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Use of Urinary β_2-Microglobulin in the Assessment of the Health Risk from Environmental Cadmium Exposure

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Abstract

Cadmium (Cd) is a ubiquitous environmental pollutant with no nutritional value or physiological role in the body. It readily accumulates in various tissues as it is easily absorbed from the diet but only poorly excreted. Due to the widespread contamination of staple foods, exposure to this toxic metal is inevitable for most people. The health risk due to dietary Cd exposure has long been underappreciated. This is primarily due to the use of urinary excretion of β_2-microglobulin (β_2M) as an indicator of an adverse health effect. This study employed advanced benchmark dose (BMD) modeling in a Thai cohort (n = 799) to reassess health risks from dietary Cd exposure. The BMD limit for urinary Cd was identified as 0.17 μg/g creatinine when using a reduction in estimated glomerular filtration rate (eGFR) as the endpoint, whereas no reliable BMD could be established using β_2-microglobulin excretion. Given that eGFR reduction is a more reliable indicator of chronic kidney disease, its use is recommended for deriving health-protective guidelines. The findings demonstrate that the current urinary Cd threshold of 5.24 μg/g creatinine is inadequate, supporting the adoption of a threshold below 0.20 μg/g creatinine in future exposure guidelines.

Keywords: β_2-microglobulin; benchmark dose; cadmium; dietary exposure; estimated glomerular filtration rate; health risk; threshold

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1. Introduction

Consumption of rice contaminated with the toxic metal cadmium (Cd) can cause itai-itai disease, marked by loss of kidney mass, osteoporosis, and osteomalacia [1–4]. Based on a lifetime intake of 2 g of Cd, and an excretion of β_2-microglobulin (β_2M) at a rate above 300 μg/g creatinine (cr), a tolerable Cd intake level was estimated to be 0.83 μg/kg b.w./d (58 μg/d for a 70 kg person), with a threshold of 5.24 μg/g cr [5]. By definition, a threshold level for an adverse effect of any food contaminant reflects the exposure level from a normal diet that can produce an adverse effect in 5% of the general populations [6–9]. However, rates of many Cd-related health effects, notably chronic kidney disease (CKD), are higher than the acceptable environmental-related disease prevalence of 5%.

The diagnosis of CKD is based on a fall of estimated glomerular filtration rate (eGFR) to one-third of a normal range (60 mL/min/1.73 m^2) and/or the presence of albuminuria for at least 3 months [10–12]. Presently, CKD affects 8–13% of the adult population worldwide, and it has now reached epidemic proportions in many parts of the world [13–15]. A

dose–response relationship between Cd exposure and CKD risk has been reported in a meta-analysis by Doccioli et al. [13]. The urinary Cd excretion levels associated with an increased risk of CKD were below a Cd excretion threshold of 5.24 μg/g cr. These human population data imply that current Cd exposure guidelines do not afford adequate health protection. Concerningly, CKD is predicted to be the fifth-leading cause of years of life lost by 2040 [14,15]. Developing strategies to prevent CKD and to reduce its progression to kidney failure is thus of great public health significance.

Dietary Cd exposure guidelines were also determined by the European Food Safety Agency (EFSA) and US Food and Drug Administration (US FDA) [6]. The EFSA guideline describes a reference dose (RfD), while the US FDA guideline describes a toxicological reference value (TRV). Notably, however, most countries adopted the JECFA guidelines [5].

The present work has three main objectives; firstly, to apply advanced benchmark dose (BMD) modeling to define the BMD limit (BMDL) value for urinary Cd excretion, which may have a negligible impact on the kidneys [16–19]. The BMDL figure for any health-hazardous substance in foodstuffs has now become a replacement for the no-observed-adverse-effect level (NOAEL) [18,19]. To the best of our knowledge, the present study is the first report of estimating a urinary Cd threshold based on a clinically relevant outcome.

The second objective it was to ascertain whether there is a significant impact of Cd exposure on CKD risk. To this end, urinary Cd excretion rate was used as an indicator of kidney burden, while changes in eGFR and β_2M excretion reflected adverse effects on the kidneys. Data were from a Thai cohort of 799 individuals, 18–87 years of age, who did not have diabetes nor had workplace exposure to metals.

The third objective was to reiterate that the practice of adjusting urinary concentrations of Cd and β_2M to creatinine excretion (E_{cr}) can obscure Cd's effects on the kidneys. This phenomenon is described as reverse causality due to the imprecision in measuring Cd exposure dose levels and its effects [20]. These biases have led to erroneous conclusions that Cd did not diminish eGFR nor did it promote progressive eGFR reduction toward kidney failure [21,22]. Accordingly, results of Cd nephrotoxicity assessment obtained from adjusting excretion rates of Cd and β_2M to E_{cr} as E_{Cd}/E_{cr} were compared with those adjusted by creatinine clearance (C_{cr}) as E_{Cd}/C_{cr} and $E_{\beta 2M}/C_{cr}$.

2. Materials and Methods

2.1. Data Sourcing

A reliable toxicological risk assessment requires a population exposed to a wide range of Cd doses to establish a clear dose–response relationship. The Mae Sot District in western Thailand, a geographic area with endemic environmental Cd pollution, provided a well-circumscribed population of people with the same level of exposure that would enable one to discern the health impact of excessive dietary Cd exposure [23]. More than 40% of residents 40 years of age and older were at risk of Cd nephrotoxicity [24].

The Cd concentration of the paddy soil samples from the Mae Sot District exceeded the standard of 0.15 mg/kg, and the rice samples collected from household storage contained four times the amount of the permissible Cd level of 0.1 mg/kg [25]. In a health survey of the Mae Sot residents (n = 5273), urinary Cd excretion levels correlated with the prevalence rates of hypertension and diabetes [26].

The present study selected individuals without diabetes from a pre-existing Thai population cohort (n = 1189) where residents of Bangkok, Nakhon Si Thammarat Province and the Mae Sot District, Tak Province were enrolled [27]. Following an exclusion of outliers and those with incomplete data, 799 individuals were analyzed in the present study. The Mae Sot residents represented the moderate-to-high Cd exposure group. The residents of Nakhon Si Thammarat Province were considered to represent low environmental Cd

exposure conditions in non-urban areas, given that levels of arsenic, chromium, lead, and Cd in samples of soils and food crops in Nakhon Si Thammarat were within permissible ranges [28]. In addition, water arsenic concentrations were not significantly related to risk of diabetes in the region [29].

All participants enrolled in the parent cohort gave informed consent prior to participation. Inclusion criteria for the Mae Sot and Nakhon Si Thammarat groups were living at their current addresses for 30 years or longer. For the Bangkok group, living at their current addresses for at least 16 years was the inclusion criterion.

Exclusion criteria were pregnancy, breastfeeding, a history of exposure to metals in workplace settings, and a hospital record or physician's diagnosis of an advanced chronic disease. The sociodemographic data, smoking status, educational attainment, occupation, health status, family history of diabetes, and use of food supplements were obtained by structured interview questionnaires.

