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Biological monitoring of cadmium exposure – an Italian experience

by Lorenzo Alessio, MD,[1] Pietro Apostoli, MD,[1] Alessandra Forni, MD,[2] Franco Toffoletto, MD[3]

ALESSIO L, APOSTOLI P, FORNI A, TOFFOLETTO F. Biological monitoring of cadmium exposure – an Italian experience. *Scand J Work Environ Health* 1993;19 suppl 1:27—33. Studies carried out over the last 12 years in northern Italy on a case list of 105 cadmium (Cd) workers showed that blood levels (B-Cd) were influenced not only by current exposure but also by body burden, while urinary levels (U-Cd) were influenced predominantly by body burden. Moreover, no advantages were gained by adjusting U-Cd for creatinine or specific gravity. The urinary beta$_2$-microglobulin levels were within the reference values when B-Cd and U-Cd were below the current threshold values. However, some indicators of early renal effect appeared to be altered. An increase in chromosome-type aberrations in lymphocytes occurred in workers with a heavy cadmium burden. Studies carried out on more than 600 subjects from the general population showed higher B-Cd levels in smokers than in nonsmokers. B-Cd levels were higher in men and in higher age groups. Identifying reference values for B-Cd through meta-analysis studies seems useful.

Key terms: blood cadmium, chromosome aberration, reference value, renal effect, urinary cadmium.

This paper summarizes the results of studies carried out on the biological monitoring of cadmium (Cd) in occupationally exposed persons and a reference population in northern Italy over the last 12 years.

Studies on occupationally exposed subjects

The case list of cadmium workers consisted of 83 men and 22 women employed in a factory producing cadmium alloys. Figure 1 shows the cadmium concentrations in the ambient air (A-Cd) of the factory, as measured over the years in different departments of the factory.

Adjustment of urinary cadmium for creatinine and specific gravity

Since the urinary cadmium concentration (U-Cd) is widely used for the biological monitoring of workers, we wanted to verify whether the adjustment of U-Cd values for urinary creatinine offered any particular advantage (1). In fact, results of earlier studies had raised doubts as to the reliability of creatinine as an adjustment parameter because of the wide inter- and intraindividual variation of its levels (2).

In this study U-Cd, determined from 24-h urine, was considered the most reliable index of cadmium exposure. We therefore investigated the relationship between U-Cd in 24-h urine and U-Cd in spot samples, considering both uncorrected values ($\mu g \cdot l^{-1}$) and values adjusted for creatinine ($\mu g \cdot g$ creatinine^{-1}) (figure 2). The following two comments can be made about the results: (i) the correlation between U-Cd in 24-h urine and U-Cd in spot samples was fairly close whether the values are uncorrected or adjusted for creatinine and (ii) the range of the corrected values was not as wide as that of the uncorrected values. However, this latter statement should not be accepted uncritically since the ranges were probably reduced, apparently as the result of a mathematical artifact. In fact, when the value of a urinary indicator is adjusted, it is usually divided by a number greater than one. This interpretation is confirmed by the fact that the relative variability showed no improvement. In fact, not only did the correlation coefficient not increase after adjustment, but the coefficient of variation (CV) of the U-Cd values was even higher for the adjusted values than for the uncorrected ones: U-Cd $\mu g \cdot g$ creatinine^{-1} = 6.2 (SD 6.7), CV 108%; U-Cd $\mu g \cdot l^{-1}$ = 9.6 (SD 7.8), CV 81%.

Identical results were also obtained when density was used as a parameter for the adjustment of U-Cd, and the two parameters appeared to be closely correlated. Thus these data seem to indicate that no practical advantage would have been gained by adjusting the U-Cd values either for creatinine or for density in our case list.

[1] Institute of Occupational Health, University of Brescia, Brescia, Italy.
[2] Institute of Occupational Health, University of Milano, Milan, Italy.
[3] Institute of Occupational Health, Hospital of Desio, Desio, Italy.

Reprint requests to: Professor L Alessio, Institute of Occupational Health, University of Brescia, P le Spedali Civili 1, Brescia, I-25100 Italy.

