
Biological Monitoring in the Epidemiology of Chronic Effects of Cadmium in Workers

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1 INTRODUCTION

Biological monitoring has gained increasing attention as a means of assessing health hazards in both the workplace and the general environment. The methods can be applied to estimate both individual and collective exposures, and can be used as tools to estimate the probability of adverse health effects either in an individual or in populations (Zielhuis, 1978; Aitio and Jarvisalo, 1984; Piotrowski, 1985).

According to Piotrowski (1985), data supporting methods for biological monitoring are available for about 50 to 60 organic substances and 17 metals. The degree of development of biological monitoring methods differs for specific toxicants, with respect to analytical procedures and to available interpretive bases. This paper presents an illustrative study of the application of biological monitoring to cadmium (Cd). The study sought to obtain data on the usefulness of Cd measurements in blood taking into account exposure duration, and to predict the risk of renal dysfunction manifested by the increased excretion of low molecular weight proteins in urine.

The study was carried out in workers exposed to Cd because high exposure levels would help verify selected assumptions. The results obtained were compared with those for two populations, neither of which was occupationally exposed to Cd, but with one living in an area adjacent to a zinc mill and the other in an area not known to be contaminated.

1.1 TOXICOKINETICS AND MECHANISM OF Cd TOXICITY

In chronic Cd exposure, the kidney is a target organ; the tubular dysfunction manifested by increased excretion of low molecular proteins in the urine is the first sign of functional changes (Piscator, 1982). In the study of Cd workers, a glomerular type of proteinuria was found to exist but was not necessarily associated with tubular proteinuria (Lauwerys *et al.*, 1979).

Cd can be absorbed through the lungs (uptake 25 percent) and gastrointestinal tract (uptake 5 percent). Assuming daily dietary intake of

25 μg , uptake will amount to about 1 μg . A similar amount can be absorbed through the lungs as a result of smoking 20 cigarettes a day (Piscator, 1982). According to current reports based on animal experiments (Suzuki, 1984; Nordberg, 1984), the chemical forms of Cd are the main controlling factors that determine the distribution of Cd either to the liver or kidneys. Initially after exposure, Cd in blood is bound to albumins and to other proteins with molecular weights higher than that of albumin. Such Cd is chiefly taken up by the liver where it induces metallothionein synthesis and is accumulated in the form of the complex with this low molecular weight protein. Liver metallothionein is released into plasma in small quantities and its concentrations in persons occupationally exposed to Cd was from below 2 to 11 ng/g (Nordberg *et al.*, 1982).

At low levels of exposure, when the quantity of Cd metallothionein released from liver and the concentration of Cd in the kidneys are relatively low, the metallothionein is taken up efficiently in the renal tubule by pinocytosis. Reabsorbed metallothionein is degraded in the lysosomes, and liberated Cd is sequestered with the copper-rich metallothionein within the normal biosynthetic capacity. According to Suzuki (1984) this normal mechanism of metallothionein synthesis has a capacity sufficient to bind Cd up to a concentration of about 100 μg of kidney tissue. When the concentration of Cd in the kidney exceeds this level, however, the quantity of non-complexed Cd may increase inducing transitory damage of the renal tubules. At the same time, Cd ions stimulate the induction of synthesis of new metallothionein which is poor in copper. The kidneys lose function, and Cd accumulates further in the kidneys.

Persistent renal damage was observed in rats when Cd had reached a maximum plateau level of about 350 $\mu\text{g/g}$ of kidney (Suzuki, 1984). At this level, the relative ratio of Cd to the coexisting metals, and the difference between total renal Cd quantity and the thermostable soluble fraction, metallothionein, were higher than before the plateau.

These data indicate that the kidney has a threshold limit above which its ability to synthesise metallothionein is insufficient, and the ion Cd binding with other proteins becomes more important for normal functioning of the kidney.