2.2. Blood and Urine Sampling and Chemical Compositional Analysis

Samples of venous blood and morning voided urine were collected after an overnight fast. Aliquots of urine, whole blood, serum, and plasma were stored at −80 °C for later analysis. To prevent the degradation of β_2M in acidic conditions, 1 M NaOH was added to adjust the pH of urine aliquots to >6.

The concentrations of creatinine in urine and plasma samples were determined by alkaline picrate Jaffe's reaction [30]. The urine concentration of β_2M was determined by the immunoagglutination method [31] or the human beta-2 microglobulin/β_2M ELISA pair set (Sino Biological Inc., Wayne, PA, USA).

Urinary Cd concentration was determined by graphite furnace atomic absorption spectrometry [32]. To calibrate the instrument, multielement standards (Merck KGaA, Darmstadt, Germany) were used. Reference urine metal controls (Lyphocheck, Bio-Rad, Hercules, CA, USA) or urine standard reference material No. 2670 (National Institute of Standards, Washington, DC, USA) was used for quality control, accuracy, and precision assurance of Cd analysis.

The limit of detection (LOD) value for urinary Cd was defined as the standard deviation values of at least ten blank sample measurements multiplied by 3. For any urine sample containing Cd below the LOD, the Cd concentration assigned was the LOD value divided by the square root of 2 [33].

2.3. Calculation of eGFR and Normalization of Cd and β_2M Excretion Rates

The eGFR was computed with equations of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) [34]. CKD stages 1, 2, 3, 4, and 5 corresponded to eGFR of 90–119, 60–89, 30–59, 15–29, and <15 mL/min/1.73 m^2, respectively [34].

Because urine samples were collected at a single time point (voided urine), a correction for interindividual differences in urine volume (dilution) was undertaken. Accordingly, the excretions of Cd (E_{Cd}) and β_2M ($E_{\beta 2M}$) were adjusted to creatinine excretion (E_{cr}) and creatinine clearance (C_{cr}), using the below equations.

$E_x/E_{cr} = [x]_u/[cr]_u$, where x = Cd or β_2M; $[x]_u$ = urine concentration of x (mass/volume) and $[cr]_u$ = urine creatinine concentration (mg/dL). E_x/E_{cr} was expressed as an amount of x excreted per g of creatinine.

$E_x/C_{cr} = [x]_u[cr]_p/[cr]_u$, where x = Cd or β_2M; $[x]_u$ = urine concentration of x (mass/volume); $[cr]_p$ = plasma creatinine concentration (mg/dL); and $[cr]_u$ = urine creatinine concentration (mg/dL). E_x/C_{cr} was expressed as an amount of x excreted per volume of the glomerular filtrate [35].

2.4. Benchmark Dose Modeling

We used the PROAST software version 71.1 (https://proastweb.rivm.nl, accessed on 28 May 2025) to estimate benchmark dose limit (BMDL) values for urinary Cd excretion rate associated with two toxic endpoints: eGFR reduction and abnormal β_2M excretion rates. The BMD was estimated through fitting an entire exposure–effect dataset using multiple dose–response models with a pre-defined specific effect size, referred to as the benchmark response (BMR) [16–19].

For continuous endpoints (eGFR reduction and abnormal β_2M excretion rates), a lower 95% confidence bound of BMD is referred to as the BMDL value, and the BMDL value derived when BMR is set at 5% can represent the NOAEL equivalent [18,19]. An upper 95% confidence bound of BMD is referred to as BMDU. The BMDU/BMDL ratio represents the degree of uncertainty in BMD estimates [18,19]. The mathematical equations applied to continuous endpoints were inverse exponential, natural logarithmic, exponential, and Hill dose–response models [36,37].

For quantal endpoints (% CKD and % abnormal β_2M excretion rates), respective BMDL5 and BMDL10 values refer to the lower 95% confidence bound of BMD values derived when the prevalence of any outcome is set at 5% and 10% [18,19]. Similarly, respective BMDU5 and BMDU10 refer to the upper 95% confidence bound of BMD values derived when the prevalence of any outcome (% CKD and % abnormal β_2M excretion rates) is set at 5% and 10%. The BMDL5 could be considered as a population exposure threshold level. The mathematical equations applied to quantal endpoints were two-stage, logarithmic logistic, Weibull, logarithmic probability, gamma, exponential, and Hill dose–response models [16,38,39].

For both continuous and quantal response endpoints, we evaluated model performance using the Akaike information criterion (AIC), which balances goodness of fit against model complexity to reduce the risk of overfitting and underfitting. Model weights, reflecting the relative information retained by each model, were higher for models with less information loss, indicating better quality.

2.5. Statistical Analysis

Data were analyzed with IBM SPSS Statistics 21 (IBM Inc., New York, NY, USA). The variability of any continuous variable and differences in percentages across the eGFR groups were assessed by the Kruskal–Wallis test and the Pearson chi-squared test, respectively. The one-sample Kolmogorov–Smirnov test was used to assess departure from a normal distribution of any continuous variable. Logarithmic transformation was applied to the excretion rates of Cd and β_2M that showed rightward skewing before they were subjected to parametric statistics analyses.

The prevalence odds ratio (POR) values for CKD and abnormal β_2M excretion levels 1, 2, and 3 were determined by multivariable logistic regression modeling with adjustment for covariates, namely age, body mass index (BMI), gender, smoking, and hypertension.

3. Results

3.1. Cd, eGFR, and β_2M Excretion Levels in Study Subjects

Subjects were grouped according to their eGFR values, where 426, 303, and 70 persons had eGFR $\geq$ 90, 61–89, and $\leq$60 mL/min/1.73 m^2, respectively (Table 1).

Table 1. Characteristics of study subjects grouped by eGFR.

Variables	All Subjects *n* = 799	eGFR, mL/min/1.73 m2			*p*
		≥90, *n* = 426	61−89, *n* = 303	≤60, *n* = 70	
Age, years	49.2 (11.5)	43.7 (9.5) *	53.5 (8.9) *	63.9 (11.1) *	<0.001
Age range, years	18−87	16−73	28−80	44−87	–
BMI, kg/m^2	23.3 (3.9)	23.6 (3.8)	23.2 (3.9)	22.3 (4.3)	0.088
eGFR, mL/min/1.73 m^2	90 (21)	106 (10)	77 (8)	47 (11)	<0.001
Women, %	62.5	59.9	66.3	61.4	0.202
Hypertension, %	33.5	30.3	36.2	41.4	0.087
Smoking, %	39.7	34.3 *	42.9 *	58.6 *	<0.001
E_{Cd}/E_{cr}, µg/g creatinine	2.15 [2.09]	1.58 [2.53]	2.55 [1.90]	6.58 [1.23]	<0.001
$E_{\beta 2M}/E_{cr}$, µg/g creatinine	46.0 [1.0]	21.1[1.11]	63.17 [1.03]	1341.1 [1.00]	<0.001
$E_{\beta 2M}/E_{cr}$, µg/g creatinine, %					
≥100	31.8	20.0	37.0	81.4	<0.001
≥300	16.1	6.3	18.5	65.7	<0.001
≥1000	8.9	0.9	9.2	55.7	<0.001
$(E_{Cd}/C_{cr}) \times 100$, µg/L filtrate	1.82 [2.47]	1.15 [3.25]	2.38 [2.00]	9.00 [1.18]	<0.001
$(E_{\beta 2M}/C_{cr}) \times 100$, µg/L filtrate	38.9 [1.1]	21.13 [1.11]	63.17 [1.04]	1341 [1.00]	<0.001
$(E_{\beta 2M}/C_{cr}) \times 100$, µg/L filtrate, %					
≥100	28.2	14.8	34.3	82.9	<0.001
≥300	16.0	5.2	18.8	70.0	<0.001
≥1000	8.6	0.5	8.9	57.1	<0.001