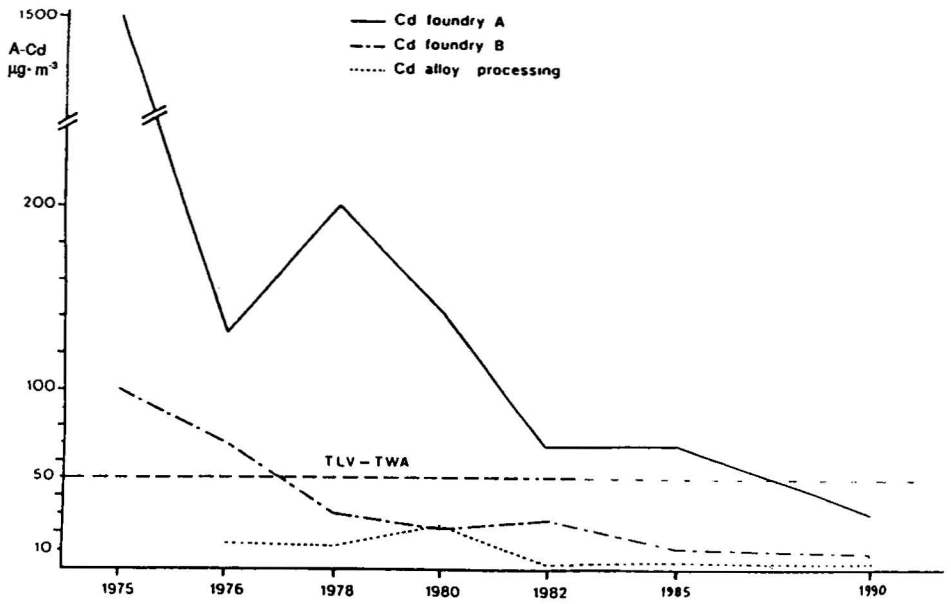

Figure 1. Concentrations of cadmium in the ambient air (A-Cd) in the period 1975—1990, as measured with air samplers (before 1982) and with personal samplers (after 1982). (TLV = threshold limit value, TWA = time-weighted average)

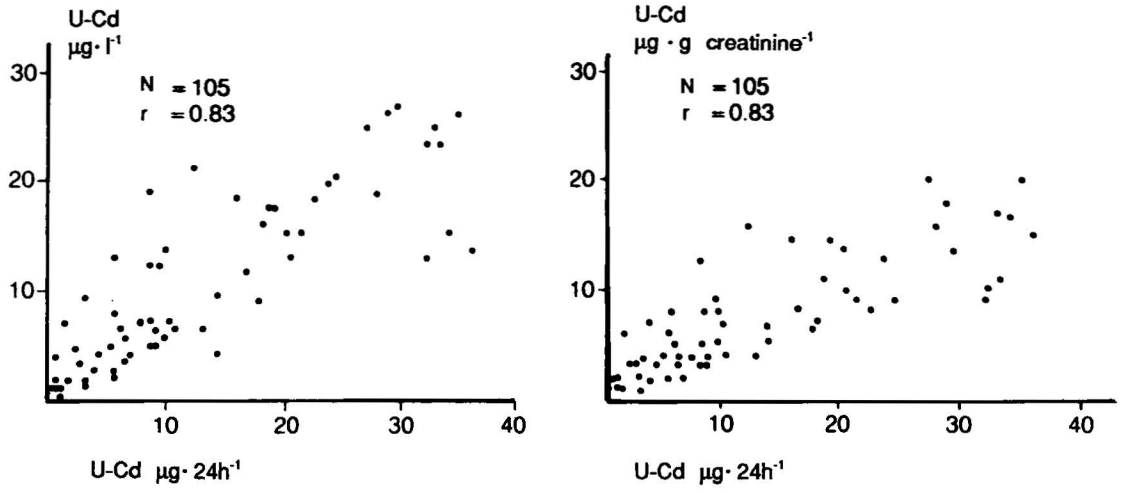

Figure 2. Relationship between the urinary cadmium concentration (U-Cd) for 24-h urine and the U-Cd for spot samples.

Cadmium in blood and urine in relation to exposure

With the same case list, we attempted to check whether the meaning generally assigned to the two cadmium indicators of dose can be as clearly differentiated as is generally reported (3). In fact, the cadmium concentration in blood (B-Cd) is generally considered useful in evaluating current exposure, and U-Cd, in low-level exposure, is considered to reflect body burden, while under high-absorption conditions it is considered to be an indicator of current exposure (4).

