Study on diagnostic standard of cadmium concentration in urine for chronic cadmium poisoning caused by environmental cadmium pollution in China

Authors: HAN Jingxiu, SHANG Qi, ZHANG Wenli

Authors Note: Institute for Environmental Health and Related Product Safety, Chinese Center for Disease Control and Prevention, Beijing 100021, China
Han Jingxiu, associate professor, research direction: environmental epidemiology, E-mail: hanjxcdc@139.com

Corresponding author: Shang Qi, Professor, master tutor, E-mail: qshangq@163.com

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Abstract: Objective To study the diagnostic standard of urinary cadmium for chronic cadmium poisoning in China.
Methods 1369 participants (male 644, female 752) with non-occupational cadmium exposure history aged 25-54 years were selected randomly from five non-environmental cadmium pollution areas. The participants filled out a self-questionnaire and underwent sampling of urine. Urinary cadmium and urinary creatinine (Cr) concentration was tested, and urinary Cr <0.3 g/L and >3 g/L was excluded. All samples were stratified by influence factors of urinary cadmium, normal range of urinary cadmium in general population was calculated using 95% quantile (P95) after adjustment for urinary Cr, reference limit of cadmium poisoning is proposed.
Results Cadmium concentration in urine ascends with increased age and is significantly higher in women than in men, significantly higher in smokers than in non-smokers (P<0.01). Samples were categorized by gender, smoking status, and age (25~34 and 35~54 years), upper limit of cadmium in urine was analyzed, the 95% upper limit of urinary cadmium among female participants (<9.73μg/gCr) and male smokers aged 35~54 years (8.08 μg/gCr) was significantly higher compared to the others(<6.0 μg/gCr). In addition to the male non-smokers aged 25~34 years, the 95% upper limit of urinary cadmium of other participants is higher than the limits (5 μg/gCr) specified in the Diagnosis of Occupational Cadmium Poisoning (GB Z17—2015) in China. However, the limit is lower than the kidney damage biological...
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Threshold value (10 μg/gCr) recommended by WHO, and also lower than that specified in the criteria of health hazard area of environmental cadmium pollution (GB / T 17221-1998) (15 μg/gCr).

Conclusion Standard limits for urine cadmium in GBZ17-2015 and GB / T 17221-1998 should be unified, and adjusted to <10 μg / gCr. More researches are needed to explore the limit smoking women over 30 year-old.

Key Words: cadmium poisoning, urinary cadmium, standard value
Cadmium (Cd) is a heavy metal element, often exist in a state of compound in nature, widely used in electroplating, dyes, chemicals, electronics and nuclear industry and other fields. Cadmium can damage on human kidneys, bones and other organs, increase population mortality and reduce life expectancy \[1\]. Cd is on the list of Key environmental pollutants by United Nations Environment Programme (UNEP) and the International Commission on Occupational Health to heavy metal, also is a prime food contaminant to be research by World Health Organization (WHO). Agency for Toxic Substances and Disease Registry (ATDR) ranked Cd the 6th toxic substance with adverse effect to human health. Environmental cadmium pollution hazards is more serious in recent years in China. With increasingly arising of mass incident associated with domestic environmental cadmium pollution, such as Guangdong Beijiang in 2005, Zhuzhou Xiangjiang in 2006, Liuyang river in Hunan in 2009, and Guangxi Long river in 2012, more and more attention has been aid on the health hazards caused by environmental cadmium pollution. Kidney is the main target and the most sensitive organ of cadmium toxicity \[2\]. Urinary cadmium (U-Cd) is one of indicators of cadmium level in the body. There is a big difference about U-Cd limit values among standards and documents, such as Chinese Diagnosis of Occupational Cadmium Poisoning (GB Z17-2015) \[3\], Criteria of Health Hazard Area of Environmental Cadmium Pollution (GB / T 17221-1998) \[4\]in China, and WHO relevant documents. Urinary cadmium criteria limit of mild chronic cadmium poisoning is defined as 5μg / g creatinine (Cr) in the GB Z17-2015. The existing criteria of urinary cadmium for body hazard was 15μg / g Cr in GB / T 17221-1998. The standard of U-Cd of environmental cadmium pollution health hazards is much higher than the occupational cadmium poisoning diagnostic standards. According to experience, environmental pollution should has generally less effect than occupational exposure. Therefore, some doubts were raised about these two standards of U-cd. In order to provide evidence to support the formulation of the standard limits, We chose five typical areas, 2 areas with high Cd concentration in soil and three areas mainly live on rice (Cd enrichment), and investigated urinary cadmium levels of the general population (no occupational exposure) in non-cadmium-polluted environment to observe the distribution characteristic and normal ranges of urinary cadmium of general population which will provide evidence for identifying diagnostic value of chronic environmental cadmium poisoning.  

