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ABSTRACT The benchmark dose (BMD) method has been proposed as an alternative to the no-observed-adverse-effect level (NOAEL) approach for assessing noncancer risks associated with hazardous compounds. The benchmark dose method is a more powerful statistical tool than the traditional NOAEL approach and represents a step in the right direction for a more accurate risk assessment. The benchmark dose method involves fitting a mathematical model to all the dose-response data within a study, and thus more biological information is incorporated in the resulting estimates of guidance values. The BMD and the lower confidence limit on BMD (BMDL) of blood lead to cause renal dysfunction were determined in the population exposure to lead. The blood lead level was used as an exposure biomarker, while total protein (TP), β_2-microglobulin (β_2-MG), and N-Acetyl-β-D-glucosaminidase (NAG) in the urine were considered as effect biomarkers. The dichotomized data were used as effect endpoints. The BMD and BMDL of blood lead were determined at the 10% benchmark response for the effect biomarkers by using BMDS Version 1.3.1. The results showed that BMD and BMDL of blood lead for NAG, TP, and β_2-MG ranged from 323.6 to 754.3 μg/L and 274.2 to 541.5 μg/L, respectively. The BMDL for blood lead was ranked from high to low as TP, β_2-MG, and NAG. Urinary NAG activity could be served as a sensitive indicator to detect early renal dysfunction.

KEYWORDS Benchmark Dose; Lead Exposure; Renal Dysfunction

INTRODUCTION

Lead has been one of the most common heavy metals with wide applications for many centuries (Levin and Goldberg 2000). Occupational and environmental lead exposures are among the most significant public health problems (Fels et al. 1998; Levin and Goldberg 2000; Gurer-Orhan et al. 2004). It is well known that long-term exposure to lead in the occupational environment causes renal dysfunction (Ehrlich et al. 1998; Staessen et al. 1992; Wang et al. 2002). The first sign of renal effects is tubular damage, characterized by increased urinary excretion of low-molecular-weight proteins (β_2-microglobulin) or intracellular tubular enzymes (N-acetyl-β-D-glucosaminidase).

To protect workers from the adverse effects of lead exposure, it is important to determine threshold exposure levels for lead using different indices of lead exposure and health effects.

Risk assessment is the process of characterizing and quantifying potential adverse effects on humans of exposure to chemicals or physical agents that pose a human health hazard (McClellan 1999). Risk assessment of chemicals has two main objectives, either to establish permissible exposure levels for humans or to assess the health risks in connection with a particular exposure. The traditional method for risk assessment was involved in the establishment of a no-observed-adverse-effect level (NOAEL), and then applied

an uncertainty factor to account for species differences in response and interindividual variability. There are several limitations to use this method. Firstly, the NOAEL does not consider the shape of the dose-response curve and the variability of the data. Secondly, the NOAEL has a tendency to increase as the sample size reduces. Thirdly, the number and spacing of dose level in a study influence the close level chosen for the NOAEL (Foster and Auton 1995).

A benchmark dose (BMD) was recently proposed to determine reference dose (RfD) in quantitative risk assessment of toxicology. It is a more powerful statistical tool than the traditional approach of NOAEL and represents a step in the right direction for a more accurate risk assessment (Crump 1995). BMD, first described by Crump (1984) and Dourson et al. (1985), was developed in an attempt to remedy some notable shortcomings of the use of a NOAEL in the default approach described above. As defined by Crump (1984), BMD is the dose that corresponds to a specified level of increased response. A statistical lower confidence bound on the BMD (BMDL) has been specifically proposed as a replacement for the NOAEL in setting acceptable levels of human exposure, for added health protectiveness, and to account for statistical uncertainties (Barnes et al. 1995). The objective of the current paper is to use an example of occupational epidemiology data to explore the application of the BMD approach to determine the exposure limit of the renal dysfunction by lead.

SUBJECTS AND METHODS

Subjects

One hundred thirty-five workers exposed to lead for more than 1 year from a storage battery plant in the north part of China were selected to be the exposure group, while 143 workers (mechanics) without occupational exposure to lead or any other toxin were used as the control group. All subjects agreed to participate in the study with written informed consent and filled in the investigational forms including occupational history, disease history, medication history, and lifestyle (consumption of tobacco). Subjects with other renal diseases were excluded from the study. The status in smoking and employment period was similar between the two groups.

