The Reference Dose for Subchronic Exposure of Pigs to Cadmium Leading to Early Renal Damage by Benchmark Dose Method

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Pigs were exposed to cadmium (Cd) (in the form of CdCl₂) concentrations ranging from 0 to 32 mg Cd/kg feed for 100 days. Urinary cadmium (U-Cd) and blood cadmium (B-Cd) levels were determined as indicators of Cd exposure. Urinary levels of β₂-microglobulin (β₂-MG), α₁-microglobulin (α₁-MG), N-acetyl-β-D-glucosaminidase (NAG), cadmium-metallothionein (Cd-MT), and retinol binding protein (RBP) were determined as biomarkers of tubular dysfunction. U-Cd concentrations were increased linearly with time and dose, whereas B-Cd reached two peaks at 40 days and 100 days in the group exposed to 32 mg Cd/kg. Hyper-metallothionein-urinary (HypermTuria) and hyper-N-acetyl-β-D-glucosaminidase-urinary (hyperNA-Guria) emerged from 80 days onwards in the group exposed to 32 mg Cd/kg, followed by hyper-β₂-microglobulin-urinary (hyperβ₂-MGuria) and hyper-retinol-binding-protein-urinary (hyperRBPuria) from 100 days onwards. The relationships between the Cd exposure dose and biomarkers of exposure (as well as the biomarkers of effect) were examined, and significant correlations were found between them (except for α₁-MG). Dose–response relationships between Cd exposure dose and biomarkers of tubular dysfunction were studied. The critical concentration of Cd exposure dose was calculated by the benchmark dose (BMD) method. The BMD₁₀/BMDL₁₀ was estimated to be 1.34/0.67, 1.21/0.88, 2.75/1.00, and 3.73/3.08 mg Cd/kg feed based on urinary RBP, NAG, Cd-MT, and β₂-MG, respectively. The calculated tolerable weekly intake of Cd for humans was 1.4 μg/kg body weight based on a safety factor of 100. This value is lower than the currently available values set by several different countries. This indicates a need for further studies on the effects of Cd and a re-evaluation of the human health risk assessment for the metal.

Key Words: cadmium; renal dysfunction; biomarkers; benchmark dose; subchronic exposure.

Cadmium (Cd) is a relatively rare element that occurs naturally in ores together with zinc and lead. Industrial uses of Cd and agricultural uses of phosphate fertilizers have caused widespread dispersion of the metal at trace levels into the environment and human foodstuffs (Satarug et al., 2003; WHO, 1992). Cd is efficiently retained in the kidney and liver in human body through dietary exposure, with a very long biological half-life ranging from 10 to 30 years (Nordberg, 2007; Staessen, 1994; WHO, 1992). Cd can cause several adverse health effects. Together with other heavy metals such as lead and mercury, Cd is considered to be a threat to human health (Jarup, 2003; WHO, 2000).

Kidney is the critical target organ for dietary exposure to Cd. Renal damage is characterized by Cd accumulation in convoluted proximal tubules followed by glomerular damage (Åkesson et al., 2005; Jarup and Åkesson, 2009; Suwazono Y., 2010; WHO, 1996). On the basis of epidemiological data, the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives set the provisional tolerable weekly intake (PTWI) of Cd at 7 μg/kg body weight, which corresponds to 1 μg/kg body weight for each day of the week (i.e., 70 μg/day for a person of 70-kg body weight). Recently, the European Food Safety Authority (EFSA) carried out a meta-analysis applying the benchmark dose (BMD) approach (EFSA, 2009). Based on the one-compartment model, translating urine cadmium (U-Cd) into dietary exposure (Amzal et al., 2009), EFSA arrived at a tolerable weekly intake (TWI) of 2.5 μg Cd/kg body weight, which was much lower than the provisional TWI of 7 μg Cd/kg body weight established by the Joint FAO/WHO Expert Committee on Food Additives. Furthermore, emissions of Cd from various industries and the combustion of waste and fossil fuels have resulted in a large increase in the concentrations of Cd in soils over the last century (Jarup et al., 1998; Thomas et al., 2009). The high transfer rate of Cd from soil to plants, coupled with continuing mobilization of small amounts of the metal from non-bioavailable geologic matrices into biologically accessible media, led to the prediction that human exposure to dietary Cd will gradually increase in the next 10–20 years (Satarug and Moore, 2004). Exposure to Cd in the general population is already close to the critical level, particularly in susceptible population groups and populations living close to polluting industries. However, the concentration
above which early effects occur is unknown, and the dose–response relationships at low-level exposure have not been well established. Interestingly, several epidemiological studies on Cd-induced renal effects in the 1990s and 2000s focused on the early effects of Cd on the kidney based on a low-level subchronic exposure, but very few animal studies were carried out.