Early attempts to determine a critical level in man were based on the material collected at autopsies and biopsies (WHO, 1977). In recent years, a technique for *in vivo* measurement of Cd in human kidney and liver has become available by means of neutron activation. According to Roels *et al.* (1983) the critical level of Cd in renal cortex of 216 $\mu\text{g/g}$ protects probably more than 90 percent of male workers from developing Cd-induced renal dysfunction. This level corresponds to the excretion of Cd in the urine amounting to 10.8 $\mu\text{g/g}$ of creatinine. The dose/response curve presented by Ellis *et al.* (1984) indicates that the 10 percent probability of occurrence

of disturbances in tubular reabsorption in kidney can be expected at the concentration of Cd in the kidney cortex amounting to about 230 $\mu\text{g/g}$.

1.2 BIOLOGICAL MONITORING OF Cd EXPOSURE

The selection of the method of evaluation of exposure to Cd depends on the effect which is to be monitored. The occurrence of acute and chronic lung dysfunction, which is an effect of local action, can be correlated with the concentration of Cd oxide airborne dust in the respirable particle size fraction and to the duration of exposure (WHO, 1980).

To evaluate effects of chronic exposure on kidney function, biological monitoring offers advantages over other approaches. Existing data suggest that when equilibrium is reached, Cd in blood is a good indicator of the average industrial exposure during recent months. In newly employed workers in a Cd battery factory in Sweden where the average concentration of Cd oxide airborne dust was 50 $\mu\text{g Cd/m}^3$, Cd levels in blood increased with a half-life of 77 days to an equilibrium level of about 30 $\mu\text{g/l}$ (Kjellstrom and Nordberg, 1978). In similar investigations carried out by Lauwerys *et al.* (1979), concentrations of Cd in air ranged from 110 to 2120 $\mu\text{g/m}^3$. Cd levels in blood increased linearly up to 120 days and then levelled to the mean concentration of about 150 $\mu\text{g/l}$. According to Piscator (1973), half-life of Cd in blood ranged from two to about five months after termination of exposure.

However, although it is generally assumed that at long-term, low level exposure Cd in urine reflects Cd quantity accumulated in an organism (WHO, 1980), interpretation of results is much more complicated than in the case of Cd in blood. Observations made by Lauwerys and co-authors (1979) showed, in newly employed workers, four phases of excretion including two equilibria: (1) at the concentration of about 15 $\mu\text{g/g}$ creatinine lasting up to 120 days from the beginning of exposure, and (2) at the concentration of about 200 $\mu\text{g/g}$ creatinine starting about 220 days after the beginning of exposure. According to Lauwerys and co-authors (1979), the first equilibrium is related to saturation of the kidney and to binding of Cd to metallothionein, whereas the second reflects the current level of exposure. When kidney dysfunction occurs, there is also a sudden increase in Cd excretion probably due to the lower reabsorption of the Cd metallothionein complex in the tubules and to excretion of kidney deposits.

Recommendations for safe levels of Cd in biological material include: 10 $\mu\text{g Cd/l}$ of blood and 10 $\mu\text{g/g}$ creatinine in urine (WHO, 1980). This last value corresponds with the value given by Roels *et al.*, i.e., 10.8 $\mu\text{g/g}$ creatinine and cited the data of Lauwerys *et al.* (1979).

Apart from determinations of the concentrations of Cd in blood and urine, it is recommended that early signs of kidney injury including

determination of low and high molecular weight proteins should be evaluated. Detailed information and methods are to be found in the paper by Herber (1984). β_2 -Microglobulin (β_2 M) is commonly regarded as the most sensitive, and is the most popular, index for early detection of excessive Cd exposures. However, β_2 M can be subject to rapid degradation at pH values below 5.5, which to a certain extent makes the applicability of this parameter questionable. Generally, 200 to 300 μg of β_2 M/g creatinine is considered as a critical concentration (Herber, 1984). According to Kowal and Zirkes (1983), excretion of β_2 M in the urine increases with age. Using the upper limit of the 95 percent confidence interval, they determined the normal upper limit of urinary β_2 M of 413, 653 and 1451 $\mu\text{g/l}$ for age groups 20 to 29, 50 to 59 and 70 to 74, respectively. According to Pharmacia Diagnostics, the normal upper limit of μ_2 M in the urine is 370 $\mu\text{g/l}$ for ages 15 to 63. Besides μ_2 M, retinol binding protein, amino acids, and glucose are used as common indices of tubular dysfunction. Determinations of albumin and total proteins in the urine serve as indices of effects on glomerular filtration.