Methods

Blood samples were collected. Blood lead was determined using an atomic absorption spectrophotometer with a graphite furnace. All of the syringes and containers were checked for lead. The result of blood lead was within the standard value $\pm$ uncertainty degree.

Urine samples obtained from all subjects were immediately stored at $-20\,^{\circ}C$ until analysis. Urinary N-acetyl-β-D-glucosaminidase (UNAG) activity was measured by colorimetric method. Urinary β_2-microglobulin (Uβ_2-MG) was analyzed by radio immunoassay. Urinary total protein (UTP) and urinary creatinine were determined in an auto-analyzer (OLYMPUS AU2700, Japan). The concentrations of urinary NAG, urinary TP, and urinary β_2-MG were adjusted by the urinary creatinine value.

Statistics

The data were analyzed using Statistical Package for Social Sciences (SPSS, version 10.0). The distributions of values of NAG and β_2-MG were normalized through logarithmic transformation of their observed values. Geometric means of the two groups were compared. BMD was calculated using the software of BMD (Version 1.3.1; U.S. EPA 2001).

Benchmark Dose Calculation

Blood lead was considered as an exposure biomarker, while urinary NAG, urinary β_2-MG, and urinary TP were considered as effect biomarkers reflecting the damage of renal function. The BMD method was used to estimate BMD and BMDL of blood lead.

The concrete calculation procedures (U.S. EPA 2000) were as follows:

1. Determination of appropriate endpoints on which to base BMD calculation: Urinary NAG, urinary β_2-MG, and urinary TP were considered as observable variables. The dichotomized (binary) data were used as effect endpoints. The normal cut-off point was defined based on the 95th percentile of three indices of renal dysfunction in the control group. If the value was above the cut-off point, renal function was defined as abnormal (positive), otherwise as normal (negative).
2. Analysis of dose-response relationship: All research subjects were divided into six groups according to the blood lead level. The prevalence of every effect endpoint was calculated, and then the linear trend was examined. The variables only with dose-response trend could be analyzed.
3. Identification of a benchmark response (BMR) value: A 10% response level is conventionally used (for quantal endpoints) to define effective doses.
4. Selection of model to use in computing the BMD: The mathematics model, which U.S. Environmental Protection Agency (EPA) uses for quantal data, is as follows at present: Logistic, Gamma, Probit, Quantal-Linear, Quantal-Quadratic, Multi-Stage, and Weibill models. Models are ranked based on the values of their Akaike Information Criterion (AIC), a measure of the deviance of the model fit adjusted for the degrees of freedom, and the model with the lowest AIC was used to calculate the BMDL.
5. Test of goodness of fit: A value of $\alpha = 0.1$ was used to determine a critical value for goodness of fit. p values were obtained from the Chi-square test with Pearson goodness-of-fit test; if $p > 0.05$, the equation is a good fit.
6. Calculation of BMD and BMDL.

RESULTS

Changes of Renal Function at Different Levels of Blood Lead

The 40 μg/dL of blood lead was the biological exposure limit in China. The exposure group was divided into two groups: (1) high blood lead level $\geq$40 μg/dL (87 people) and (2) low blood lead level $<$40 μg/dL (48 people). Table 1 shows that the levels

TABLE 1 **Renal function in control and exposure groups**

Group	N	UTP (mg/g Cr, $\bar{x} \pm s$)	Uβ_2-MG (μg/g Cr, G $\pm$ s)	UNAG (U/g Cr, G $\pm$ s)
Control	143	44.49 $\pm$ 26.35	64.98 $\pm$ 2.35	7.49 $\pm$ 2.04
low blood Pb	48	66.18 $\pm$ 57.86*	104.98 $\pm$ 1.83*	12.64 $\pm$ 1.53*
high blood Pb	87	65.51 $\pm$ 25.96*	124.34 $\pm$ 2.14*	14.74 $\pm$ 1.65*
F		13.75	19.87	37.03

*compared with control group, $p < 0.05$.

of blood lead, urinary TP, β_2-MG, and NAG in either the high or low blood lead group were higher than those in the control group. However, there was no significant difference between the two exposing groups.

