Bull. Environ. Contam. Toxicol. (2006) 76:8–15
© 2006 Springer Science+Business Media, Inc.
DOI: 10.1007/s00128-005-0883-9

Bulletin of Environmental Contamination and Toxicology

Tolerable Level of Lifetime Cadmium Intake Estimated as a Benchmark Dose Low, Based on Excretion of β_2-Microglobulin in the Cadmium-Polluted Regions of the Kakehashi River Basin, Japan

E. Kobayashi,[1] Y. Suwazono,[1] M. Uetani,[1] T. Kido,[2] M. Nishijo,[3] H. Nakagawa,[3] K. Nogawa[1]

[1] Department of Occupational and Environmental Medicine (A2), Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuohku, Chiba, 260-8670 Japan
[2] Department of Community Health Nursing, Kanazawa University School of Health Sciences, Japan
[3] Department of Public Health, Kanazawa Medical University, Kanazawa, Japan

Received: 15 July 2005/Accepted: 12 October 2005

Recently, a benchmark dose (BMD) approach defined by Crump (Crump et al, 1984, 1995) was used to estimate the threshold levels of toxic substances (Gaylor et al, 1998). BMD is a statistical lower confidence limit to a dose producing some predetermined increase in response rate, such as 0.01-0.1 in the abnormal rates of substances. The benchmark dose low (BMDL) is defined as the value corresponding to the lower 95% confidence interval of the BMD and can be used instead of the no observed adverse effect level (NOAEL) or the lowest observable adverse effect level (LOAEL) (Gaylor et. al, 1998) in the evaluation of a dose-response relationship.

Over the past several years, we have demonstrated a dose-response relationship between lifetime cadmium intake (LCD) indicators of renal dysfunction such as urinary $ß_2$-microglobulin ($ß_2$-MG), metallothionein (MT), protein, glucose, amino acid etc. in the Kakehashi River basin and the Jinzu River basin. We have reported that the tolerable level of LCD for both sexes in the Kakehashi River basin is approximately 2.0 g (Nogawa et al, 1989; Kido et al, 1991, 1993; Kido and Nogawa 1993; Hochi et al, 1995) and less than 1.6 g in the Jinzu River basin (Chiyoda et al, 2003; Watanabe et al, 2004). In these studies the rate of abnormal urinary analysis in controls was substituted into every regression formula calculated with LCD and the rate of abnormal urinary analysis in the cadmium (Cd)-exposed subjects and tolerable levels of LCD were obtained. Thus, the threshold value of LCD corresponds to the rate of abnormal urinary analysis of the control subjects. Our studies have previously demonstrated, even in areas in Japan not polluted with Cd, a dose-response relationship between urinary Cd and urinary substances such as low molecular weight proteins indicative of renal dysfunction mediated by Cd exposure (Yamanaka et al, 1998; Oo et al, 2000; Suwazono et al, 2000). Therefore, it may be inappropriate to use the rate of abnormal urinary analysis of people living in areas unpolluted by Cd to estimate the threshold value of LCD.

Therefore, in the present study we estimated the tolerable levels of LCD as BMDL, employing BMD procedures for the same data used in the previous studies (Nogawa et al, 1989; Kido et al, 1991, 1993; Kido and Nogawa 1993; Hochi et al, 1995) and compared BMDL values and the tolerable values obtained in the previous studies (Nogawa et al, 1989; Kido et al, 1991, 1993; Kido and Nogawa 1993; Hochi et al, 1995). We believe that this comparison is useful to estimate the accurate tolerable values of LCD.

MATERIALS AND METHODS

The target population of the present study was the same one as described in various previous studies (Nogawa et al, 1989; Kido et al, 1991, 1993; Kido and Nogawa 1993; Hochi et al, 1995),

Correspondence to: E. Kobayashi

namely it was drawn from the population evaluated in the 1981 and 1982 health survey conducted among the entire population over 50 years of age residing in the Kakehashi River basin. The numbers of participants in the Cd-polluted and non-polluted areas were 3178 (1424 men and 1754 women, participation rate 91%) and 294 (133 men and 161 women, participation rate 94%), respectively. Among them the subjects with a complete data set for age, residence history, urinary β_2-MG concentration and Cd concentration in the rice of their hamlet were selected as the target population of the present study. The data concerning age and history of residence were gathered on the basis of self-reported replies confirmed afterwards by interview. The population that underwent analysis consisted of 1838 participants (874 men and 964 women). The numbers of subjects examined according to sex and age are shown in Table 1.