For this reason, the biological indicators of cadmium in 83 male cadmium workers were studied in relation to current exposure, length of service, and cumulative exposure, computed as mean yearly concentrations of cadmium at the workplace multiplied by years of exposure. (See tables 1, 2, and 3.)

The B-Cd values were significantly higher in the subgroups of subjects with higher current cadmium exposure and in those with greater cumulative exposure, but the test levels were not influenced by duration of exposure. The U-Cd levels were significantly higher in the groups of subjects with greater

Table 1. Geometric means and standard deviations of biological indicators of cadmium in 83 male workers divided into four groups according to current exposure.

Cadmium concentration in ambient air (µg · m⁻³)	Subjects (N)	Cadmium concentration (µg · l⁻¹)			
		Blood		Urine	
		Mean	SD	Mean	SD
0—1	27	1.64	2.3	5.02	3.2
1—10	31	2.91	2.2	5.70	2.5
10—50	16	5.91	1.7	11.15	2.1
>50	9	6.70	2.0	10.49	2.3
F		12.69		3.01	
P		<0.001		0.036	

Table 2. Geometric means and standard deviations of biological indicators of cadmium in 83 male workers divided into three groups according to length of exposure. (NS = not significant)

Length of exposure	Subjects (N)	Cadmium concentration (µg · l⁻¹)			
		Blood		Urine	
		Mean	SD	Mean	SD
<5 years	17	2.43	2.5	3.27	2.7
6—15 years	44	2.96	2.6	7.20	2.6
>15 years	22	4.94	2.1	10.73	2.1
F		1.93		6.92	
P		NS		0.002	

cumulative exposure, but were less influenced by current exposure or duration of exposure. When the entire group of subjects was considered, a fairly good correlation was found between B-Cd and U-Cd ($r = 0.69$, $P<0.001$, B-Cd = 1.29 + 0.30 U-Cd). These two biological indicators were also correlated in four groups of subjects with varying degrees of current exposure. Nevertheless, for identical U-Cd values, the B-Cd levels were higher in the subjects with heavier current exposure (figure 3).

The overall data showed that the two indicators at least partially reflect identical phenomena. In fact, the B-Cd values did not depend solely on current exposure, but also increased with body burden, which however, had a greater influence on U-Cd .

Table 3. Geometric means and standard deviations of biological indicators of cadmium in 83 male workers divided into five groups according to cumulative exposure.

Cumulative exposure (µg · m⁻³ · years)	Subjects (N)	Cadmium concentration (µg · l⁻¹)			
		Blood		Urine	
		Mean	SD	Mean	SD
<50	23	1.52	1.9	2.53	2.2
51—250	18	2.26	1.9	5.19	2.2
251—500	17	5.12	2.2	8.92	2.0
501—3000	14	4.54	2.3	11.78	1.9
>3000	11	6.84	1.9	20.59	1.4
F		12.01		19.98	
P		<0.001		<0.001	

Indicators of early cadmium renal effect

The indicators of early cadmium renal effect were considered in three studies, in the first of which (3) urinary beta$_2$-microglobulin was determined in samples not rendered alkaline, while in the other two studies (5, 6) urine samples were adjusted to pH 7 with sodium hydroxide.

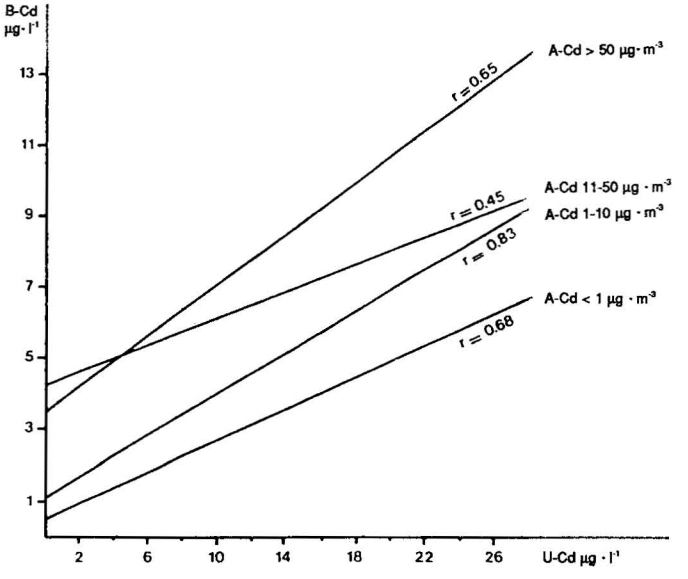

Figure 3. Relationship between the cadmium concentration in the blood (B-Cd) and urine (U-Cd) of 83 male workers divided into four groups according to current exposure. (A-Cd = cadmium in the ambient air)