n, sample size; eGFR, estimated glomerular filtration rate; BMI, body mass index; Cd, cadmium; β_2M, β_2-microglobulin; cr, creatinine; C_{cr}, creatinine clearance. Data for BMI were from 773 subjects. All other data were from 799 subjects. Excretion rates of Cd and β_2M are presented as geometric mean [geometric standard deviation, SD] values. All other continuous variables are expressed as arithmetic mean (SD) values. For all tests, $p \leq 0.05$ identifies statistical significance, determined with the Pearson Chi-Square test for differences in percentages and the Kruskal–Wallis test for the variation across the eGFR groups. * Significant between group differences at *p* levels ≤ 0.05.

The overall mean age was 49.2 years (range: 18−87 years), and the percentages (%) of women, smokers, and those with hypertension were 62.5, 32.5, and 42.7, respectively. The low-eGFR group was the oldest. It had the highest % of hypertension (42.9) and abnormal excretion of β_2M; $E_{\beta 2M}/E_{cr} \geq 300$ µg/g cr (65.7) and $(E_{\beta 2M}/C_{cr}) \times 100 \geq 300$ µg/L filtrate (70).

Respective arithmetic (geometric) means for E_{Cd}/E_{cr} and $(E_{Cd}/C_{cr}) \times 100$ were 5.24 (2.15) µg/g creatinine and 5.05 (1.82) µg/L filtrate. Mean $E_{\beta 2M}/E_{cr}$ and mean $(E_{\beta 2M}/C_{cr}) \times 100$ values were the highest, moderate, and lowest in the groups with eGFR values ≤ 60, 61–89, and ≥90 mL/min/1.73 m^2, respectively. The BMI variability in the three eGFR groups did not differ statistically.

3.2. Determinants of the Prevalence Odds for CKD (eGFR ≤ 60 mL/min/1.73 m^2)

Logistic regression modeling was employed to assess effects of Cd exposure on the prevalence odds ratio (POR) for CKD (eGFR criterion) with adjustment for covariates: age, BMI, gender, smoking, and hypertension. Two models were constructed; ECd was incorporated in models A and B as E_{Cd}/E_{cr} and E_{Cd}/C_{cr}, respectively. All other independent variables in both models A and B were identical. Results are presented in Table 2.

Table 2. Determinants of the prevalence odds ratios for CKD.

Independent Variables/Factors	CKD (eGFR ≤ 60 mL/min/1.73 m^2)				
	β Coefficients (SE)	POR	95% CI		*p*
			Lower	Upper	
Model A					
Age, years	0.160 (0.020)	1.173	1.128	1.220	<0.001
$Log_2[(E_{Cd}/E_{cr})]$, μg/g creatinine	0.684 (0.142)	1.981	1.500	2.615	<0.001
Gender	0.126 (0.107)	1.135	0.533	2.415	0.743
Hypertension	0.659 (0.355)	1.933	0.965	3.874	0.063
Smoking	0.131 (0.392)	1.140	0.529	2.456	0.738
BMI, kg/m^2					
12−18	Referent				
19−23	0.140 (0.489)	1.150	0.441	3.002	0.775
≥24	1.387 (0.554)	4.002	1.351	11.86	0.012
Model B					
Age, years	0.156 (0.022)	1.168	1.119	1.219	<0.001
$Log_2[(E_{Cd}/C_{cr})]$, μg/L filtrate	1.142 (0.169)	3.132	2.249	4.361	<0.001
Gender	−0.330 (0.422)	0.719	0.315	1.643	0.434
Hypertension	0.977 (0.392)	2.656	1.231	5.727	0.013
Smoking	0.098 (0.417)	1.103	0.487	2.495	0.815
BMI, kg/m^2					
12−18	Referent				
19−23	0.126 (0.528)	1.134	0.403	3.189	0.812
≥24	1.565 (0.603)	4.784	1.468	15.59	0.009

CKD, chronic kidney disease; POR, prevalence odds ratios; CI, confidence interval; BMI, body mass index; Cd, cadmium; cr, creatinine; C_{cr}, creatinine clearance. CKD was defined as eGFR ≤ 60 mL/min/1.73 m^2. Cd excretion rate was normalized to E_{cr} and C_{cr} in models A and B, respectively. All other independent variables in the two models were identical. Data were from 691 subjects (420 women, 271 men).

As shown in model A, POR for CKD was affected by age, BMI ≥ 24 kg/m^2, and E_{Cd}/E_{cr}, while an effect of hypertension did not reach statistically significant levels (p = 0.068). The POR for CKD rose 1.98-fold and 4-fold by a 2-fold increase in E_{Cd}/E_{cr} and BMI ≥ 24 kg/m^2. It rose 7.3% for every one-year increase in age.

As shown in model B, POR for CKD was affected by hypertension, age, BMI ≥ 24 kg/m^2, and E_{Cd}/C_{cr}. POR for CKD rose 4.78-fold, 3.13-fold, and 2.66-fold by BMI ≥ 24 kg/m^2, doubling E_{Cd}/C_{cr}, and hypertension, respectively. It rose 6.8% for every one-year increase in age.

3.3. Incremental β_2M Excretion Rates in Relation to Cd Excretion Levels

Additional logistic regression models were conducted to examine whether the incremental levels of β_2M excretion could be related to Cd excretion levels in a dose-dependent manner (Table 3).

As shown in Table 3, Cd excretion levels were incorporated in the regression model as categorial independent variables together with CKD (eGFR ≤ 60 mL/min/1.73 m^2), age, BMI, gender, hypertension, and smoking. In both models A and B, the POR values for the β_2M excretion levels 1, 2, and 3 were more prevalent in those with CKD. As expected, β_2M excretion level 3 appeared to occur in the highest frequency in those with CKD in both models.

Notably, however, in model A, a dose–effect relationship could not be established between Cd excretion levels and the graded incremental β_2M excretion levels. POR values for β_2M excretion levels 1, 2, and 3 in the subjects with the highest Cd exposure category did not differ. Thus, β_2M excretion levels 1, 2, and 3 occurred in the same high frequencies as E_{Cd}/E_{cr} rose above 2.50 μg/g creatinine.

Table 3. Determinants of the prevalence odds ratios for abnormal β_2M excretion rates.