1. Subjects and Methods
1.1 Survey population
The survey was carried out in five areas, Bijie City GuiZhou Province, Dayu County Jiangxi Province, Jiangyan City Jiangsu Province, Xupu County Hunan Province and Jiangshan City Zhejiang Province. All these are natural rural areas, no environmental cadmium pollution behaviors, such as mining, ferrous metal smelting and others. 2 to 5 administrative villages were selected randomly in each area as the investigation sites by a random number according to geographical location. The participants local permanent residents live on local grains and vegetables, no
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1.0 Introduction

Kidney and urinary system diseases, and persons with occupational cadmium exposure experience are excluded. The persons who lived in other areas before and married local residents with environmental cadmium exposure are excluded too. The participants were divided into 3 groups by age: 25~ 34, 35~ 44, and 45~ 54 years. Participants were randomly selected from all eligible local residents by using random numbers. Women avoid the menstrual period. Excluding urine creatinine (Cr) concentration of <0.3 g / L and > 3 g / L of investigation records [5]. The survey investigated 1396 people, including 644 males, 752 females.

1.1 Survey methodology

Each participant was asked to fill out "Crowd Urine Collection Registration Form" and "Crowd Urine Screening Questionnaire" face to face before urine collection. The survey includes address, gender, date of birth, height, weight, marital status, living history of local respondents, migrant workers, smoking, and previous history of chronic diseases and other related information.

1.2 Survey methodology

1.2.1 Survey methodology

Urine collection and pretreatment

All participants were collected randomly disposable urine. Sampling containers and dispensing preservation container are colorless polyethylene plastic products, soaked 12 hours or more with a 1: 1 nitric acid solution, and then rinsed with deionized water, natural dry before sampling. Blank values of cadmium in containers which sampled should lower than the detection limit. Take 5 ~ 10 ml of urine dispensed into the glass tube, tested the urinary creatinine on that day. Take 15 ~ 20 ml of urine dispensed to a plastic tube with lid closed, urinary pH adjusted with HNO₃ (excellent pure) of about 2 (about 10 ml urine sample add 0.1 ml of concentrated nitric acid), immediately after mixing to save -20°C, within a month to complete the determination of cadmium in urine.

1.2.2 The laboratory tests

Urine creatinine determination method: spectrophotometry, using picric acid (no except protein) method kits from Beihua Kangtai Company, reagents used within the validity period. 721 spectrophotometer as detection equipment was metrology before use. Urine creatinine test was done in the same day of urine collection.

Urinary cadmium determination method: The urinary cadmium from Jiangxi survey site detected by graphite furnace atomic absorption spectrometry method (AAS PE ANALYST800), the detection limit was 0.001 mg / kg. The remaining four survey sites are using inductively coupled plasma mass spectrometry (ICP-MS, the instrument model Thermo x2 / X0330). Urinary Cd detection limit is 0.004 μg / L, below the detection limit values are replaced with a detection limit of 1/2. Quality control was conducted by parallel testing of all samples, and using a standard substance simultaneously. The data from five survey sites combined analysis because of the consistent results of the two methods of urinary cadmium according to the laboratory quality control data.