1.3 HEALTH-BASED LIMITS IN EXPOSURE TO Cd

According to the kinetic model of Friberg *et al.* (1984) biological half-lives of Cd in kidney ranged from 19 to 9.5 years; the daily Cd intake via food required to reach 200 $\mu\text{g/g}$ in kidney cortex at age 50 was calculated to be between 352 and 616 $\mu\text{g/day}$. According to the model of Nordberg and Kjellstrom (1979), the corresponding daily intake amounts to 440 μg (biological half-life in kidney = 12 years).

For shorter exposure of industrial workers, the one compartment model of Friberg predicted at concentrations of 11.1 to 15.8 $\mu\text{g Cd/m}^3$ of air, a half-life of 9.5 to 19 years; and with a 25 percent adsorption by inhalation, a level of 200 $\mu\text{g Cd/g}$ of kidney cortex should be reached within 25 years. The corresponding value in the eight-compartmental model (Nordberg and Kjellstrom, 1979) is approximately 40 $\mu\text{g/m}^3$ for $t_{1/2} = 12$ years with 18 percent adsorption by inhalation, in order to reach 220 $\mu\text{g/g}$ in renal cortex after a 25 year exposure to Cd. The difference between these data is almost threefold.

Accurate estimation of the safe duration time at work in contact with Cd is especially important since, according to current thinking, mild renal injuries caused by Cd can be reversible, provided that the duration is not too long (Suzuki, 1984; Nomiya and Nomiya, 1984). Therefore, the concept of integrated exposure (i.e., average concentration of Cd in the air in mg/m^3 multiplied by years of exposure) seems to be promising (Lauwerys *et al.*, 1979; Falck *et al.*, 1983). In a follow-up study of a group of 11 workers, Lauwerys and coauthors (1979) found that kidney function disturbances occurred when the integrated exposure reached 1500 to 3000 $\text{mg/m}^3 \times$ the number of years. However, these data have not included

absorption from the alimentary tract which might have occurred from swallowing dust.

According to Falck and co-authors (1983), the average cumulative time-weighted exposure needed to cause renal dysfunction is about $1140 \mu\text{g}/\text{m}^3 \times$ the number of years of exposure. In a group of subjects with normal renal functions, this value was significantly lower, about $460 \mu\text{g}/\text{m}^3 \times$ the number of years. If the value of $1200 \mu\text{g}/\text{m}^3 \times$ the number of years was adopted as a threshold of integrated exposure, then, at a concentration of $50 \mu\text{g}/\text{m}^3$, the tolerable period of work would be 24 years. The $1200 \mu\text{g}/\text{m}^3 \times$ the number of years of integrated exposure is close to the value of $1000 \mu\text{g}/\text{m}^3$ (25 years, $40 \mu\text{g}/\text{m}^3$) estimated to cause saturation of the kidney cortex with Cd using the eight compartment kinetic model (Nordberg and Kjellstrom, 1979).

The main obstacle to the application of the concept of integrated exposure is the difficulty in precisely evaluating Cd concentration both in air to which workers are exposed and the true amounts absorbed through the respiratory system and the gastrointestinal tract. Taking into account toxicokinetic data, the determination of Cd in blood can constitute a much better parameter for evaluation of integrated exposure than concentration of Cd in air.

2 INVESTIGATIONS IN WORKPLACE CONDITIONS

Investigations were carried out in an alkaline battery factory and in a nonferrous metals foundry. The population examined consisted of several study groups: 102 employees of the battery factory and 30 employees of a division producing Cd in the foundry, and two control groups, 50 persons from the administration of the battery factory, and 32 persons from a textile industry with no known industrial Cd exposure.

Determinations of Cd in blood (Cd-B) and in urine (Cd-U) were performed by means of flameless atomic absorption spectroscopy (AAS). In addition, determinations of retinol binding protein (RBP) in urine, latex immunoassay, β_2 -microglobulin in urine, $\beta_2\text{M-U}$, radioimmunoassay, and total amino acids in urine (TA-U) were conducted. The laboratories participating in the external quality control system were: for Cd-B, UK EQAS, Wolfson Research Laboratories, UK; and Cd-U, European Q.C. Programme for Toxic Metals and Organic Compounds in Urine, Helsinki. Cd concentrations in air were measured by flame AAS.