Independent Variables	β_2M Excretion Level 1 [a]		β_2M Excretion Level 2		β_2M Excretion Level 3	
	POR (95% CI)	***p***	**POR (95% CI)**	***p***	**POR (95% CI)**	***p***
Model A						
Age, years	1.045 (1.021, 1.070)	<0.001	1.044 (1.017, 1.073)	0.002	1.035 (1.000, 1.071)	0.049
BMI, kg/m^2	0.931 (0.880, 0.986)	0.015	0.905 (0.842, 0.974)	0.008	0.963 (0.878, 1.055)	0.413
Gender	0.781 (0.501, 1.218)	0.276	2.031 (1.192, 3.459)	0.009	1.235 (0.624, 2.444)	0.545
Hypertension	1.353 (0.887, 2.065)	0.161	1.399 (0.815, 2.401)	0.223	1.436 (0.718, 2.874)	0.306
Smoking	0.728 (0.470, 1.126)	0.153	1.150 (0.662, 1.998)	0.619	0.653 (0.313, 1.359)	0.254
CKD [b]	4.475 (2.051, 9.763)	<0.001	8.125 (3.399, 16.93)	<0.001	12.51 (5.879, 26.64)	<0.001
E_{Cd}/E_{cr}, μg/g creatinine						
≤0.37	Referent					
0.38−2.49	1.976 (1.180, 3.308)	0.010	1.163 (0.566, 2.390)	0.680	1.419 (0.430, 4.688)	0.566
≥2.50	5.285 (2.929, 9.536)	<0.001	3.470 (1.629, 7.394)	0.001	4.540 (1.374, 14.99)	0.013
Model B	POR (95% CI)	*p*	POR (95% CI)	*p*	POR (95% CI)	*p*
Age, years	1.049 (1.023, 1.075)	<0.001	1.048 (1.018, 1.078)	0.001	1.050 (1.013, 1.088)	0.007
BMI, kg/m^2	0.918 (0.865, 0.975)	0.005	0.893 (0.826, 0.965)	0.004	0.988 (0.896, 1.089)	0.805
Gender	1.273 (0.806, 2.009)	0.300	2.551 (1.457, 4.466)	0.001	1.605 (0.779, 3.310)	0.200
Hypertension	1.074 (0.699, 1.652)	0.744	1.245 (0.721, 2.149)	0.431	1.070 (0.519, 2.205)	0.855
Smoking	0.833 (0.529, 1.311)	0.430	1.044 (0.587, 1.856)	0.883	0.527 (0.238, 1.165)	0.113
CKD [b]	5.823 (2.601, 13.04)	<0.001	10.07 (4.657, 21.78	<0.001	12.13 (5.588, 26.35)	<0.001
$(E_{Cd}/C_{cr}) \times 100$, μg/L filtrate						
≤1.49	Referent					
1.50−4.99	2.740 (1.460, 5.143)	0.002	2.296 (0.913, 5.774)	0.077	2.606 (0.297, 22.85)	0.387
≥5	5.831 (3.095, 10.99)	<0.001	6.351 (2.617, 15.41)	<0.001	15.35 (1.976, 119.3)	0.009

POR, prevalence odds ratios; CI, confidence interval; BMI, body mass index; Cd, cadmium; cr, creatinine; C_{cr}, creatinine clearance. [a] The β_2M excretion levels 1, 2, and 3 corresponded to $E_{\beta 2M}/E_{cr} \geq 100$, ≥ 300, and ≥ 1000 μg/g cr in model A and $(E_{\beta 2M}/C_{cr}) \times 100 \geq 100$, ≥ 300, and ≥ 1000 μg/L filtrate in model B. [b] CKD was defined as eGFR ≤ 60 mL/min/1.73 m^2.

In comparison, a dose–effect relationship between of Cd excretion and β_2M excretion levels was apparent in model B. POR values for β_2M excretion levels 1, 2, and 3 rose 5.8-fold, 6.4-fold, and 15.4-fold in the highest Cd exposure category. Thus, β_2M excretion level 3 occurred in the highest frequency in the group with the highest Cd exposure [$(E_{Cd}/C_{cr}) \times 100 \geq 5$ μg/L filtrate] as did CKD.

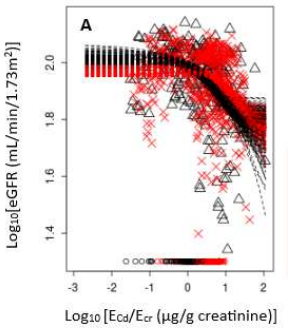
3.4. Cd Excretion Benchmarks Derive from Continuous eGFR and β_2M Endpoints

Figure 1 presents the BMD modeling of two eGFR/E_{Cd} datasets, including bootstrap model averaging curves (Figure 1A,D), model weights (Figure 1B,E), and BMDU/BMDL values of E_{Cd} (Figure 1C,F).

For E_{cr}-adjusted datasets, the inverse exponential model had the highest weight (0.3674), followed by the natural logarithmic model (0.2695) (Figure 1B). In all subjects, E_{Cd}/E_{cr} of 0.17 μg/g cr produced a 5% reduction in eGFR (Figure 1C). The BMDL value of E_{Cd}/E_{cr} producing the same BMR in women was 4.8-fold higher than in men (1.52 vs. 0.32 μg/g cr). The BMDU/BMDL ratio for the benchmark E_{Cd}/E_{cr} of 0.17 μg/g cr in all subjects was 16.9.

For C_{cr}-adjusted datasets, the weights for the natural logarithmic model (0.2787) and the exponential model (0.2624) were close (Figure 1E). In all subjects, $(E_{Cd}/C_{cr}) \times 100$ of 0.88 μg/L filtrate (Figure 1F) produced a 5% reduction in eGFR. BMDL value of $(E_{Cd}/C_{cr}) \times 100$ for the same BMR was only 21% higher in women compared to men (0.79 vs. 1 μg/L filtrate) (Figure 1F). The BMDU/BMDL ratio for the C_{cr}-based benchmark was 3.4. This indicated a higher degree of certainty, compared to the E_{cr}-based BMD estimates.

Figure 2 presents the BMD modeling of two $E_{\beta 2M}/E_{Cd}$ datasets, including bootstrap model averaging curves (Figure 2A,D), model weights (Figure 2B,E), and BMDU/BMDL values of E_{Cd} (Figure 2C,F).

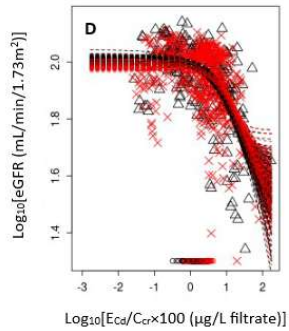


B

Dose-response model	Weight
Inverse exponential	0.3674
Natural logarithmic	0.2695
Hill	0.1825
Exponential	0.1806

C

eGFR fall by 5%	E_{Cd}/E_{cr}, µg/ g creatinine		
	BMDL	BMDU	BMDU/BMDL ratio
Men	0.32	2.12	6.6
Women	1.52	7.05	4.6
All	0.17	2.88	16.9



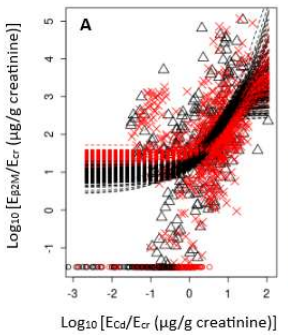
E

Dose-response model	Weight
Natural logarithmic	0.2787
Exponential	0.2624
Hill	0.2375
Inverse exponential	0.2214