1.3 Urine collection method and laboratory tests

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1.4 Statistical methods

SAS 9.3 statistical software was applied for statistical analysis. Urinary cadmium concentration was corrected by urinary creatinine. Background values of crowd is showing with 95% confidence limits. Constituting ratio between the groups and
the average urinary cadmium respectively compared by using chi-square and rank sum test.

2 Results
2.1 Cadmium concentrations in the surveyed area
Environmental media includes air, water and soil. The concentration of cadmium in the air was not considered since the survey areas do not have industrial and mining emissions of cadmium pollution. And cadmium has low solubility in water, the concentration of cadmium in water is mainly related to the concentration of soil cadmium, so the concentration of cadmium in the soil environment was used to verify the absence of cadmium contamination. Local residents eat the food and vegetables grown in the local area. Cadmium enrichment capability of rice is higher than vegetables in the same kind of environment, therefore, the rice no existing cadmium pollution can be representative of no environmental cadmium pollution and exposure in the local.

According to "Soil Environmental Quality Standards" (GB15618-2008) secondary standard (pH < 6.5), total cadmium concentration limits of the farmland is 0.30mg / kg. In addition to Guizhou, the cadmium content in soil in these five areas is lower than the national standard. Local crops in Guizhou survey point is corn. Corn enrichment of cadmium is less than rice. According to "Food Contaminants Limited" (GB2762-2005) in the provision of rice cadmium (0.2 mg / kg) and corn (0.1 mg / kg) cadmium limits (MLS), the main crops cadmium content of each survey sites were lower than national standards, a non-cadmium polluted (Table 1).

<table>
<thead>
<tr>
<th>Area</th>
<th>Environmental media</th>
<th>n</th>
<th>Cd</th>
<th>crop</th>
<th>n</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guizhou</td>
<td>soil</td>
<td>21</td>
<td>0.655±0.282</td>
<td>corn</td>
<td>20</td>
<td>0.030±0.020</td>
</tr>
<tr>
<td>Jiangxi</td>
<td>soil</td>
<td>15</td>
<td>0.229±0.095</td>
<td>rice</td>
<td>74</td>
<td>0.098±0.065</td>
</tr>
<tr>
<td>Jiangsu</td>
<td>soil</td>
<td>15</td>
<td>0.158±0.055</td>
<td>rice</td>
<td>29</td>
<td>0.038±0.019</td>
</tr>
<tr>
<td>Zhejiang</td>
<td>soil</td>
<td>15</td>
<td>0.135±0.023</td>
<td>rice</td>
<td>30</td>
<td>0.060±0.030</td>
</tr>
<tr>
<td>Hunan</td>
<td>soil</td>
<td>15</td>
<td>0.064±0.024</td>
<td>rice</td>
<td>30</td>
<td>0.035±0.015</td>
</tr>
</tbody>
</table>

2.2 Demographic situation of surveyed population

Male to female ratio of the survey respondents is 1: 1.17. Age and gender composition was no significant difference ($\chi^2 = 0.62, P>0.05$). The population of each survey point has no significant difference in gender and age constitute (P>0.05). In this survey, 28.22% of the people smoke, mostly male. Male smoking rate was 59.32% (382/644), female smoking rate was 1.60% (12/752).
2.3 Urinary cadmium levels 2.08% (29 cases) of the detected value is below the detection limit. Because urinary cadmium data corrected by creatinine were not normally distributed, therefore, average of the U-cd using median (P50), was 1.62 μg / gCr, P25 ~ P75 was 0.44 ~ 3.82 μg / gCr, P95 was 8.28μg / gCr, a maximum of 30.08μg / gCr. Because gender, age, and smoking have a significant impact on the urinary cadmium levels of general population [7, 8], we analyzed urine cadmium distribution by using these factors as group factors.