Table 1. Number of subjects examined according to sex and age.

Age (ys)	Males	Females
50-59	381	381
60-69	282	316
70-79	171	197
$\geq$ 80	40	70
Total	874	964

As described in the previous studies, morning urine samples were collected from the participants and kept frozen at -20^0C until analysis. The specimens for β_2-MG determination were adjusted to a pH above 6 and β_2-MG was measured using the Pharmacia β_2-micro radioimmunoassay (RIA) method (Pharmacia Diagnostics AB, Sweden). Urinary creatinine concentration was determined using the Jaffe reaction (Bonsnes and Taussky, 1945).

The calculation of LCD was based on the formula of Nogawa (1989): (mean Cd concentration in rice of hamlet × 333.5 g + 34 µg) × 365 days × number of years of residence in present hamlet + 50 µg × 365 days × number of years living in non-polluted region). In the above formula, 333.5 g represents the daily mean rice intake (Ishikawa Prefecture, 1976), 34 µg is the mean daily Cd intake from foods other than rice in the Cd-polluted region (Ishikawa Prefecture, 1976). The method of estimating the daily mean rice intake (333.5 g) or the mean daily Cd intake from foods other than rice (34 µg) in the Cd-polluted Kakehashi River basin was the collection of a duplicate sample of the meals consumed. While 50 µg is the mean daily Cd intake in regions not polluted with Cd (Yamagata, 1978).

In 1976 (Ishikawa Prefecture, 1976), rice was collected from the farmers in all the polluted hamlets and was divided into 23 groups according to location with 35,451 rice bags being stored in warehouses. Random samples from the bags of rice from each polluted hamlet were extracted and mixed well before being assayed for Cd. Cadmium concentrations in the rice were measured by atomic absorption spectrometry after wet ashing with H_2SO_4, HNO_3, $HClO_4$ and extraction with ammonium pyrrolidine dithiocarbamate-methyl-isobutyl ketone (APDC-MIBK). The mean Cd concentration in the rice was therefore ascertained. The mean Cd concentration of rice from non-polluted areas was not measured in this manner because the rice was not collected in a similar fashion. The mean Cd concentration in the rice harvested from control hamlets was < 0.1 μg/g.

The concentrations of urinary substances were expressed in a corrected value normalized for creatinine excretion (g/cr). LCD was divided into 8 categories and a rate of abnormal urinary β_2-MG excretion in each subgroup was calculated. Cut-off values for urinary β_2-MG excretion were defined as corresponding to the 84% (geometric means × geometric standard deviations) and 95% (geometric means × 1.96 geometric standard deviations) upper limit values calculated from 2034 non-smoking persons living in the non-polluted areas, and 1000 μg/g cr. The values defined as the 84% and 95% upper limit values are shown according to sex in Table 2. We then calculated the BMD and BMDL. BMD_{10} or BMD_5 is the LCD level that can be expected to yield an excess prevalence of an abnormal level of urinary β_2-MG excretion of 10 or 5%. BMDL is LCD of the lower limit of the 95% confidence intervals of the BMD (Crump et al., 1984, 1995). The analysis of regression and curve estimation was performed with Benchmark Dose Software (version 3.1.1) available from the U.S. Environmental Protection Agency (EPA). The BMD and BMDL values were calculated by a log-logistic model defined as follows: P [response] = background + [1 - background] / [1 + EXP (-intercept – slope × log (dose)).

Table 2. Cut-off values of β_2-microglobulin-uria.

Cut-off value	84%		95%	
Sex	Male	Female	Male	Female
(μg/g creatinine)	507.0	399.9	993.7	783.9

Cut-off values are defined as the 84% (geometric means × geometric standard deviations) or 95% (geometric means × 1.96 × geometric standard deviations) upper limit values, which are calculated from 2034 non-smoking persons living in the non-polluted areas.

RESULTS AND DISCUSSION

The prevalence calculated for each cut-off value (84% and 95% upper limit values and 1000 μg/g cr) increased with increasing LCD in both men and women (Table 3 and Table 4).