Prevalence of Abnormality of Renal Function at Different Levels of Blood Lead Groups

On the basis of the 95th percentile of three parameters of renal function in the control group, the normal cut-off value was defined. If the value was above the cut-off value, renal function was defined as abnormal (positive), otherwise as normal (negative). The cut-off values of urinary TP, β_2-MG, and NAG were 89.70 mg/g Cr, 386.19 μg/g Cr, and 19.26 U/g Cr, respectively.

The prevalence of abnormality of urinary TP, β_2-MG, and NAG in all subjects at different levels of blood lead was calculated and is shown in Table 2. The prevalence of abnormality of urinary TP, β_2-MG, and NAG was proportional to blood lead levels. There was statistical significant difference.

Determination of BMD and BMDL

BMDS Version 1.3.1 was used to calculate BMD and BMDL of blood lead based on urinary TP, β_2-MG, and NAG parameters. Urinary NAG used Logistic model; urinary TP and β_2-MG used Probit model. The results are shown in Table 3 and Figure 1. The BMD and BMDL of blood lead to affect renal function were estimated to be 323.6 to 754.3 μg/L and 274.2 to 541.5 μg/L. Urinary NAG might be a sensitive biomarker.

TABLE 2 **Prevalence of renal dysfunction indices at different concentrations of blood lead groups**

Blood Pb (μg/ L)	N	UTP	Uβ_2-MG	U NAG
−110	52	1.92	1.92	3.85
110∼−	77	6.49	5.19	7.79
210∼−	37	18.92	8.11	16.22
310∼−	22	4.55	0.00	9.09
410∼−	35	8.57	5.71	20.00
510∼−	55	20.00	12.73	38.18
Linear trend test				
χ^2		7.654	3.872	26.286
p		$p < 0.01$	$p < 0.05$	$p < 0.01$

DISCUSSION

A primary objective in quantitative risk assessment is characterization of the severity and likelihood of an adverse effect caused by lead. The BMD method has been recommended to replace the NOAEL approach in health risk assessment of chemical substances. In the present article, we used the method of BMD developed by the EPA to estimate BMD and BMDL of blood lead that resulted in renal toxicity of workers occupationally exposed to lead. BMD and BMDL of blood lead were calculated as 10% response level of renal damage using BMDS Version 1.3.1. The study indicated that the values of BMD and BMDL of blood lead were 323.6 to 754.3 μg/L and 274.2 to 541.5 μg/L, respectively. The value of BMDL in urinary NAG was the smallest among the three indices of renal damage. Therefore, urinary NAG activity could serve as a sensitive indicator for detecting early renal damage. It was suggested that the biological exposure limit of renal injury in workers occupationally exposed to lead was 270 μg/L. This value is lower than 400 μg/L as reported by Osterloh et al. (1989). Therefore, it is more accurate and sensitive in the risk assessment of workers exposed to lead.

In studies of health effects caused by exposure to lead, blood lead concentration is often used as an indicator of internal dose. Blood lead concentration is closely related to the lead poisoning degree (WHO 1980). There is a close relationship between blood lead concentration and other indices. Blood lead is usually used as a criterion of assessing other indices. Therefore, we consider blood lead to be a useful indicator of the internal dose of lead exposure.

TABLE 3 **BMD and BMDL of blood lead of renal dysfunction indices**

Index	b_0	b_1	BMD (μg/L)	BMDL (μg/L)	χ^2	*P*
UTP	−1.810	0.0014	525.6	397.6	8.31	0.08
Uβ_2-MG	−3.494	0.0021	754.3	541.5	3.04	0.55
UNAG	−1.736	0.0020	323.6	274.2	2.05	0.73

p values were obtained from the Chi-square test with Pearson goodness-of-fit test; if $p > 0.05$, the equation is a good fit.