F

eGFR fall by 5%	$(E_{Cd}/C_{cr}) \times 100$, µg/L filtrate		
	BMDL	BMDU	BMDU/BMDL ratio
Men	0.79	2.59	3.3
Women	1.00	2.95	3.1
All	0.88	2.95	3.4

Figure 1. Cadmium excretion benchmarks for a 5% reduction in eGFR. Bootstrap dose–response model averaging for eGFR-E_{Cd}/E_{cr} (**A**) and eGFR-E_{Cd}/C_{cr} (**D**); Model weights for E_{Cd}/E_{cr} (**B**) and E_{Cd}/C_{cr} (**E**); BMDL/BMDU values for E_{Cd}/E_{cr} (**C**) and E_{Cd}/C_{cr} (**F**). × and Δ represent male and female participants, respectively. eGFR: Estimated glomerular filtration rate; Cd: cadmium; E_{cr}: creatinine excretion; BMDL: benchmark dose limit; E_{Cd}: urinary Cd; BMDU: upper 95% confidence bound of BMD.

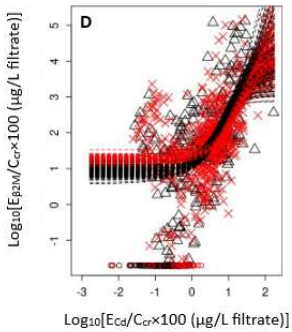


B

Dose-response model	Weight
Natural logarithmic	0.5239
Hill	0.1927
Exponential	0.1744
Inverse exponential	0.1090

C

Increment of β_2M excretion by 5%	E_{Cd}/E_{cr}, µg/ g creatinine		
	BMDL	BMDU	BMDU/BMDL ratio
Men	0.003	0.472	154
Women	0.012	1.400	112
All	0.004	0.758	182



E

Dose-response model	Weight
Natural logarithmic	0.3734
Inverse exponential	0.3660
Exponential	0.1387
Hill	0.1218

F

Increment of β_2M excretion by 5%	$(E_{Cd}/C_{cr}) \times 100$, µg/L filtrate		
	BMDL	BMDU	BMDU/BMDL ratio
Men	0.0378	0.451	12
Women	0.0803	0.852	11
All	0.0273	0.701	25

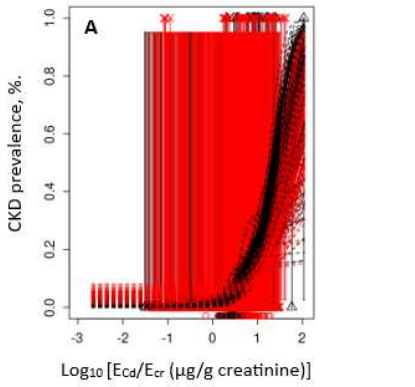
Figure 2. Cadmium excretion benchmarks for a 5% increase in β_2M excretion levels. Bootstrap dose–response model averaging for $E_{\beta 2M}/E_{cr}$-E_{Cd}/E_{cr} (**A**) and $E_{\beta 2M}/C_{cr}$-E_{Cd}/C_{cr} (**D**); Model weights for $E_{\beta 2M}/E_{cr}$ (**B**) and $E_{\beta 2M}/C_{cr}$ (**E**); BMDL/BMDU values E_{Cd}/E_{cr} (**C**) and E_{Cd}/C_{cr} (**F**). × and △ represent male and female participants, respectively. β_2M: β_2-microglobulin; $E_{\beta 2M}$: urinary β_2M; Cd: cadmium; E_{cr}: creatinine excretion; BMDL: benchmark dose limit; E_{Cd}: urinary Cd; BMDU: upper 95% confidence bound of BMD.

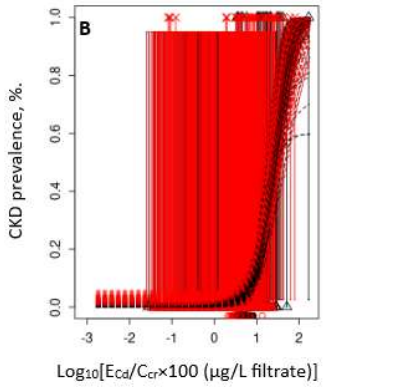
For E_{cr}-adjusted datasets, the weights of the natural logarithmic model (0.3734) and inverse exponential model (0.3660) were nearly the same (Figure 2B). The BMDL value of E_{Cd}/E_{cr} producing a 5% increment in $E_{\beta 2M}/E_{cr}$ were unreliable because the BMDU/BMDL ratios (an uncertainty index) were higher than 100 (Figure 2C). The uncertainty indices for men, women, and all subjects were 154, 112, and 182, respectively.

For C_{cr}-adjusted datasets, the natural logarithmic model had the highest weight (0.5239), followed by the Hill model (0.1927) (Figure 2B). The BMDL values of $(E_{Cd}/C_{cr}) \times 100$ producing a 5% $E_{\beta 2M}/C_{cr}$ increment endpoint were 0.04, 0.08, and 0.03 μg/L filtrate in men, women, and all subjects, respectively (Figure 2F). These E_{Cd} benchmarks were of high certainty, reflected by BMDU/BMDL ratios of 12, 11, and 15 for men, women, and all subjects, respectively.

3.5. Cd Excretion Benchmarks Derived from CKD and Abnormal β_2M Excretion Prevalences

Figure 3 presents the quantal BMD modeling of two CKD prevalence/E_{Cd} datasets, including bootstrap model averaging curves (Figure 3A,B), model weights (Figure 3C,D), and BMDU/BMDL values of E_{Cd} (Figure 3E,F).





C

Dose-response model	Weight
Hill	0.3369
Logarithmic probability	0.2106
Logarithmic logistic	0.1440
Gamma	0.1025
Weibull	0.0975
Two-stage	0.0882
Exponential	0.0203

D

Dose-response model	Weight
Gamma	0.2406
Weibull	0.2287
Logarithmic logistic	0.1911
Logarithmic probability	0.1430
Two-stage	0.1307
Hill	0.0332
Exponential	0.0329

E

[a] Prevalence of CKD at 5%	E_{cd}/E_{cr}, μg/ g creatinine		
	$BMDL_5$	$BMDU_5$	$BMDU_5/BMDL_5$ ratio
Men	1.76	4.54	2.58
Women	2.24	9.30	4.15
All	2.23	6.29	2.82