The result of the BMD and BMDL for each cut-off value was calculated using a log-logistic model setting an abnormal value at either 10% or 5% (Table 5). When an abnormal value of 10% was employed, the BMDLs of LCD for the 84% and 95% upper limit values and 1000 μg/g cr for β_2-MG were 2.254, 2.836 and 2.836 g in men and 1.526, 2.075 and 2.227 g in women. When an abnormal value of 5% was employed, the BMDLs of LCD for each cut-off values were 1.379, 1.858 and 1.858 g in men and 0.876, 1.263 and 1.373 g in women.

In studies of the health effects caused by exposure to Cd, urinary Cd concentration is often used as an indicator of the internal dose. Reports on animals (Nordberg and Nishiyama, 1972; Nomiyama, 1974; Bernard et al, 1980) and Cd workers (Lauwerys et al, 1979; Roels et al, 1981; Hasseler et al, 1982) have shown a close relationship between urinary Cd excretion and the total body burden of Cd. Kido and his co-workers (2004) investigated the relationship between urinary Cd concentration corrected for creatinine and LCD of 198 persons (100 men and 98 women) living in the most polluted hamlets in the Kakehashi River basin. Statistically

Table 3. Prevalence of β_2-microglobulin-uria (μg/g cr) at different levels of life-time Cd intake in males living in Cd-polluted areas of the Kakehashi River basin.

LCD (g)		Total number of subjects	Cut-off value 84%		Cut-off value 95%	
Range	GM		n	%	n	%
<2.00	1.663	137	16	11.7	8	5.8
2.00-2.49	2.265	118	17	14.4	9	7.6
2.50-2.99	2.729	133	23	17.3	17	12.8
3.00-3.49	3.258	127	28	22.0	21	16.5
3.50-4.249	3.882	139	36	25.9	24	17.3
4.25-5.49	4.765	164	48	29.3	36	22.0
5.50-6.49	6.067	41	29	70.7	23	56.1
≥6.50	6.966	15	12	80.0	10	66.7
Total		874	209	23.9	148	16.9

LCD (g)		Total number of subjects	Cut-off value 1000 μg/g cr	
Range	GM		n	%
<2.00	1.663	137	8	5.8
2.00-2.49	2.265	118	9	7.6
2.50-2.99	2.729	133	17	12.8
3.00-3.49	3.258	127	21	16.5
3.50-4.249	3.882	139	24	17.3
4.25-5.49	4.765	164	36	22.0
5.50-6.49	6.067	41	23	56.1
≥6.50	6.966	15	10	66.7
Total		874	148	16.9

LCD: Life-time Cd intake (g). GM: Geometric mean. n: Number of subjects with β_2-MG-uria. %: Prevalence of β_2-MG-uria (%). cr: Creatinine.

significant correlation coefficients between urinary Cd concentration and LCD of 0.879 and 0.835 were obtained in men and women, respectively. We also investigated the relationship between calculated LCD and the corresponding individual urinary Cd concentration. The target population consisted of 1815 subjects (865 men and 950 women) in a Cd-polluted area and 1764 residents (686 men and 1078 women) in other non-polluted regions in the same prefecture. Using individual LCD calculated from the formula of Nogawa (Nogawa et al, 1989), high correlation coefficients ($p < 0.001$) of 0.61 in men and 0.59 in women were obtained with urinary Cd levels (corrected for creatinine) of the corresponding individuals (Kobayashi et al, 2005). Moreover, using the regression formula to calculate urinary Cd concentration corresponding to the 2.0 g, which we previously determined to be the maximum allowable limits of LCD (Nogawa et al, 1989; Kido et al, 1991, 1993; Kido and Nogawa 1993; Hochi et al, 1995), values of 3.7 μg/g cr and 5.9 μg/g cr were obtained for men and women, respectively (Kobayashi et al, 2005). These values are in close agreement with the tolerable urinary Cd concentration of 1.6-3.0 μg/g cr for men and 2.3-4.6 μg/g cr for women calculated by Hayano in

Table 4. Prevalence of β_2-microglobulin-uria (μg/g cr) at different levels of life-time Cd intake in females living in Cd-polluted areas of the Kakehashi River basin.