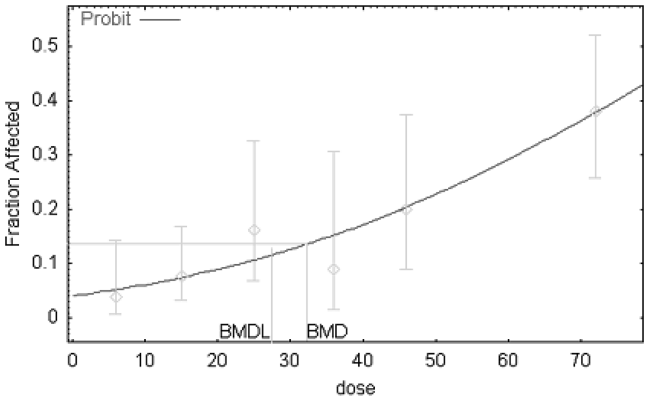

FIGURE 1 **Probit Model with 0.95 Confidence Level for BPb on UNAG.**

Urinary total protein is considered to reflect glomerular and tubular dysfunction induced by lead exposure, and urinary NAG and β_2-MG are considered to reflect renal tubular dysfunction. Cui et al. (2005) also demonstrated that these three parameters were useful as indicators of renal dysfunction in an investigation of renal effects caused by exposure to lead. Thus, we used these three parameters as indicators of renal dysfunction.

At present, there is a trend to use the BMD method to determine RfD. The main advantage of the BMD method is to utilize more available dose-response information by fitting a mathematical model to the data. It uses the whole range of experimental dose-response data in its determination of the RfD as compared to the NOAEL, in which only a single investigator-selected data point can be used (Gaylor et al. 1998). Secondly, the benchmark reflects sample size more appropriately than a NOAEL. Smaller studies tend to result in a smaller BMDL, whereas the opposite is true for NOAEL (Edler et al. 2002). The EPA has employed this method to determine RfD and reference air concentration (RfC) for a number of substances. Numerous investigators have used the BMD method to analyze dose-response toxicity data for diverse effects including neurotoxicity, developmental toxicity, and nephrotoxicity (Reiss and Gaylor 2005; Rabovsky et al. 2001; Jin et al. 2004; Uno et al. 2005).

To some noncancer health effects, NOAEL was divided by an uncertainty factor to derive a safe level for human exposure such as RfD. Generally speaking, the BMD method offering estimation tended to be lower compared with NOAEL results. The research materials were rooted in worker population. The assumption of an uncertainty factor of 10 was excluded for interspecies extrapolation from experimental animals to humans. After a suitable dose-response curve has been fit to the experimental data, the BMDL is defined as a lower confidence limit on the exposure level that corresponds to a specified excess risk (e.g., 10%) above background. The exposure level itself is the RfD, or the biological exposure limit.

CONCLUSION

The BMD method attempts to use more of the available dose-response information by fitting a mathematical model to the data and then determining the dose associated with a specified response level. It is feasible to use the BMD approach in setting up RfD and RfC. The BMD approach provides a new and better way to determine the RfD/RfC. However, a large amount of additional epidemiological data is needed to prove its practicability before being widely accepted.

REFERENCES

Barnes, D. G., Daston, G. P., Evans, J. S., Jarabek, A. M., Kavlock, R. J., Kimmel, C. A., Park, C., and Spitzer, H. L. 1995. Benchmark Dose Workshop: criteria for use of a benchmark dose to estimate a reference dose. *J. Regul. Toxicol. Pharmacol.* 21:296–306.

Crump, K. S. 1984. A new method for determining allowable daily Intakes. *J. Fundam. Appl. Toxicol.* 4:854–871.

Crump, K. S. 1995. Calculation of benchmark doses from continuous data. *J. Risk Anal.* 15(1):79–89.

Cui, Y., Zhu, Y. G., Zhai, R., Huang, Y., Qiu, Y., and Liang, J. 2005. Exposure to metal mixtures and human health impacts in a contaminated area in Nanning, China. *J. Environ. Int.* 31(6):784–790.

Dourson, M. L., Hertzberg, R. C., Hartung, R., and Blackburn, K. 1985. Novel methods for the estimation of acceptable daily intake. *J. Toxicol. Ind. Health* 1(4):23–33.