2.3.1 Urinary cadmium levels of different gender and age groups.

There was a significant difference among age groups (Kruskal-Wallis test χ² = 33.27 P <0.01). By pairwise comparison, urinary cadmium level of 25~34 age group was significantly lower than the 35~54 year-olds. Urinary cadmium level of Women was significantly higher than men (Kruskal-Wallis test χ² = 26.51 P <0.01). The average urinary cadmium among 35~44 age women is the highest, followed by urinary cadmium levels decreased. Urinary cadmium increased with age in men, the higher urinary cadmium is in the 45~54 age group. Compares with the urinary cadmium limit in Diagnosis of Occupational Cadmium Poisoning (GBZ17-2015) (5 μg / gCr), we found that the 95% limit (P95) of cadmium in urine of survey population were higher than this standard in addition to 25~34 age men group, but lower than the urinary cadmium limit in Criteria of Health Hazard Area of Environmental Cadmium Pollution (GB / T 17221-1998) (15 μg / gCr) (Table 2).

Table 2 Urinary cadmium levels of age-gender groups (μg/gCr)

<table>
<thead>
<tr>
<th>age group</th>
<th>n</th>
<th>male</th>
<th>female</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>P₂₅~P₇₅</td>
<td>P₉₅</td>
<td>n</td>
</tr>
<tr>
<td>25~34</td>
<td>192</td>
<td>0.30</td>
<td>4.3</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>2.16</td>
<td>4</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>35~44</td>
<td>231</td>
<td>0.29</td>
<td>7.8</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>3.56</td>
<td>4</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>45~54</td>
<td>221</td>
<td>0.55</td>
<td>6.7</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>3.66</td>
<td>7</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>total</td>
<td>644</td>
<td>0.36</td>
<td>6.5</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>3.15</td>
<td>6</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

2.3.2 Smokers and non-smokers urinary cadmium levels

By Wilcoxon rank sum test, the urinary cadmium levels of the smokers and non-smoking are no significant difference (P > 0.05). As the female urinary cadmium levels were significantly higher than males, female smoking rate is low, so we stratified by gender. The results show that (Table 3) male smoking significantly increased urinary cadmium average, while because the sample size of woman smokers is too small, there was no significant effect on the average, but the U-Cd minimum and P95 limits of smoking female were significantly higher than other groups.
Table 3 The effect of smoking on urinary cadmium levels (μg/gCr)

<table>
<thead>
<tr>
<th>gender</th>
<th>smoking</th>
<th>n</th>
<th>P25</th>
<th>P50</th>
<th>P75</th>
<th>P95</th>
<th>min</th>
<th>max</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>total</td>
<td>smoking</td>
<td>394</td>
<td>0.44</td>
<td>1.61</td>
<td>3.61</td>
<td>7.84</td>
<td>0.001</td>
<td>19.21</td>
<td>0.04</td>
<td>0.843</td>
</tr>
<tr>
<td></td>
<td>No-smoking</td>
<td>1002</td>
<td>0.44</td>
<td>1.63</td>
<td>3.87</td>
<td>8.32</td>
<td>0.002</td>
<td>30.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>smoking</td>
<td>382</td>
<td>0.44</td>
<td>1.63</td>
<td>3.66</td>
<td>7.59</td>
<td>0.001</td>
<td>19.21</td>
<td>19.45</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>No-smoking</td>
<td>262</td>
<td>0.24</td>
<td>0.98</td>
<td>2.20</td>
<td>4.99</td>
<td>0.003</td>
<td>13.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>smoking</td>
<td>12</td>
<td>0.33</td>
<td>1.34</td>
<td>2.96</td>
<td>13.47</td>
<td>0.136</td>
<td>13.47</td>
<td>0.66</td>
<td>0.415</td>
</tr>
<tr>
<td></td>
<td>No-smoking</td>
<td>740</td>
<td>0.52</td>
<td>2.01</td>
<td>4.42</td>
<td>9.39</td>
<td>0.002</td>
<td>30.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.4 Normal upper limit of urinary cadmium of the general population

Because survey sample scale of women smokers is small, unrepresentative, this study does not suggest a urinary cadmium limit value for this crowd. Cadmium is a non-essential trace element in the human body \[9, 10\] in the current scientific understanding. We take unilateral urinary cadmium reference range calculated on the P95 value. Comprehensive analysis of the results of the foregoing, the present study analyzed urinary cadmium limits stratified by sex and smoking, according to the 25~34 and 35~54 age groups. Table 5 shows that average urinary cadmium of 35~54 year-old female (<2.29 μg / gCr) higher than the average in other populations (<1.80 μg / gCr). In addition to the 25~34 year-old male non-smoker, the urinary cadmium of the rest of the crowd exceeded the upper urinary cadmium limit of GBZ17-2015 (5 μg / gCr), but were lower than the WHO recommended standard limits (10 μg / gCr).