F

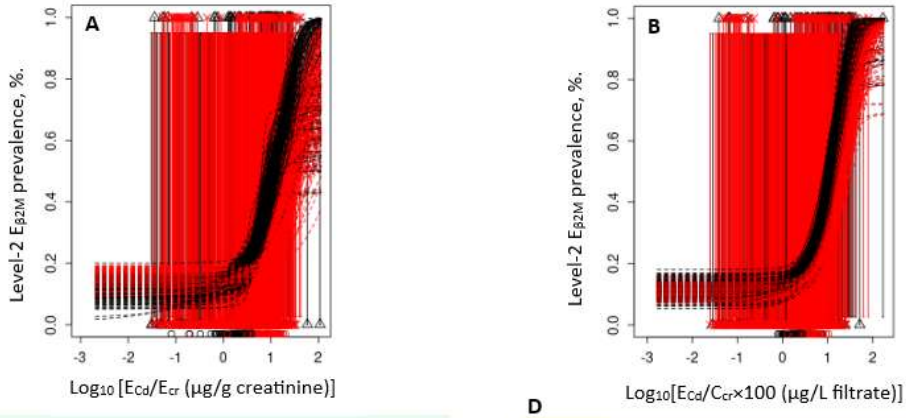
[a] Prevalence of CKD at 5%	$(E_{cd}/C_{cr}) \times 100$, μg/L filtrate		
	$BMDL_5$	$BMDU_5$	$BMDU_5/BMDL_5$ ratio
Men	3.33	6.76	2.03
Women	2.95	6.30	2.14
All	3.10	6.19	2.00

Figure 3. Cadmium excretion levels associated with 5% prevalences of CKD. Bootstrap dose–effect model averaging for CKD prevalence/E_{Cd}/E_{cr} (**A**) and CKD prevalence/E_{Cd}/C_{cr} (**B**). Model weights for E_{Cd}/E_{cr} (**C**) and E_{Cd}/C_{cr} (**D**). BMDL5/BMDU5 values of E_{Cd}/E_{cr} (**E**) and E_{Cd}/C_{cr} (**F**). [a] CKD was defined as eGFR $\leq$ 60 mL/min/1.73 m^2. CKD: chronic kidney disease; Cd: cadmium; E_{Cd}: urinary Cd; Ecr: creatinine excretion; BMDL: benchmark dose limit; BMDU: upper 95% confidence bound of BMD.

For the E_{cr}-adjusted datasets, the weight of the Hill model (0.3369) was the highest, followed by the logarithmic probability (0.2106) and the logarithmic logistic (0.1440) models (Figure 3C). Respective E_{Cd}/E_{cr} benchmarks for a 5% CKD prevalence in men, women, and all subjects were 1.76, 2.24, and 2.23 μg/g cr (Figure 3E). The uncertainty indices for the E_{Cd}/E_{cr} benchmark estimates for men, women, and all subjects were 2.58, 4.15, and 2.82.

For the C_{cr}-adjusted datasets, the weight of the gamma model (0.2406) was close to that of the Weibull model (0.2287) (Figure 3D). Respective E_{Cd}/E_{cr} benchmarks for a 5% CKD prevalence in men, women, and all subjects were 3.33, 2.95, and 3.10 μg/g cr (Figure 3F). The uncertainty indices for the E_{Cd}/E_{cr} benchmark estimates for men, women, and all subjects were 2.03, 2.14, and 2.00.

Figure 4 presents the quantal BMD modeling of two abnormal β_2M excretion prevalence/E_{Cd} datasets, including bootstrap model averaging curves (Figure 4A,B), model weights (Figure 4C,D), and BMDU/BMDL values of E_{Cd} (Figure 4E,F).



C

Dose-response model	Weight
Exponential	0.5679
Hill	0.3310
Logarithmic probability	0.0623
Logarithmic logistic	0.0356
Weibull	0.0011
Gamma	0.0011
Two-stage	0.0010

D

Dose-response model	Weight
Logarithmic probability	0.3104
Logarithmic logistic	0.2753
Gamma	0.1189
Exponential	0.1097
Hill	0.1054
Two-stage	0.0659
Weibull	0.0144

E

[a] Prevalence of $E_{β2M}$ level 2 at 5%	E_{Cd}/E_{cr}, μg/ g creatinine		
	$BMDL_5$	$BMDU_5$	$BMDU_5/BMD_5$ ratio
Men	0.869	4.48	5.16
Women	2.340	14.3	6.11
All	0.812	5.51	6.79

F

[b] Prevalence of $E_{β2M}$ level 2 at 5%	$(E_{Cd}/C_{cr}) \times 100$, μg/L filtrate		
	$BMDL_5$	$BMDU_5$	$BMDU_5/BMDL_5$ ratio
Men	1.10	4.40	4.00
Women	2.01	6.78	3.37
All	1.91	5.13	2.69

Figure 4. Cadmium excretion rates associated with 5% prevalence β_2M excretion level 2. Bootstrap dose–effect model averaging for $E_{β2M}/E_{cr}$ (**A**) and $E_{β2M}/C_{cr}$ (**B**). Model weights for $E_{β2M}/E_{cr}$ (**C**) and $E_{β2M}/C_{cr}$ (**D**). $BMDL_5/BMDU_5$ values of E_{Cd}/E_{cr} (**E**) and E_{Cd}/C_{cr} (**F**). [a] $E_{β2M}/E_{cr}$ level 2 was defined as $E_{β2M}/E_{cr} \geq 300$ μg/g creatinine. [b] $E_{β2M}/C_{cr}$ level 2 was defined as $(E_{β2M}/C_{cr}) \times 100 \geq 300$ μg/L filtrate. β_2M: β_2-microglobulin; $E_{β2M}$, urinary β_2M; Cd, cadmium; E_{Cd}, urinary Cd; E_{cr}, creatinine excretion; BMDL, benchmark dose limit; BMDU, upper 95% confidence bound of BMD.

For the E_{cr}-adjusted datasets, the weight of the exponential model (0.5679) was the highest, followed by the Hill model (0.3310) (Figure 4C). Respective E_{Cd}/E_{cr} benchmarks for a 5% prevalence of $E_{β2M}/E_{cr}$ values ≥ 300 μg/g cr in men, women, and all subjects were 0.869, 2.340, and 0.812 μg/g cr (Figure 4E). The uncertainty indices for the E_{Cd}/E_{cr} benchmark estimates were 4, 14.3, and 5.51 for men, women, and all subjects, respectively.

For the C_{cr}-adjusted datasets, the weights of the logarithmic probability model (0.3104) and the logarithmic logistic model (0.2753) were close (Figure 4D). Respective E_{Cd}/E_{cr} benchmarks for a 5% prevalence of $(E_{β2M}/C_{cr}) \times 100$ values ≥ 300 μg/L filtrate in men, women, and all subjects were 1.10, 2.01, and 1.91 μg/L filtrate (Figure 4F). All estimated benchmarks were of the high statistical certainty, given that the uncertainty indices were as little as 1.10, 2.01, and 1.91 for men, women, and all subjects, respectively.

Equivalent quantal BMD modeling for prevalences of abnormal $E_{\beta 2M}$ excretion values were conducted using two additional cut-off values: ≥100 and ≥1000 μg/g cr for E_{cr}-adjusted datasets and ≥100 and ≥1000 μg/L filtrate for C_{cr}-adjusted datasets. Full results are provided in Figures S1 and S2. Urinary Cd excretion benchmarks estimated from these cut-off values are displayed in Table 4 together with those reported in Figures 3E,F and 4E,F.

Table 4. Quantal Cd excretion benchmarks derived from abnormal eGFR values and abnormal β_2M excretion rate.