Edler, L., Poirier, K., Dourson, M., Kleiner, J., Mileson, B., Nordmann, H., Renwick, A., Slob, W., Walton, K., and Wurtzen, G. 2002. Mathematical modeling and quantitative methods. *J. Food Chem. Toxicol.* 40:283–326.

Ehrlich, R., Robins, T., Jordaan, E., Miller, S., Mbuli, S., Selby, P., Wynchank, S., Cantrell, A., De Broe, M., D'Haese, P., Tood, A., and Landrigan, P. 1998. Lead absorption and renal dysfunction in a South African battery factory. *J. Occup. Environ. Med.* 55:453–460.

Fels, L. M., Wunsch, M., Baranowski, J., Norska-Borowka, I., Price, R. G., and Taylor, S. A. 1998. Adverse effects of chronic low level lead exposure on kidney function—a risk group study in children. *J. Nephrol. Dial. Transplant* 13:2248–2256.

Foster, P. M., and Auton, T. R. 1995. Application of benchmark dose risk assessment methodology to developmental toxicity: an industrial view. *J. Toxicol. Lett.* 82–83:555–559.

Gaylor, D., Ryan, L., Krewski, D., and Zhu, Y. 1998. Procedures for calculating benchmark doses for health risk assessment. *J. Regul. Toxicol. Pharmacol.* 28(2):150–164.

Gurer-Orhan, H., Sabir, H. U., and Ozgunes, H. 2004. Correlation between clinical indicators of lead poisoning and oxidative stress parameters in controls and lead-exposed workers. *J. Toxicol.* 195:147–154.

Jin, T., Wu, X., and Tang, Y. 2004. Environmental epidemiological study and estimation of benchmark dose for renal dysfunction in a cadmium-polluted area in China. *J. Biometals* 17:525–530.

Levin, S. M., and Goldberg, M. 2000. Clinical evaluation and management of lead-exposed construction workers. *J. Am. J. Ind. Med.* 37:23–43.

McClellan, R. O. 1999. Human health risk assessment. A historical overview and alternative paths forward. *J. Inhal. Toxicol.* 11:477–518.

Osterloh, J. D., Selby, J. V., Bernard, B. P., Becker, C. E., Menke, D. J., Tepper, E., Ordonez, J. D., and Behrens, B. 1989. Body burdens of lead in hypertensive nephropathy. *J. Arch. Environ. Health* 44: 304.

Rabovsky, J., Fowles, J., Hill, M. D., and Lewis, D. C. 2001. A health risk benchmark for the neurologic effects of styrene: comparison with NOAEL/LOAEL approach. *J. Risk Anal.* 21(1):117–126.

Reiss, R., and Gaylor, D. 2005. Use of benchmark dose and meta-analysis to determine the most sensitive endpoint for risk assessment for dimethoate. *J. Regul. Toxicol. Pharmacol.* 43:55–65.

Staessen, J. A., Robert, R., Lauwerys, R. R., Buchet, J. P., Bulpitt, C. J., Rondia, D., Vanrenterghem, Y., and Amery, A. 1992. Impairment of renal function with increasing blood lead concentrations in the general population. *N. Engl. J. Med.* 327:151–156.

Uno, T., Kobayashi, E., Suwazono, Y., Okubo, Y., Miura, K., Sakata, K., Okayama, A., Ueshima, H., Nakagawa, H., and Nogawa, K. 2005. Health effects of cadmium exposure in the general environment in Japan with special reference to the lower limit of the benchmark dose as the threshold level of urinary cadmium. *J. Scand. J. Work Environ. He* 31:307–315.

U. S. EPA. 2000. Benchmark dose technical guidance document. *J. Risk Assessment Forum.*1–87.

U. S. EPA. 2001. BMDS Benchmark Dose Software. For software background and tutorial see: http://www.epa.gov/NCEA/bmds_training/index.htm.

Wang, V. S., Lee, M. T., Chiou, J. Y., Guu, C. F., Wu, C. C., and Lai, J. S. 2002. Relationship between blood lead levels and renal function in lead battery workers. *J. Int. Arch. Occup. Environ. Health* 75:569–575.

WHO. 1980. Recommended health-based limits in occupational exposure to heavy metal. Tech. Res. Ser No 647, Geneva: WHO.