Relationship between urinary cadmium and urinary beta$_2$-microglobulin in a cross-sectional study. In all of the 83 male workers studied the urinary beta$_2$-microglobulin levels were below the upper limit of our reference group (260 $\mu g \cdot l^{-1}$). However, there was a clear trend for beta$_2$-microglobulin to increase as both the length of exposure and cumulative exposure increased. If the subjects treated were divided into three groups according to increasing U-Cd levels, the cumulative frequency distributions of the urinary beta$_2$-microglobulin values showed that the highest values of microproteinuria occurred more frequently in subjects with U-Cd >10 $\mu g \cdot l^{-1}$ (figure 4).

These findings led us to hypothesize that the behavior of urinary beta$_2$-microglobulin could be the expression of an initial effect induced by cadmium in the kidney (3).

Ten-year follow-up of urinary beta$_2$-microglobulin. After a 10-year follow-up, the case list was divided into groups according to whether the B-Cd and U-Cd values exceeded 10 $\mu g \cdot l^{-1}$ and 10 $\mu g \cdot g$ creatinine^{-1}, respectively, values which have been generally adopted as biological threshold values (5). Among workers with B-Cd and U-Cd levels always below the threshold, the proportion exceeding the upper "normal" limit of urinary beta$_2$-microglobulin was less than 3% (ie, a percentage comparable to that of the reference population). When B-Cd exceeded 10 $\mu g \cdot l^{-1}$ and U-Cd was over 10 $\mu g \cdot g^{-1}$ creatinine on one or more occasions, the frequency of subjects with an increased urinary excretion of beta$_2$-microglobulin was considerably higher, namely, 8.4 and 7.5%, respectively.

These results seem to indicate that, when B-Cd and U-Cd are always below the proposed threshold levels, they are "safe" from the point of view of tubular function, at least as indicated by urinary beta$_2$-microglobulin levels. One should however raise the question of whether this test is sufficiently sensitive to detect early tubular alterations.

We therefore verified the urinary beta$_2$-microglobulin levels, as well as the levels of N-acetyl-glucosaminidase, retinol binding protein, and albumin, in 42 subjects included in our case list (6).

Validity of different urinary tests in the study of early renal effects

The 42 cadmium workers were subdivided into two groups on the basis of the median values of U-Cd over the last 10 years: <10 and >10 $\mu g \cdot l^{-1}$. For all four of the renal indicators, the highest levels with respect to the upper limit of the general population were documented in the group of subjects with the highest exposure to cadmium; however, altered levels were noted in the other group, in particular for N-acetyl-glucosaminidase (figure 5).

Moreover, the results of this study led us to believe that, when the renal effects of cadmium are monitored, it is advisable to use several indicators simultaneously. In fact the number of tests that were simultaneously altered differed in relation to the exposure levels. In the first group there were two duplicates, and in the most heavily exposed group there were four duplicates, two triplicates, and three quadruplicates.

Moreover, the results cast some doubts on whether the current threshold for U-Cd is really "safe" from the point of view of tubular function.

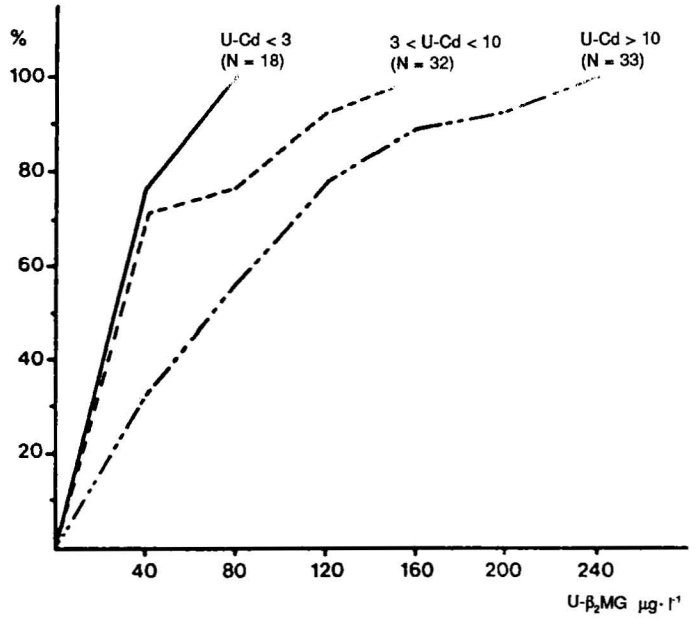

Figure 4. Cumulative frequency distributions of urinary beta$_2$-microglobulin (β_2MG) in three groups of workers with increasing urinary cadmium (U-Cd) levels