LCD (g)		Total number of subjects	Cut-off value 84%		Cut-off value 95%	
Range	GM		n	%	n	%
< 1.749	1.469	93	13	14.0	5	5.4
1.75-1.99	1.866	70	11	15.7	6	8.6
2.00-2.249	2.128	74	14	18.9	12	16.2
2.25-2.749	2.495	163	48	29.4	31	19.0
2.75-3.249	2.985	129	45	34.9	28	21.7
3.25-3.749	3.491	126	49	38.9	33	26.2
3.75-4.449	4.156	130	68	52.3	49	37.7
≥ 4.50	5.370	179	111	62.0	87	48.6
Total		964	359	37.2	251	26.0

LCD (g)		Total number of subjects	Cut-off value 1000 μg/g cr	
Range	GM		n	%
< 1.749	1.469	93	5	5.4
1.75-1.99	1.866	70	5	7.1
2.00-2.249	2.128	74	11	14.9
2.25-2.749	2.495	163	26	16.0
2.75-3.249	2.985	129	25	19.4
3.25-3.749	3.491	126	31	24.6
3.75-4.449	4.156	130	45	34.6
≥ 4.50	5.370	179	80	44.7
Total		964	228	23.7

LCD: Life-time Cd intake (g). GM: Geometric mean. n: Number of subjects with β_2-MG-uria. %: Prevalence of β_2-MG-uria (%). cr: Creatinine.

the inhabitants of the Cd-polluted region in the Kakehashi River basin using the rate of abnormal urinary ß$_2$-MG excretion (Hayano et al, 1996). Accordingly, these results demonstrated clearly that the LCD calculated by this formula is sufficiently accurate and suitable for calculating the threshold levels of LCD.

In this study three kinds of cut-off values for ß$_2$-MG excretion were used, that is the 84% and 95% upper limit values which were calculated from controls and 1000 μg/g cr of urinary ß$_2$-MG concentration. The 95% upper limit value is often employed as the cut-off value. As shown in Table 2, the 95% upper limit values were 994 μg/g cr for men and 784 μg/g cr for women in this study.

Kido et al. (1988) investigated the reversibility of ß$_2$-MG-uria in 74 inhabitants (32 males and 42 females) in the Cd-polluted regions of the Kakehashi River basin. The subjects participated in two examinations conducted just after the cessation of Cd exposure and again 5 years later when Cd exposure ceased. In cases where ≥ 1000 μg/g cr of ß$_2$-MG-uria was observed in the first investigation, almost all individuals exhibited a deterioration of β_2-MG-uria whereas no significant changes were observed in cases where values β_2-MG-uria were < 1000 μg/g cr. Thus, the threshold level of urinary β_2-MG excretion associated with the transition to irreversible renal damage was determined to be 1000 μg/g cr. Other studies in Japan also showed similar results (Iwata et al, 1993; Cai et al, 2001). Therefore, when the 95% upper limit value, which is nearly equal to 1000 μg/g cr, was used as a cut-off value, this represented the value at which renal damage may become irreversible.

Nakagawa et al. (1993) conducted a 9-year follow-up survey of 3178 inhabitants of the Cd-polluted Kakehashi River basin aged ≥ 50 years. They divided the subjects into 4 groups according to β_2-MG concentrations (i.e. < 300 μg/g cr, 300 to < 1000 μg/g cr, 1000 to < 10,000 μg/g cr, and ≥ 10,000 μg/g cr), and the relationship between mortality and the level of β_2-MG-uria was studied. Mortality ratios in both females and males were found to increase as urinary β_2-MG excretion increased compared with the group with the lowest level of β_2-MG-uria (< 300 μg/g cr). Thus, increasing tubular dysfunction adversely impacts upon long term mortality, even at moderate levels of urinary β_2-MG excretion (300 to < 1000 μg/g cr). In the present study, the 84% upper limit values for β_2-MG were 507 μg/g cr for men and 400 μg/g cr for women. It should be noted that the value was higher than the 300 μg/g cr value used in the Nakagawa study. Therefore, we thought that the cut-off values for urinary β_2-MG defined as corresponding to the 84% upper limit values are reasonable.

Table 5. BMDL estimates of life-time cadmium intake for β_2-microblobulin-uria (cut-off values = 84% and 95% upper limit values and 1000 μg/g creatinine) using log-logistic model.