Independent Variables	Elevated β_2M Excretion Levels			Low eGFR [a]
	Level 1: ≥100	Level 2: ≥ 300	Level 3: ≥1000	
E_{cr}-normalized dataset [b]	$BMDL_5$ ($BMDU_5$)	$BMDL_5$ ($BMDU_5$)	$BMDL_5$ ($BMDU_5$)	$BMDL_5$ ($BMDU_5$)
Men	0.451 (2.65)	0.869 (4.48)	1.52 (4.96)	1.76 (4.54)
Women	0.668 (4.49)	2.340 (14.3)	3.11 (13.8)	2.24 (9.30)
All	0.640 (3.68)	0.812 (5.51)	2.35 (6.76)	2.23 (6.29)
C_{cr}-normalized dataset [c]	$BMDL_5$ ($BMDU_5$)	$BMDL_5$ ($BMDU_5$)	$BMDL_5$ ($BMDU_5$)	$BMDL_5$ ($BMDU_5$)
Men	0.91 (3.58)	1.11 (4.40)	2.93 (7.41)	3.33 (6.76)
Women	0.91 (3.18)	2.01 (6.78)	4.50 (9.74)	2.95 (6.30)
All	0.93 (3.30)	1.91 (5.13)	3.62 (7.80)	3.10 (6.19)

[a] Cut-off values for the low eGFR was ≤60 mL/min/1.73 m^2. [b] Cut-off values for β_2M excretion levels 1, 2, and 3 were $E_{\beta 2M}$/Ecr rates ≥ 100, ≥ 300, and ≥ 1000 μg/g cr. [c] Cut-off values for elevated β_2M excretion levels 1, 2, and 3 were ($E_{\beta 2M}/C_{cr}$) × 100 rates ≥100, ≥ 300, and ≥ 1000 μg/L filtrate.

For the E_{cr}-normalized dataset, benchmark Cd excretion rates producing 5% prevalences of low eGFR with $E_{\beta 2M}/E_{cr}$ values ≥ 100, ≥ 300, and 1000 μg/g cr were 0.640, 0.812, and 2.35 μg/g cr, respectively. Notably, the benchmark Cd excretion for 5% prevalence of $E_{\beta 2M}/E_{cr}$ levels ≥ 1000 μg/g cr was higher than the figure for low eGFR.

For the C_{cr}-normalized dataset, benchmark Cd excretion rates producing 5% prevalences of low eGFR with ($E_{\beta 2M}/C_{cr}$) × 100 values ≥ 100, ≥300, and 1000 μg/L filtrate were 0.93, 1.91, and 3.62 μg/L filtrate, respectively. Similarly, the benchmark Cd excretion for 5% prevalence of ($E_{\beta 2M}/C_{cr}$) × 100 values ≥ 1000 μg/L filtrate was higher than the figure for low eGFR.

3.6. Validity Analysis of a Proposed Urinary Cd Threshold

In a 4 × 4 cross-tabulation analysis of 799 subjects, the % of low eGFR (CKD) in those with E_{Cd}/E_{cr} below a new threshold figure was 5.1%. The CKD prevalence rate rose to 9.2% in those with E_{Cd}/E_{cr} ≥ 0.2 μg/g cr. The chi-square test of the prevalence rate ratio (9.2/5.1) did not reach statistical significance levels (p = 0.232). However, in a logistic regression analysis adjusting for age, BMI, smoking, gender, and hypertension, E_{Cd}/E_{cr} at 0.2 μg/g cr still showed an association with an increased risk of CKD (p = 0.020) (Table S1).

4. Discussion

4.1. Cd Exposure as a Risk Factor for CKD and Understated Toxicity by E_{cr} Adjustment

Consistent with a large body of literature, and a recent meta-analysis evaluating an effect of Cd exposure on CKD risk [10], age, BMI, blood pressure, and chronic exposure to Cd independently influenced risk for CKD, defined as eGFR falling to 60 mL/min/1.73 m^2 or below. For every one-year increase in age, CKD risk rose 6.8%, while BMI ≥ 24 kg/m^2, doubling E_{Cd}/C_{cr}, and hypertension raised risk of CKD 4.78-fold, 3.13-fold, and 2.66-fold, respectively (model B, Table 2).

As revealed by model A (Table 2), an independent effect of hypertension on CKD risk was not apparent because of adjusting urinary Cd excretion to E_{cr}. For the same reason, the effect size of Cd on CKD dropped 68%; the POR for CKD rose 1.98 vs. 3.13 by two-fold increments of E_{Cd}/E_{cr} vs. E_{Cd}/C_{cr}.

Another notable result is that adjusting E_{Cd} and $E_{\beta 2M}$ to E_{cr} nullified the dose–response relationship between Cd exposure and the severity of Cd toxicity, reflected by β_2M excretion (Table 3). The POR for β_2M excretion levels 1, 2, and 3 in the highest exposure group ($E_{Cd}/E_{cr} \geq 2.50$ μg/g cr) increased 5.3-fold, 3.5-fold, and 4.5-fold. These data were in line with a study from Japan, where differences in the prevalence of abnormal β_2M excretion rates were unrelated to Cd exposure but were related to age [40]

In comparison, however, a dose–response relationship was observed in C_{cr}-normalized datasets; the POR for β_2M excretion levels 1, 2, and 3 rose 5.8-fold, 6.4-fold, and 15.4-fold in the highest exposure group ($(E_{Cd}/C_{cr}) \times 100 \geq 5$ μg/L filtrate). Thus, for accurate measurements of Cd exposure levels and kidney effects, urinary concentrations of Cd and biomarkers of kidney effects, e.g., β_2M, should be normalized to creatinine clearance (C_{cr}).

4.2. Reduction in eGFR Due to Cd-Induced Nephron Destruction

Notably, β_2M excretion level 3 was most prevalent in those with low eGFR (CKD) (Table 3), thereby suggesting that a massive β_2M excretion could be a consequence of nephron destruction due to the cytotoxicity of cumulative Cd in the proximal tubular cells of the kidneys [41]. A fall in eGFR at a high rate (≥3 mL/min/1.73 m^2 per year) among cohort participants has been causally linked to Cd excretion in a longitudinal study from Switzerland [42]. In another prospective cohort study from Japan, a large decrease in eGFR was observed over a 5-year observation period among participants who had β_2M excretion rates above 300 μg/g cr, but environmental exposure to toxic metals was not investigated [43].

4.3. Urinary Cd Benchmarks for the β_2M Excretion and eGFR Reduction Endpoints

Most conventional BMD methods rely on a single dose–response model and arbitrary cut-off values to define departure from normalcy (a point of departure, POD). In toxicological risk assessment, POD represents the dose at which a specific adverse effect is first observed or at which a response deviates from a baseline or control [18,21]. It is a starting point to evaluate the potential health impact of exposure to any health-hazardous substance. PODs are used to estimate permissible exposure levels for chemicals in both environmental and occupational exposure settings.

Herein, we report urinary Cd excretion levels that link to the conventional toxic endpoint, an elevation of β_2M excretion, and a newly proposed toxic endpoint, a reduction in eGFR, through advanced BMD modeling, involving multiple dose–response models, model weights, and averaging (Section 2.3). The use of AIC to assess performance of different models is another advantage of advanced BMD modeling. In addition, exposure benchmark levels can be derived for continuous and quantal endpoints.