The purpose of this study was to determine the dose–response relationship and lower 95% confidence limit of the benchmark dose (BMDL) based on Wuzhishan pigs (WZSPs) that were subchronically exposed to relatively low and environmentally realistic concentrations of Cd. Pigs were chosen as the model because they are omnivores and their renal toxic metabolic function is similar to that of humans (Feng et al., 1999). Low doses of Cd were added to the feed during subchronic exposure. We focused on the concentration of cadmium in blood (B-Cd) and urine (U-Cd) referred to as the “internal dose.” Urinary β2-microglobulin (β2-MG), α1-microglobulin (α1-MG), N-acetyl-β-D-glucosaminidase (NAG), cadmium-metallothionein (Cd-MT), and retinol binding protein (RBP) were detected as biomarkers of tubular dysfunction. The BMD method was used to quantitatively estimate the BMDL based on the data of β2-MG, α1-MG, NAG, Cd-MT, and RBP. Determination of these values may help in the detection of early events of chronic intoxication by Cd and provide a scientific basis for monitoring Cd to prevent overexposure.

MATERIALS AND METHODS

Ethical approval of the study protocol. The study protocol was approved by the Ethics Committee of the Institute of Agro-Food Science and Technology (Beijing, China).

Experimental setup. Twenty WZSPs (F18, i.e., 2.5–3 months) of inbred strain (inbreeding coefficient, 0.979) were purchased from the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (Beijing, China). They were housed under conventional conditions at 24–32°C, with natural light and humidity of 45–65%. They were fed twice a day (7:30 am and 4:30 pm). The feed intake was 3% of body weight (Feng et al., 1999). The feed was mainly composed of 67.6% corn, 5% wheat, 20% soybean meal, and 7.4% concentrates. The concentrates contained fish meal, cottonseed meal, soy protein concentrate, copper (Cu), iron (Fe), zinc (Zn), manganese (Mn), vitamin A, vitamin C, vitamin E, and lysine. The concentrations of Cu, Fe, Zn, Mn, Pb are 144.02, 55.26, 161.27, 135.28, and 0.046 mg/kg feed, respectively. Tap water (pH, 6.5–6.8; Cd concentration < 0.001 μg/L) was supplied continuously. Pigs were weighed randomly and subdivided into five groups (four each group). They were exposed to 0, 0.5, 2.8, and 32 mg Cd/kg in the feed for 100 days, respectively. Cd was added to the feed in the form of cadmium chloride (CdCl2 > 99%; Shanghai Chemical Reagent Company, Shanghai, China). Pigs were weighed before exposure and placed in a metabolic cage for urine collection. Drinking water was available ad libitum in the metabolic cage. Body weight was measured every 20 days. The amount of feed was changed according to the total body weight of each group. During the exposure period, pigs were regularly placed in metabolic cages (every 20 days) and blood samples were obtained from the anterior vena cava.

Preparation and bioanalyses of samples. Urine samples were collected from pigs, placed in polyethylene bottles and stored at −70°C until analyses. Before collection of samples, all the empty bottles were washed with dilute nitric acid followed by deionized water to ensure they were metal-free prior to use. Each urine sample was split into four parts (5 ml each) immediately after being collected. The first was acidified with 2% (V/V) concentrated nitric acid and used for a Cd assay; the second was used to measure β2-MG levels after being adjusted pH to 7–9 with NaOH; the others were used to determine levels of creatinine, α1-MG, NAG, Cd-MT, and RBP without pretreatment. Levels of β2-MG, α1-MG, NAG, Cd-MT, and RBP were measured by an enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN).