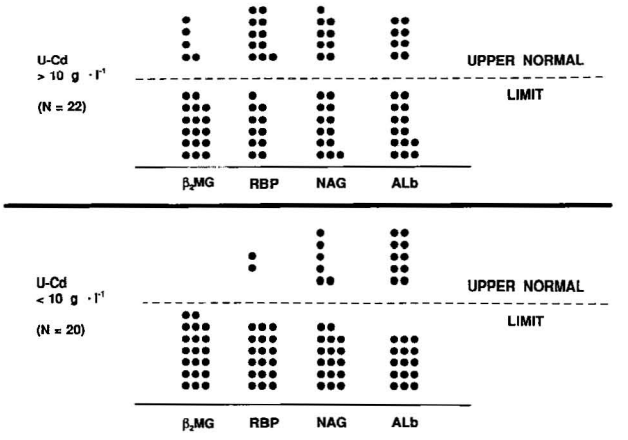

Figure 5. Beta$_2$-microglobulin (β_2MG), retinol binding protein (RBP), N-acetyl-glucosaminidase (NAG), and albumin (ALb) in the urine of two groups of cadmium workers with different degrees of exposure. (U-Cd = concentration of cadmium in urine)

Table 4. Rates of abnormal metaphases (excluding gaps)[a] and of cells with chromosome-type aberrations in cadmium workers, subdivided according to a cumulative exposure index (CEI), and in their matched referents.

	Number of exposed workers	Abnormal metaphases (%)		Chromosome-type aberrations (%)	
		Exposed workers	Referents	Exposed workers	Referents
Total	40	2.60	1.70	1.15	0.45
		P = 0.0125[b]		P = 0.0075[b]	
CEI[c]					
100	10	1.80	1.60	0.80	0.70
101—500	13	2.61	1.54	0.76	0.15
501—1000	9	2.44	2.33	1.00	0.55
>1000	8	3.75	1.37	2.37[d]	0.50
		P<0.005[b]		P<0.005[b]	

[a] 100 metaphases scored for each sample.
[b] Wilcoxon matched pair test.
[c] CEI determined as micrograms per cubic meter multiplied by years of exposure.
[d] Different from the other subgroups (χ^2 13.2, P<0.001).

Table 5. Geometric means and standard deviations of the cadmium concentrations in the blood of 404 men from Brescia according to their smoking habits.

	Men (N)	Cadmium concentration in blood (µg · l⁻¹)	
		Mean	SD
Nonsmokers and exsmokers	275	0.36	2.06
Smokers			
<10 cigarettes · d⁻¹	63	0.59	1.67
10—20 cigarettes · d⁻¹	50	1.11	1.40
>20 cigarettes · d⁻¹	16	1.58	1.32

Chromosome effects in cadmium workers

The chromosome effects of cadmium exposure in humans is a controversial issue (7). Our experience with the relationship between cytogenetic findings and cadmium exposure concerns 40 healthy male workers belonging to our case list (8).

In lymphocytes cultured for 2 d, the rates of total abnormal metaphases (including gaps) did not significantly differ between the exposed subjects and the referents, matched for gender, age, and smoking habits (3.50 versus 3.07%), while the total number of abnormal metaphases excluding gaps was significantly higher for the exposed subjects than for the referents. The difference was due solely to chromosome-type aberrations (table 4). When the group was divided into four subgroups with different cumulative exposures, only the subgroup with the highest cumulative exposure had a rate of abnormal metaphases and chromosome-type aberrations significantly higher than that of the referents and the three subgroups with lower cumulative exposure (table 4).

Moreover, the 22 workers with a mean U-Cd of >10 µg · l^{-1} had significantly higher rates of chromosome-type aberrations (1.55%) than the matched referents (0.41%) and the 18 workers with a mean U-Cd <10 µg · l^{-1} (0.67%), who did not differ from their matched referents (0.50%).