	Cut-off	Intercept	Slope	P	BMD_{10}	$BMDL_{10}$	BMD_5	$BMDL_5$
M	84%	-3.094	0.541	0.092	2.417	2.254	1.518	1.379
	95%	-3.756	0.589	0.320	3.041	2.836	2.059	1.858
	1000 μg	-3.756	0.589	0.320	3.041	2.836	2.059	1.858
F	84%	-2.454	0.573	0.588	1.626	1.526	0.952	0.876
	95%	-3.058	0.578	0.512	2.218	2.075	1.387	1.263
	1000 μg	-3.175	0.572	0.626	2.380	2.227	1.509	1.373

M: Males. F: Females. BMD_{10}: Excess risk at BMD of 0.10. BMD_5: Excess risk at BMD of 0.05.
P[response] = 1-EXP(-slope×log (dose)).

Previously, we demonstrated a dose-response relationship between LCD and urinary β_2-MG excretion in the Kakehashi River basin using the same data as the present study (Nogawa et al, 1989). In this study we calculated the tolerable values of LCD by substituting the rate of abnormal urinary β_2-MG excretion in controls into all regression formulae calculated in the Cd-exposed subjects (Nogawa et al, 1989). When 1000 μg/g cr was used as the cut-off value, the value was 1.678 g in men and 1.763 g in women. In the present study, we obtained the tolerable values of LCD as $BMDL_5$ with 1.858 g in men and 1.373 g in women using 1000 μg/g cr as a cut-off value. Thus, the tolerable values of LCD are comparable between the previous and present studies. Therefore, when the rate of abnormal urinary β_2-MG excretion was used as an indicator of renal dysfunction caused by environmental Cd-exposure, it can be said that the tolerable values of LCD are sufficiently accurate and a BMD approach is useful in estimating a tolerable value of LCD.

REFERENCES

Bernard A, Goret A, Buchet JP, Roels H, Lauwerys RR (1980). Significance of cadmium levels in blood and urine during long-term exposure of rats to cadmium. J Toxicol Environ Health 6: 175-184

Bonsnes RW, Taussky HH (1945). On the calorimetric determination of creatinine by the Jaffe reaction. J Biol Chem 158: 581-591

Cai Y, Aoshima K, Katoh T, Teranishi H, Kasuya M (2001). Renal tubular dysfunction in male inhabitants of a cadmium-polluted area in Toyama, Japan--an eleven-year follow-up study. J Epidemiol 11: 180-189

Chiyoda N, Kobayashi E, Okubo Y, Suwazono Y, Kido T, Nogawa K (2003). Allowable level of lifetime cadmium intake calculated from the individuals in the Jinzu River basin of Japan. Biol Trace Elem Res 96: 9-20

Crump KS (1984). A new method for determining allowable daily intakes. Fundam Appl Toxicol 4: 854-871

Crump KS (199). Calculation of benchmark dose from continuous data. Risk Analysis 15: 79-89

Gaylor D, Ryan L, Krewski D, Zhu Y (1998). Procedures for calculation of benchmark doses for health risk assessment. Reg Toxicol Pharmacol 28: 150-164

Hasseler F, Lind B, Piscator M (1983). Cadmium in blood and urine related to present and past exposure. A study of workers in an alkaline battery factory. British J Ind Med 40: 420-425

Hayano M, Nogawa K, Kido T, Kobayashi E, Honda R, Tsuritani I (1996). Dose-response relationship between urinary cadmium concentration and β_2-microglobulinuria using logistic regression analysis. Arch Environ Health 51: 162-167

Hochi Y, Kido T, Nogawa K, Kito H, Shaikh ZA (1995) Dose-response relationship between life-time cadmium intake and prevalence of renal dysfunction using general linear models. J Appl Toxicology 15: 109-116

Ishikawa Prefecture, Department of Health (1976) Report of the health examination on the inhabitants in the Kakehashi River basin. Kanazawa p6 and 79-84 [in Japanese]

Iwata K, Saito H, Moriyama M, Nakano A (1993). Renal tubular function after reduction of environmental cadmium exposure: a ten-year follow-up. Arch Environ Health 48: 157-163

Kido T, Honda R, Tsuritan I, Yamaya H, Ishizaki M, Nogawa K (1988). Progress of renal dysfunction in inhabitants environmentally exposed to cadmium. Arch Environ Health 43: 213-217