Samples of urine and blood for Cd determination were digested using a microwave accelerated reaction system (MARS; CEM Company, Newark, CA). About 1 ml of the urine or blood, 6 ml of 65% metal-oxide-semiconductor grade (MOS-grade) HNO3 (Beijing Institute of Chemical Reagents, Beijing, China) and 2 ml of 30% MOS-grade H2O2 (Beijing Institute of Chemical Reagents) were added into a teflon digestion vessel and digested for 50 min by increasing the power to 1600 W and the temperature to 175°C in a stepwise fashion. The digested solution was quantitatively transferred into a 50-ml teflon volumetric flask and made up to volume with 18.2 MΩ-cm water (Milli-Q; Millipore, Bedford, MA). Cd concentrations in urine and blood were measured by a graphite furnace atomic absorption spectrometry (GF-AAS) with an AA-7000 machine (Shimadzu, Japan) using standard calibration curves. Creatinine was measured by the Jaffe reaction method. All urinary parameters were adjusted for creatinine in urine.

BMD method. Crump et al. (1984) defined the BMD as the lower confidence limit to a dose producing a predetermined increase in response such as 1–10%. The BMDL is gradually replacing the no-observed-adverse-effect level (NOAEL) in risk assessment (EFSa, 2009; U.S. EPA, 1995). The BMD approach involves the use of a statistical method that is based on the information in the complete dataset instead of making pairwise comparisons using subsets of data. Therefore, the BMD approach utilizes the entire dose–response curve and gives more information than a NOAEL by explicitly indicating the upper bound of effect at that dose as defined by the benchmark response (BMR), and BMR values of 1%, 5%, and 10% extra risk have been proposed to be used for quantal data from animal studies. Use of a 5% BMR is practicable for some but not all toxicity datasets and 10% appears more appropriate for quantal data because the BMDL can become substantially dependent on the choice of the dose-response model at lower BMRs (EFSa, 2009). In this study, a level of risk of 10% was used based on the data of β2-MG, α1-MG, NAG, Cd-MT, and RBP. The model is accepted from a nested family of models by comparing the log-likelihoods of various members in that family using likelihood ratio tests. A member with fewer parameters is selected if it does not show a significantly poorer fit (EFSa, 2009). The lowest BMDL among all the endpoints was chosen in the present study.

RfD calculated method. Traditionally, a safety factor (SF) of 100 would be used for reference dose (RfD) calculations to extrapolate from a well-conducted animal bioassay (10-fold factor animal to human) and to account for human variability in response (10-fold factor human-to-human variability) (Klaassen, 2001). The RfD was calculated using the formula:

\[ \text{RfD} = \frac{\text{BMDL}}{\text{Feed intake} \times \text{Body weight} \times \text{SF}} \]

Statistical methods. Statistical analyses were undertaken using SPSS statistical package version 18.0 (SPSS Inc., Chicago, IL, USA). Correlation between pairs of variables was analyzed by Pearson’s correlation test. One-way ANOVA was used to examine if there were significant differences between different groups at the 95% confidence level. Dunnett’s multiple comparison was then applied to compare means. The BMD and BMDL were calculated using Benchmark Dose Software (BMDS), Version 2.1.1 (U.S. EPA).

RESULTS

Gain in Body Weight

During subchronic exposure, all pigs were increased in weight (Fig. 1). From 40 days on, the group exposed to 0.5 mg
Cd/kg appeared to grow faster than the control group, and the differences were significant at the 0.05 level. The p values for 40, 60, 80, and 100 days were 0.046, 0.023, 0.048, and 0.031, respectively. The differences in other dose groups were not significant compared with the control group.

Daily Intake of Cd

Feed consumption was not reduced due to the fact that all feed given have been consumed during the entire experiment. The actual concentrations of Cd in feed and mean daily intake of Cd are shown in Table 1.