Table 4 Reference range of urinary cadmium of non-cadmium exposed population (μg/gCr)

<table>
<thead>
<tr>
<th>gender</th>
<th>smoking</th>
<th>age</th>
<th>n</th>
<th>mean</th>
<th>SD</th>
<th>median</th>
<th>P90</th>
<th>P95</th>
</tr>
</thead>
<tbody>
<tr>
<td>male</td>
<td>yes</td>
<td>25~34</td>
<td>95</td>
<td>1.94</td>
<td>2.95</td>
<td>0.90</td>
<td>3.74</td>
<td>5.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35~54</td>
<td>287</td>
<td>2.71</td>
<td>2.69</td>
<td>1.80</td>
<td>6.32</td>
<td>8.08</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>25~34</td>
<td>97</td>
<td>1.30</td>
<td>1.26</td>
<td>1.03</td>
<td>3.14</td>
<td>3.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35~54</td>
<td>165</td>
<td>1.69</td>
<td>1.95</td>
<td>0.92</td>
<td>4.41</td>
<td>5.32</td>
</tr>
<tr>
<td>female</td>
<td>yes</td>
<td>25~34</td>
<td>5</td>
<td>0.94</td>
<td>0.74</td>
<td>0.94</td>
<td>2.03</td>
<td>2.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35~54</td>
<td>7</td>
<td>3.74</td>
<td>4.60</td>
<td>2.84</td>
<td>13.47</td>
<td>13.47</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>25~34</td>
<td>208</td>
<td>2.67</td>
<td>2.54</td>
<td>1.30</td>
<td>5.79</td>
<td>7.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35~54</td>
<td>532</td>
<td>3.28</td>
<td>3.47</td>
<td>2.29</td>
<td>7.58</td>
<td>9.73</td>
</tr>
</tbody>
</table>

According to the survey results, the suggested urinary cadmium standard limits is 10μg / g creatinine either occupational poisoning or environmental hazard determination. In these survey, the urine cadmium of 16.83 % of general population are more than 5μg / gCr, while less than 5% of the proportion are more than 10μg / gCr, less than 1% are more than 15μg / gCr (Table 5). It is usually taken 95% confidence interval of general population as the normal range.
Table 5  Distribution of urinary cadmium levels of the population

<table>
<thead>
<tr>
<th>U-Cd levels (µg/gCr)</th>
<th>n</th>
<th>percentage (%)</th>
<th>Cumulative Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;15</td>
<td>10</td>
<td>0.72</td>
<td>0.72</td>
</tr>
<tr>
<td>10-15</td>
<td>23</td>
<td>1.65</td>
<td>2.36</td>
</tr>
<tr>
<td>5-10</td>
<td>202</td>
<td>14.47</td>
<td>16.83</td>
</tr>
<tr>
<td>&lt;5</td>
<td>1161</td>
<td>83.17</td>
<td>100.00</td>
</tr>
<tr>
<td>total</td>
<td>1396</td>
<td>100.00</td>
<td>——</td>
</tr>
</tbody>
</table>