We were unable to determine urinary Cd excretion benchmarks for the β_2M endpoint (Figure 2), but we have found a urinary Cd excretion rate of 0.17 μg/g cr to be the benchmark level for an early effect of Cd on eGFR (Figure 1). Our findings cast considerable doubts on the use of β_2M excretion in the assessment of the health risk from environmental Cd exposure. As discussed above, β_2M excretion rates ≥ 300 μg/g cr did not appear to be an early effect of the nephrotoxicity of Cd but a consequence of severe tubular dysfunction leading to nephron destruction and a fall in eGFR. Furthermore, a recent indebt analysis on the homeostasis of β_2M has indicated that an elevated urinary excretion β_2M is not a reliable indicator of tubular dysfunction [44]. Apparently, it is inappropriate to use β_2M excretion rates above 300 μg/g cr in the assessment of the health risk from environmental Cd exposure.

4.4. Dietary Cd Exposure Carrying a Negligible Health Risk

It is recommended that eGFR reduction is used as an indicator of an adverse effect of Cd on the kidney. A reduction in eGFR is a clinically relevant outcome, and a Cd excretion benchmark of 0.17 μg/g cr could serve as a basis to compute a meaningful dietary exposure limit for Cd. This figure is 3.2% of the threshold level at 5.24 μg/g cr of the JECFA derived from the β_2M endpoint. A crude analysis of the prevalence rates of CKD among those with E_{Cd}/E_{cr} above and below 0.2 μg/g cr showed 9.2% and 5.1%, respectively. Logistic regression with adjusting for confounders revealed a significant elevation in odds of CKD even at E_{Cd}/E_{cr} of 0.2 μg/g cr (Table S1).

Typically, a translation of a threshold to exposure limits involves reverse dosimetry with the physiologically based pharmacokinetic model [45,46]. In the absence of a translational figure, Cd exposure levels associated with an increased risk of CKD in a Chinese population study are highlighted herein. Compared with a dietary Cd exposure level of 16.7 μg/d, CKD risk rose 1.73-fold, 2.93-fold, and 4.05-fold at Cd consumption rates of 23.2, 29.6, and 36.9 μg/d, respectively [47]. A Cd exposure level of 23.2 μg/d was only 40% of the JECFA's tolerable exposure level. For a 60 kg person, a Cd exposure of 23.2 μg/d is comparable to the EFSA's reference dose (RfD) of 0.36 μg/kg b.w./d [48] and the US FDA's toxicological reference value (TRV) of 0.21–0.36 μg/kg b.w./d [49].

Existing dietary Cd exposure guidelines based on the β_2M endpoint do not appear to be protective of the kidneys. By 2040, CKD is projected to be the fifth-leading cause of years of life lost [14,15]. Cd-induced nephron destruction which causes eGFR to fall is irreversible [50]. Minimization of Cd exposure from all sources may slow eGFR deterioration to < 15 mL/min/1.73 m^2, which marks dialysis or a kidney transplant for survival.

4.5. Strengths and Limitations

This cross-sectional design with a large Thai cohort (n = 799) employs advanced BMD modeling to reassess the utility of β_2M excretion as a basis to derive the reference dose for dietary Cd exposure. It has provided evidence that a reduction in eGFR is a suitable endpoint for such a purpose.

A cohort of participants exposed to a wide range of Cd doses represented its major strength. E_{Cd}/C_{cr} values ranged between 0.0002 and 1.70 μg/L filtrate (geometric mean 0.018 μg/L filtrate), while E_{Cd}/E_{cr} values ranged between 0.03 and 106 μg/g cr (geometric mean 2.15 μg/g cr). A wide range of Cd exposure doses allowed the establishment of a clear dose–response relationship as did a wide age range (18–87 years). Furthermore, age and BMI histograms of cohort participants conformed to a normal distribution. This group could thus be considered to represent the general Thai population exposed to Cd in a normal diet. The BMD estimates are generalizable because the basic mechanism of the cytotoxicity of Cd is the same and because sources of Cd make no difference to its toxicity. The use of multiple mathematical dose–response models for both continuous and quantal endpoints is an additional strength.

The limitations include a one-time-only assessment of Cd exposure and outcomes and inability to adequately adjust potential confounders that included prediabetes status, alcohol consumption, and history of non-steroidal anti-inflammatory drug use. Notably, however, the Swiss prospective cohort study has implicated Cd exposure, even in low doses, as a cause of falling eGFR at a high rate [42]. A Japanese longitudinal cohort study observed an increased risk of falling eGFR at 10 mL/min/1.73 m^2 over 5 years among those with a β_2M excretion rate of 300 μg/g cr [43]. Use of β_2M excretion rate of 300 μg/g cr as a criterion to judge the nephrotoxicity of Cd is questionable.

5. Conclusions

Using multiple mathematical dose–response models and a bootstrap model averaging approach, the benchmark of Cd excretion is 0.17 μg/g creatinine, when a reduction in eGFR is used as a toxic endpoint. The BMDU/BMDL ratio for the BMDL estimate was 16.9. In comparison, the Cd excretion benchmark for the conventional β_2M endpoint is unreliable because of a high degree of uncertainty around the BMD estimates, reflected by BMDU/BMDL of 182. For C_{cr}-based data, an E_{Cd}/C_{cr} value of 0.0088 μg/L filtrate is associated with a 5% reduction in eGFR. This benchmark estimate was of high certainty, given a BMDU/BMDL ratio of 3.4.

The practice of adjusting urinary Cd and β_2M concentrations to creatinine excretion creates non-differential errors that bias the dose–response relationships toward the null [20]. Such measurement errors/imprecisions can be attributed to the variation in creatinine excretion because of the differences in muscle mass, especially between men and women. A dose–response relationship could not be established between Cd exposure and the severity of the abnormality in β_2M excretion rates (Table 3). Thus, use of β_2M excretion at a rate higher than 300 μg/g creatinine in Cd health risk computation is inappropriate.

Excretion of any urinary marker of kidney effects, Cd included, should be adjusted to creatinine clearance. This adjustment is achieved by using a generic mathematical equation: $E_x/C_{cr} = [x]_u[cr]_p/[cr]_u$. Hence, a timed urine collection is obviated (SM. This method of adjusting urinary concentration of excreted substances corrects for interindividual differences in both urine dilution and functioning nephrons, and it is not affected by muscle mass).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app152111757/s1, Figure S1: Cadmium excretion rates associated with 5% prevalence of β_2M excretion level 1; Figure S2: Cadmium excretion rates associated with 5% prevalence of β_2M excretion level 3; Table S1: Logistic regression analysis for an association between the prevalence odds for CKD and a newly identified threshold.

Author Contributions: Conceptualization, S.S. and D.A.V.; methodology, S.S. and A.B.Đ.; formal analysis, S.S. and A.B.Đ.; resources, D.A.V.; data curation, S.S.; writing—original draft preparation, S.S.; writing—review and editing, S.S., D.A.V. and A.B.Đ. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study analyzed archived data, for which the ethical approval was obtained when the original study was conducted, thereby obviating the need for additional ethical approval.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflicts of interest.

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