Cd Measurements in Blood and Urine

B-Cd levels (Fig. 2A) were increased in a dose- and time-dependent manner up to 40 days of exposure. Starting from 40 days of exposure, the group exposed to 32 mg Cd/kg reached a peak phase with a maximal group mean ($\mu g CAD/L (n = 4)$) whereupon the B-Cd level decreased. After 60 days of exposure, B-Cd levels increased linearly in the highest concentration group, reaching the second peak value (Mean ± SD) of 19.0 ± 1.3 $\mu g CAD/L (n = 4)$ at the 100-day time point of exposure.

Cd administration resulted in a dose- and time-dependent increase in U-Cd (Fig. 2B), reaching maximal values of 74.7 ± 4.3 $\mu g/g (n = 4)$ creatinine after 100 days of exposure in the highest concentration group. Correlation analyses showed high correlations between Cd exposure dose, B-Cd, and U-Cd (Table 2).

Relationship Between Cd Exposure and Indicators of Renal Dysfunction

UCd-MT, NAG, $\beta_2$-MG, $\alpha_1$-MG, and RBP were used as biomarkers of Cd-induced renal damage. Changes in biomarker concentrations with time and dose were shown in Figure 3. Excretions of UCd-MT and NAG significantly increased (2.3 times for Cd-MT, 1.9 times for UNG) from pigs exposed to the 32 mg Cd/kg from 80 days onwards ($p = 0.02$ and $p = 0.014$, respectively, compared with the control group). Levels of $\beta_2$-MG and RBP in the highest dose group significantly increased (2.2 times for $\beta_2$-MG, 2.1 times for RBP) from 100 days onwards ($p = 0.004$ and $p = 0.002$, respectively, compared with the control group).

Correlation analyses showed that there were significant correlations between the dose of Cd exposure and levels of UCd-MT, NAG, $\beta_2$-MG, and RBP. There were also high correlations between the biomarkers except for $\alpha_1$-MG. The correlation coefficients are shown in Table 2. The results indicated strong relationships among these variables.

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### TABLE 1

<table>
<thead>
<tr>
<th>Dose group (mg Cd/kg)</th>
<th>Actual dose (mg CdCl/kg)</th>
<th>Cd intake (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control group)</td>
<td>0.181 ± 0.096</td>
<td>0.07</td>
</tr>
<tr>
<td>0.5</td>
<td>0.430 ± 0.083</td>
<td>0.32</td>
</tr>
<tr>
<td>2</td>
<td>2.171 ± 0.465</td>
<td>1.26</td>
</tr>
<tr>
<td>8</td>
<td>8.192 ± 0.705</td>
<td>5.40</td>
</tr>
<tr>
<td>32</td>
<td>31.543 ± 1.948</td>
<td>18.72</td>
</tr>
</tbody>
</table>

**Note.** Mean daily intake of cadmium per dose group was calculated using the formula: \[(BW1 + \ldots + BW5) \times 3\% \times \text{actual concentration}/5\]; BW1–BW5 represents body weight measured at 0, 20, 40, 60, and 80 days, respectively. Data are means ± SEM (n = 4).
Dose–Response Relationships Between Cd Exposure and Indicators of Renal Dysfunction

As described previously, there was a significant increase in the levels of biomarkers of renal damage Cd dose in a dose-dependent manner from 100 days onwards. According to the BMD method, dose–response relationships were described between the Cd administered dose and indicators of renal dysfunction, and the BMD was calculated. The estimated parameters and corresponding values of the BMDL of Cd exposure dose for a 10% level of risk above the background level (BMDL_{10}) are presented in Table 3. When levels of UCd-MT, NAG, β_{2}-MG, and RBP were used as indicators of renal dysfunction, the estimated BMDL_{10} was 1.00, 0.88, 3.08, and 0.67 mg Cd/kg feed, respectively. The RBP gave the lowest value for exposure dose of Cd, which is the overall BMDL_{10} for the present study. The calculated RfD of Cd for humans was 0.2 μg/kg. The dose–response curves for various indicators were plotted in Figure 4.

\[
RfD = \frac{(BMDL_{10} \times \text{Feed intake})/(\text{Body weight} \times \text{SF})}{100} = 0.2 \text{ μg/kg}
\]

DISCUSSION

Animal Model

We attempted to elucidate the effects of environmentally relevant doses of Cd in a subchronically exposed animal model and tried to clarify the dose–response relationship between the development of renal damage and dose of Cd exposure.
FIG. 3. Model of urinary Cd-MT (3A), NAG (3B), RBP (3C), and β2-MG contents as a function of time. Each point represents the mean value. SEM is not marked in the plot for clarity. Animal exposed to 32 mg Cd/kg showed signs of hyperMTuria and hyperNAGuria from 80 days of exposure, and hyperβ2-MGuria and hyperRBPuria from 100 days of exposure. *p < 0.05, **p < 0.01.