Kido T, Nogawa K (1993) Dose-response relationship between life-time cadmium intake and β_2-microgrobulinuria using logistic regression analysis. Toxicol Lett 69: 113-120

Kido T, Shaikh ZA, Kito H, Honda R, Nogawa K (1991) Dose-response relationship between dietary cadmium intake and metallothioneinuria in a population from a cadmium-polluted area in Japan. Toxicology 66: 271-278

Kido T, Sunaga K, Nishujo M, Nakagawa H, Kobayashi E, Nogawa K (2004). The relation of individual cadmium concentration in urine with total cadmium intake in Kakehashi River basin, Japan. Toxicol Lett 152: 57-61

Kido T, Shaikh ZA, Kito H, Honda R, Nogawa K (1993) Dose-response relationship between life-time cadmium intake and metallothioneinuria using logistic regression analysis. Toxicology 80: 207-215

Kobayashi E, Suwazono Y, Uetani M, Inaba T, Oishi M, Kido T, Nakagawa H. Nogawa K (2005). Association between life-time cadmium intake and cadmium concentration in individual urine. Bull Environ Contamin Toxicol 74: 817-821

Lauwerys RR, Roels H, Regniers M, Buchet P, Bernard A, Goret A (1979). Significance of cadmium concentration in blood and in urine in workers exposed to cadmium. Environ Res 20: 375-391

Nakagawa H, Nishijo M, Morikawa Y, Senma M, Kawano S, Ishizaki M, Sugita N, Nishi M, Kido T, Nogawa K (1993). Urinary β_2-microglobulin concentration and mortality in a cadmium-polluted area. Arch Environ Health 48: 428-435

Nakashima K, Kobayashi E, Nogawa K, Kido T, Honda R (1997). Concentration of cadmium in rice and urinary indicators of renal dysfunction. Occup Environ Med 54: 750-755

Nogawa K, Honda R, Kido T, Tsuritani I, Yamada Y, Ishizaki M, Yamaya H (1989) A dose-response analysis of cadmium in the general environmental with special reference to life-time cadmium intake limit. Environ Res 48: 7-16

Nogawa K, Ishizaki A (1979) A comparison between cadmium in rice and renal effects among inhabitants of the Jinzu River basin. Environ Res 18: 410-420

Nomiyama K (1974). Experimental study on cadmium intoxication. J Japanese Vet. Med Assoc 72: 955-966

Nordberg GF, Nishiyama K (1972). Whole-body and hair retention of cadmium in mice. Arch Environ Health 24: 209-214

Oo Y.K, Kobayashi E, Nogawa K, Okubo Y, Suwazono Y, Kido T, Nakagawa H (2000). Renal effects of cadmium intake of a Japanese general population in two areas unpolluted by cadmium. Arch Environ Health 55: 98-103

Roels H, Lauwerys RR, Buchet JP, Bernard A, Chettle DR, Harvey TC, AL-Haddad IK (1981). In vivo measurement of liver and kidney cadmium in workers exposed to this metal. Environ Res 26: 217-240

Suwazono Y, Kobayashi E, Nogawa K, Okubo Y, Kido T, Nakagawa H (2000). Renal effects of cadmium exposure in cadmium non-polluted areas in Japan. Environ Res 84: 44-55

Tsuchiya K, Iwao S (1978) Results and evaluation on cadmium intake of Cd-exposed inhabitants in Akita, Ishikawa and Nagasaki Prefectures. Kankyo Hoken Report 44: 86-115 [in Japanese]

Yamagata N (1978). Cadmium in the environment and in humans. In: Tsuchiya K (ed) Cadmium studies in Japan-A review. Tokyo/Amsterdam/New York/Oxford. Elsevier/North-Holland Biomedical Press 19-37

Yamanaka O, Kobayashi E, Nogawa K, Suwazono Y, Sakurada I, Kido T (1998). Association between renal effects and cadmium exposure in cadmium-nonpolluted area in Japan. Environ Res 77: 1-8

Watanabe K, Kobayashi E, Suwazono Y, Okubo Y, Kido T, Nogawa K (2004). Tolerable lifetime cadmium intake calculated from the inhabitants living in the Jinzu River basin, Japan. Bull Environ Contam Toxicol 72: 1091-1097