 μg/g creatinine). In brief, the LBMD value of renal tubular dysfunction was in the range 1.13–1.24 μg/g creatinine for cadmium coexposure to arsenic, range 136.98–144.44 μg/g creatinine for arsenic coexposure to cadmium, based on a comprehensive analysis in the present study of two urinary parameters (NAG and β_2MG). It is implied that there was an increased 10% prevalence of renal tubular dysfunction of general population with coexposure to UCd 1.13–1.24 μg/g creatinin and UAs 136.98–144.44 μg/g creatinin, if the background is zero. Järup *et al.* (1998) suggested that cadmium levels in urine should be kept below 2.50 μg/g creatinine in order to prevent renal tubular damage that can proceed to clinical disease and perhaps contribute to early death. However, in our present findings that coexposure to cadmium and arsenic, it is suggested that the UCd level and UAs level at which the lowest detectable renal effect should be observed as much lower than previously evaluated, even less than 2 μg/g creatinine and 150 μg/l for cadmium, arsenic, respectively.

Our present results show that the LBMD value of renal glomerular (UALB) and tubular (Uβ_2MG) dysfunction was 142.35 μg/g creatinine for arsenic

Table 5. BMD and LBMD Estimates of UAs and UCd (μg/g creatinine) for urinary parameters of renal dysfunction.

Parameters	Renal parameter	10% level of risk	
		BMD	LBMD
UAs	Uβ_2MG	163.61	136.98
	UALB	121.91	102.11
	UNAG	171.88	144.44
	Uβ_2MG +UALB	170.03	142.35
	Uβ_2MG +UNAG	354.40	261.50
	UALB+UNAG	268.18	225.62
	Uβ_2MG +UALB+UNAG	354.40	261.50
UCd	Uβ_2MG	1.36	1.13
	UALB	1.05	0.88
	UNAG	1.48	1.24
	Uβ_2MG +UALB	1.35	1.12
	Uβ_2MG +UNAG	1.98	1.65
	UALB+UNAG	1.83	1.53
	Uβ_2MG +UALB+UNAG	1.98	1.65

Note: The BMDs Model is Probit.

coexposure to cadmium, 1.12 μg/g creatinine for cadmium coexposure to arsenic, It is implied that there was an increased 10% prevalence of renal glomerular and tubular dysfunction of general population with coexposure to UAs 142.35 μg/g creatinin and UCd 1.12 μg/g creatinin.

Furthermore, the present results have shown that this software gave a good-fit with the present data ($P > 0.05$).

This is the first paper to use BMDS to estimate the UCd and UAs critical concentration of renal dysfunction in a general population coexposed to cadmium and arsenic. The BMDS was mainly used for risk assessment of noncancer effects at low-level exposure of toxicant instead of NOAEL or LOAEL. There are similar situation between the BMD and the critical concentration, and both of them are calculated based on a dose-effected relationship, it is therefore feasibility to use the BMDS to estimate the PCC of a general population coexposed to cadmium and arsenic. No available data can be compared with our present findings, for no one else has done these calculation ever before. After all, it is a new method to use for estimating the critical concentration, in order to further develop the application of this procedure, further study employing these methods needs to be done in the future.

In summary, the present study demonstrates that chronic exposure to arsenic, cadmium, or arsenic plus cadmium, all produce nephrotoxicity in humans, as indicated by increased Uβ_2MG, UALB and UNAG. It demonstrates that chronic co-exposure to cadmium and arsenic causes an increased risk for renal injury. And our studies show, via statistical analysis, that the two metals in a mixture act in an additive and/or synergistic fashion at low doses. It is commonly assumed that information like the one reported in the present study is highly relevant in terms of developing accurate risk assessment strategies for these important environmental contaminants in general populations.

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