TABLE 2

<table>
<thead>
<tr>
<th>Exposure dose</th>
<th>B-Cd</th>
<th>U-Cd</th>
<th>Cd-MT</th>
<th>NAG</th>
<th>β2-MG</th>
<th>α1-MG</th>
<th>RBP</th>
</tr>
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<tbody>
<tr>
<td>B-Cd</td>
<td>0.984**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U-Cd</td>
<td>0.997**</td>
<td>0.975**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd-MT</td>
<td>0.947*</td>
<td>0.982**</td>
<td>0.928*</td>
<td>1</td>
<td>0.997**</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>NAG</td>
<td>0.966**</td>
<td>0.994**</td>
<td>0.950**</td>
<td>0.996**</td>
<td>0.927*</td>
<td>0.951*</td>
<td>1</td>
</tr>
<tr>
<td>β2-MG</td>
<td>0.991**</td>
<td>0.979**</td>
<td>0.996**</td>
<td>0.927*</td>
<td>0.951*</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>α1-MG</td>
<td>0.681</td>
<td>0.578</td>
<td>0.664</td>
<td>0.520</td>
<td>0.556</td>
<td>0.611</td>
<td>1</td>
</tr>
<tr>
<td>RBP</td>
<td>0.983**</td>
<td>0.980**</td>
<td>0.969**</td>
<td>0.977**</td>
<td>0.983**</td>
<td>0.956*</td>
<td>0.668</td>
</tr>
</tbody>
</table>

Note. *p < 0.05, **p < 0.01.

TABLE 3

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Model(1)</th>
<th>Log likelihood</th>
<th>BMDL0(2) (mg Cd/kg)</th>
<th>BMDLm (mg Cd/kg)</th>
<th>p(3)</th>
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<tbody>
<tr>
<td>Cd-MT</td>
<td>Hill(4)</td>
<td>28.86</td>
<td>2.75</td>
<td>1.00</td>
<td>0.820</td>
</tr>
<tr>
<td></td>
<td>Exponential</td>
<td>28.68</td>
<td>1.85</td>
<td>1.18</td>
<td>0.816</td>
</tr>
<tr>
<td>NAG</td>
<td>Hill</td>
<td>–57.41</td>
<td>2.50</td>
<td>0.97</td>
<td>0.795</td>
</tr>
<tr>
<td></td>
<td>Exponential</td>
<td>–58.30</td>
<td>1.21</td>
<td>0.88</td>
<td>0.399</td>
</tr>
<tr>
<td>β2-MG</td>
<td>Hill</td>
<td>–79.34</td>
<td>20.56</td>
<td>1.46</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>Exponential</td>
<td>–77.41</td>
<td>3.73</td>
<td>3.08</td>
<td>0.285</td>
</tr>
<tr>
<td>RBP</td>
<td>Hill</td>
<td>–54.80</td>
<td>1.34</td>
<td>0.67</td>
<td>0.575</td>
</tr>
<tr>
<td></td>
<td>Exponential</td>
<td>–54.84</td>
<td>1.43</td>
<td>0.81</td>
<td>0.552</td>
</tr>
</tbody>
</table>