In conclusion, increased chromosome-type aberrations in cultured lymphocytes were demonstrated in cadmium workers only when a high body burden of cadmium existed. It must be pointed out, however, that the significance of these findings in relation to the occurrence of cancer at the individual level is not known.

Identification of reference values for cadmium in blood in general population groups

The studies on reference values were aimed at identifying values based on the consideration that, in industrialized countries, the widespread use of preventive measures has led to a gradual decrease in occu-

pational exposure to toxic metals. In this situation it seems more important to use biological indicators for a group of subjects than for individual workers. To do so, it is necessary to establish reference values for the biological indicators used (9). A reference value can be defined as a "concentration interval of an element in samples from a defined part of a human soft or hard tissue or from a body fluid randomly selected from a sufficient number of healthy individuals living in a defined geographical area with defined life-style and eating, drinking and smoking habits, with defined age and sex, not being professionally exposed to the appropriate element [p 235]" (10).

Reference values for cadmium concentrations in blood in the Brescia area

Between 1990 and 1991 we studied a group of blood donors living in the province of Brescia. The results of the study, carried out to determine the influence of variables such as gender, age, type of work, and drinking and smoking habits on B-Cd levels, were as follows (11). Smoking proved to be the most important of all the variables examined. Not only were the B-Cd levels clearly higher in smokers, but they also increased in direct proportion to the number of cigarettes smoked (table 5, on page 31). In contrast, no significant differences were found between drinkers of alcoholic beverages and nondrinkers. Moreover, the B-Cd levels were significantly higher among the men than among the women, even when the two groups were subdivided into smokers and nonsmokers. Division of the subjects into three age groups revealed statistical differences in the B-Cd levels due to age. The differences between these groups (men versus women, different age groups) were less marked, however, than those between smokers and nonsmokers.

In addition, a study of our case list showed that, in the selection of reference groups, it is important to exclude subjects working in the same factory, even if they are not directly exposed to cadmium and have been selected from white-collar workers.

Identification of reference values for cadmium in blood through meta-analysis studies

Articles published from 1976 to 1990 were reviewed for this project. Quality grading was carried out taking into account the sampling conditions, the analytical treatment, and the statistical treatment (12).

The majority of the studies were not focused on the definition of reference values, but rather on the formation of reference groups for toxicologic and epidemiologic investigations. Sometimes the subjects making up the reference group were white-collar workers employed in the same factory. After 18 publications (approximately 16 400 subjects) had been graded, only four (approximately 4300 subjects) were taken into consideration.

The case list was then divided into groups with smoking and area of origin as the main variables. In particular, the subjects living in Japan were separated from the general case list (table 6). The B-Cd values of subjects in the general case list were clearly lower than those of subjects living in Japan. In both case lists the B-Cd levels were higher for smokers, but in the Japanese group the influence of smoking on the B-Cd levels was less well marked than that documented for cases from the general case list. These preliminary results show that it is possible, from the methodological viewpoint, to formulate tentative reference values for B-Cd when the statistical and the analytical treatments are checked for correctness and the case list is selected with smoking as the main interfering factor.

This conclusion, in our opinion, is of practical importance because reference values obtained through meta-analysis studies can be particularly useful even for determining whether sources of environmental pollution are present or in the event of a high dietary intake of cadmium, as confirmed by the Japanese case list.

This study is being done in the framework of the Tracy Project (10) and is still in progress. In fact, we asked the authors of the papers in which the analytical treatment was considered to be satisfactory to supply the original data together with detailed information on the sampling conditions. We will reanalyze any additional data which can subsequently be included in the studies considered to date. We nevertheless think it necessary to emphasize that, in spite of the possibility of using reference values derived from meta-analysis studies, laboratories should produce their own reference values, since they are indispensable for field investigations.

Table 6. Results of the statistical treatment of data on cadmium blood concentrations ($\mu g \cdot l^{-1}$) from the literature.

	Geometric mean	Geometric standard deviation	75th percentile	90th percentile	
General case list (N = 2385)					
Nonsmokers	0.52	1.76	0.79	1.12	(N = 1319)
Smokers	1.47	2.10	2.58	4.12	(N = 665)
Japanese case list (N = 2009)					
Nonsmokers	3.42	1.50	4.43	5.87	(N = 1539)
Smokers	4.19	1.48	5.67	7.28	(N = 470)

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