3 Discussion and conclusion

For non-environmental cadmium pollution area, the environment cadmium mainly comes from the earth itself. For the general population, the daily life of cadmium exposure comes mainly from food and smoking. Basically all human foods and beverages contain small amounts of cadmium, even for non-cadmium-polluted environment crowd there are some cadmium load\[11\]. But it usually does not affect human health because of low concentrations. There is a big difference among documents or papers defined urinary cadmium. Document believes that urinary cadmium of non-cadmium-exposure workers usually does not exceed 5 µg / L, while other scholars believe that the normal urinary cadmium at 2 µg / L or less \[12\], but when urinary cadmium in <2µg / gCr, there is a higher incidence of early renal dysfunction (＞15%, criteria: urine β2- microglobulin＞1000µg / gCr, urinary NAG enzyme＞17µg / gCr ). The possible reason might be that limits of the effect indicators are too low, that the normal range of renal function indicators are still needed to be identified. Although many studies have reported lower urinary cadmium level can be observed the increasing of renal damage index, but is generally believed that, when the body of cadmium in urine up to 10µg / g Cr, kidney damage can occur. WHO set this value for the cadmium biological threshold of induced renal injury. Chinese Diagnosis of Occupational Cadmium Poisoning in 1987 (GB7803-87) also used this threshold concentration. U-Cd criteria limit of Criteria of Health Hazard Area of Environmental Cadmium Pollution was based on 95% upper limit of the control adult in an environment cadmium polluted area in the 1990s, Considering the specific circumstances at that time \[13\]. Environmental cadmium pollution control district tend to have higher soil Cd background \[7\] so its population cadmium exposure level is overall higher than the general population. And then lower trophic levels of the population at that time will also affect the human body burden of cadmium. So the standard limit of U-Cd in GB / T 17221-1998 is relatively high. It needs to revise the urinary cadmium limit in accordance with the existing population levels in China.

In China urine cadmium limit of Occupational Cadmium Poisoning is defined as 5 µg / gCr, but in this study, in addition to 25 ~ 34 year-old male non-smoker, the urinary cadmium of rest of the crowd exceeded the limit. Scholars have summed up 95% upper limit of U-Cd of the population no-exposed to cadmium in seven
different and different periods of China and
two regions in Japan, the limits are more
than 5 μg / gCr, but less than 10 μg / gCr[11].
In view of this, the diagnostic value of
urinary cadmium taken 5 μg / gCr will
appear relatively more people diagnosed
with cadmium poisoning in occupational
exposure population. It is not conducive to
deal with occupational poisoning. The
diagnostic value of urinary cadmium taken
15 μg / gCr might miss some cadmium
pollution hazards in the cadmium-exposed
population. It is not conducive to
environmental cadmium pollution on human
health protection. It should be noted that, in
some non-ferrous metal smelting enterprises,
the crowd through the respiratory exposure
to cadmium oxide fumes. Due to health
damaging effects of cadmium oxide is
greater than cadmium sulfide form which
the population generally exposed to
environmental pollution in recent times, and
the absorption rate of human body of the
former higher than the latter, and the body's
metabolism of both are different in
dynamics. However, the possibility of
human health damage at low urinary
cadmium concentration still remains with
occupational exposure to cadmium oxide
fumes.
The median urinary cadmium of this survey
population is 1.62μg/gCr, 95% reference
value is 8.28μg / gCr, close to the median
(1.15 μg / L) and range (<0.5 ~ 10.8μg/ L)
in foreign reports, but it is higher than
domestic normal urinary cadmium range in
some areas reported in China(<4.83μg / L)
[14-16]. The survey data is recommended
appropriately that the upper limit of
cadmium in urine as 10μg/gCr. But for
female smokers over the age of 30 urine
cadmium limits need more research to
explore. Meanwhile, according to a
comprehensive analysis of gender, age and
smoking, it suggests formulate to refine
urinary cadmium limit of the crowd based
on different characteristics of the population
exposure.
Due to the investigation on passive smoking
and cooking fuel using is not included,
limitation to some extent remains.
Experiments show that 33% to 62% flue gas
from cigarette smoking goes into the
environment. Passive smokers also exposed
to cadmium freed from tobacco smoking.
Urinary cadmium of female was
significantly higher than men in this survey,
it is possible because of passive smoking. In
addition, the survey is mainly concentrated
in rural areas, cooking work is mainly done
by female adult. Indoor fuel or coal burning
pollution may also be a reason, but there are
no similar reports on it.
Moreover, metabolism of cadmium in the
body may be different between male and
female [17-19], but the exact cause still needs
further investigation.

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