Note. (1) Hill model: y = a [1 + (c – 1)r/(b^2 + r^2)]; Exponential model: y = a [c – (c – 1)exp(–bx)]. a, b, c, and d represent unknown parameters that are estimated by fitting the model to the data. The model was obtained according to the log-likelihood criterion (EFSA, 2009); (2) at a BMR of 10%; (3) optimal model needs to pass the goodness-of-fit test, if p > 0.05 then the equation is a good fit; (4) the model was accepted for the lowest BMDL.
This is one of the first studies to describe the effects of a subchronic exposure to low levels of Cd in pigs. WZSPs were chosen instead of rats or mice because WZSPs are a type of "miniature pig." The size, structure, anatomy, physiology, and toxicant metabolism of the organs of WZSPs are similar to human's (Feng et al., 1999; Min et al., 2008). WZSPs are ideal experimental models for human diseases. Ingestion is the most important route of exposure for humans, so exposure via feed was chosen. Doses of 0, 0.5, 2, 8, and 32 mg Cd/kg feed were chosen according to data on mean intake by humans as well as Cd concentration in soil from contaminated sites (Eugenio, 2008; Nordberg et al., 2007; Soisungwan et al., 2003; WHO, 2000).

Animal studies have shown a significantly smaller increase in body weight (and even weight loss) in Cd-exposed animals compared with controls (Aoyagi et al., 2003; Brzoska et al., 2003). In epidemiological studies, the body weight of individuals living in Cd-polluted regions was not different from the weight of people living in nonpolluted regions (Hogervorst et al., 2006). In this study, the Cd doses applied did not decrease the weight of pigs and, therefore, may reflect the human situation more closely, but which is inconsistent with previous animal studies, it may cause by species or exposure levels differences. The group exposed to 0.5 mg Cd/kg appeared to grow faster than the control group from 40 days onwards. A partial explanation might be that this group of pigs already had a slightly higher weight at the start of the exposure or due to insufficient sample size, the differences between animals have not been reflected. These results indicated that a low dose of Cd exposure did not affect feed consumption or inhibit growth.

Cd Contents in Blood and Urine

B-Cd levels showed a dose- and time-dependent increase for all but the group receiving 32 mg of Cd/kg. The results showed that B-Cd levels reached two peaks at certain concentrations over time. This finding is not consistent with other studies. Those studies show that B-Cd levels increased linearly upon Cd exposure until they reached a peak, and then the levels decreased, that is, only one peak appeared (Bernard et al., 1992; Thijssen et al., 2007). The main reason might be that ingested Cd is mainly bound to albumin in the early phase after single administration (via the oral route), so B-Cd levels increased with dose and time. As the administration continued, Cd bound to albumin was taken up by the liver, where the complex was split, and the synthesis of metallothionein was induced. There was an increase over time of Cd bound to metallothionein in the liver, which would cause Cd accumulation in the liver, and therefore
B-Cd levels decreased. However, after long exposure, the available Cd binding sites (metallothionein) in the liver were saturated. As the available binding sites were saturated, increased transport of Cd-MT from the liver to the kidney through the blood would occur, so that B-Cd levels increased again. A positive correlation was observed between B-Cd and exposure dose of Cd. This finding suggested that B-Cd levels, in general, reflect recent exposure rather than the accumulating burden to the body.

About 0.005–0.01% of the total Cd burden is excreted via the urine each day. U-Cd concentration is mainly influenced by the burden of Cd in the body and is proportional to the Cd concentration in the kidney. An increase in U-Cd level is thought to be caused by an increase in the death of proximal tubular cells, resulting in the release of the metal in urine (Nordberg, 2007). In previous animal studies (Aoyagi et al., 2003; Brzoska et al., 2003; Thijssen et al., 2007), a relationship between U-Cd levels and kidney dysfunction was demonstrated after 8 weeks of exposure, though U-Cd levels were high and could not be compared with data from human studies. In this study, a dose- and time-dependent increase in U-Cd level was detected, and a maximal value of 74.7±4.3 μg/g creatinine was reached after 100 days of exposure to the 32 mg Cd/kg feed, which was higher than that seen in human data and consistent with previous studies. U-Cd levels had a positive correlation with levels of U-Cd-MT, NAG, RBP, and β2-MG. This indicated that U-Cd levels reflect not only the exposure dose of Cd, but also kidney dysfunction. The U-Cd level is therefore a reliable biomarker of exposure to Cd.

Renal Dysfunction

Chronic exposure to Cd gives rise to renal tubular dysfunction (Nordberg et al., 2000; WHO, 1992), which is reflected by proteinuria. Proteinuria is principally tubular; it comprises low-molecular-weight proteins whose tubular reabsorption has been impaired following injury to the cells lining proximal tubules by Cd. Some low-molecular-weight proteins are widely used as biomarkers of early nephrotoxicity induced by Cd. The predominant proteins are β2-MG and Cd-MT, but several other low-molecular-weight proteins have been identified in urine such as RBP, lysozyme, NAG, ribonuclease, α-MG, and immunoglobulin light chains (Buchet et al., 1990; Chen et al., 2006; Klaassen, 2001; Lauwersys et al., 1994; Nordberg et al., 2007). However, none of the biomarkers of tubular dysfunction is a specific indicator. Their levels must be interpreted in association with a corresponding increase in U-Cd or used together to assess renal dysfunction. Minor increases in the excretion of biomarkers of renal tubular dysfunction may not in themselves indicate adverse health effects, but they are indicators of probable progression of renal disease if Cd exposure continues (Järup et al., 1995; Klaassen, 2001). Therefore, these indicators are used as biomarkers of early nephrotoxicity induced by Cd.

This study showed the existence of renal tubular dysfunction. HyperMTuria and hyperNAGuria emerged from 80 days or longer exposure in the group exposed to the 32 mg Cd/kg feed, followed by hyperβ2-MGuria and hyperRBPuria with 100 days of exposure. Statistically significant positive correlations were observed between increased urinary excretion of Cd, exposure dose of Cd, and urinary indicators of renal dysfunction. These findings are similar to those of Nomiyama et al. (1979), which showed that increased urinary concentrations of RBP were evident after 12 weeks in monkeys exposed to 300 mg Cd/kg food, followed by proteinuria and glucosuria after 16 weeks. Brzoska et al. (2003) also found that increased urinary activities of NAG and alkaline phosphatase emerged after 6 weeks in rats exposed to 50 mg Cd/l in drinking water with a K-Cd concentration of 24.1±1.7 μg/g.

No chelation treatment could have reduced the burden of Cd in human bodies. Therefore, understanding the adverse effects of Cd on human health is crucial for taking preventive measures. The American Conference of Governmental Industrial Hygienists and the Chinese National Health Standards have recommended a limit value of U-Cd concentration of 5.00 μg/g creatinine for Cd exposure. The Cadmium in Belgium (CadmiBel) study (a classic and frequently cited study of the effects of Cd on the general population) indicated that >10% of values would be abnormal if the excretion rate of Cd in urine was as follows (in μg/24 h): >2.87 for RBP, 3.05 for β2-MG, 4.29 for amino acids, and 1.92 for Cd (Bernard et al., 1992; Buchet et al., 1990). Chen et al. (2004), by studying an occupational population in China, demonstrated that the BMDL10 of U-Cd values (in μg/g creatinine) with hyperNAGuria was 2.7, HyperMTuria was 3.1, and hyperβ2-MGuria was 3.4. As pointed out by Suwazono et al. (2010) in a review of reference dose of Cd for renal effects, all recent findings suggested that the critical concentrations of Cd in urine and the kidney have been overestimated. All these studies used U-Cd as the indicator of the internal dose of Cd exposure to calculate the reference value for renal effects.

In this study, the external dose of Cd exposure was used to calculate the reference value for renal effects (the BMDL). This is the first estimation of the BMD for Cd-associated renal effects in an animal model. The results showed that BMD and BMDL values differed depending upon the urinary parameter of renal tubular dysfunction used. A safety factor of 100 was used to assess a tolerable daily intake (TDI) of 0.2 μg/kg body weight, which is likely to be greater than when calculated with individual data due to the fact that group means of TDI values were used (EFSA, 2009). These results of various Cd exposure for renal effects were generally lower than the reference exposure expected from earlier studies (7 μg/kg body weight), suggesting the importance of further discussion regarding comprehensive measures to decrease Cd exposure in the general population. To establish the comprehensive reference dose for Cd-induced health effects, further
application of the BMD method should be expanded to other animal models such as rats.

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**REFERENCES**