In the investigated plants, exposure to Cd was high, as shown both by results of determinations in the air, Cd-A, and in biological material, Cd-B and Cd-U. Concentrations of Cd in blood and in urine were significantly higher in all workers of the investigated divisions of both plants than in the control group.

As an upper limit of excretion of proteins and amino acids, the geometric

mean value ± 1.96 G.S.D. obtained in the control group was adopted. In spite of high exposure, the signs of kidney dysfunction were observed only in two groups of workers from the battery factory with the highest exposures. However, the lack of increased excretion of RBP-U and TA-U was unexpected in the group of workers from the furnaces division where exposure was similar to that found in the two groups of battery factory workers.

The determinations of Cd concentrations in air are of limited usefulness to evaluate actual amounts of Cd absorbed into an organism, at least in the case of plants where Cd is present in the form of dust and not fumes. Concentrations of Cd-B of workers employed in the same division revealed large differences.

The ratios of mean values of Cd-B ($\mu\text{g/l}$) to Cd-A ($\mu\text{g/m}^3$) concentrations, were about twice and in one case even seven times lower than those given by Nordberg and Kjellstrom (1979). The results obtained showed that practical application of the concept of integrated exposure (Lauwerys *et al.*, 1979; Falck *et al.*, 1983) would require, in cases of exposures similar to those found in the investigated plants, carrying on continuous individual monitoring of exposure. However, this would still fail to provide any possibility to evaluate swallowed Cd. An additional difficulty in evaluating internal exposure is represented by the use of personal protective devices.

At the same time, the data confirmed that the period of exposure to Cd is an important factor in the process of initiating kidney dysfunction. Therefore, the authors analysed their data as to the feasibility of introducing the concept of integrated exposure as a predictive value with a modification consisting of replacement of Cd-A by Cd-B concentrations. In spite of the lack of retrospective data, it has been assumed, based on the stability of the factory staff, that Cd-B concentrations did not undergo any great changes over previous exposure periods. As indices of the efficiency of the tubular reabsorption of RBP-U and $\beta_2\text{M-U}$, the upper normal levels of 130 and 380 $\mu\text{g/g}$ creatinine, respectively, have been adopted.

The dose/response curve obtained shows that the probability of kidney dysfunction in 10 percent of the population occurs at the integrated exposure of about 300 to 400 Cd-B $\mu\text{g/l} \times$ number of years exposure. These data are consistent with the recommended (WHO, 1980) Cd-B value of 10 $\mu\text{g/l}$.

Such results may explain to some extent the apparent discrepancies between the level of exposure and its effects found in workers of the two investigated factories. The data on the frequency of increased RBP excretion in the urine in consecutive ranges of Cd-B $\times t$ values show that, in spite of high exposure, 97 percent of Cd-B $\times t$ values in the nonferrous metals foundry workers was below 200 and in the range between 200 and 800.

3 INVESTIGATIONS IN THE GENERAL POPULATION

Investigations were aimed at assessing the extent to which living in an area of high exposure to Cd may affect Cd absorption and accumulation and renal function in an organism.

The investigated group consisted of the inhabitants over 50 years old. The mean age of the population selected in the control area was 64 years, from 55 to 77 years, and in the area of high exposure, 63 years, from 51 to 82 years.

The scope of investigations was extended, as compared to the previously described one, by lead determinations in blood (with flameless AAS) and albumins in urine (with LIA method). The results indicated that living for a period of about 20 years in the area of high Cd has caused no significant increase of Cd in blood when compared with controls. Urinary Cd concentration in the exposed group was significantly higher than in controls, which suggests a slight increase of quantity of Cd accumulated in the organism; however, both means were among the low ones. These data confirm the hypothesis about the importance of the alimentary tract as the main site of Cd absorption in the general population, with a slight impact on locally produced food (Bennett, 1981).

For lead, the differences in blood concentrations seem to be chiefly due to the differences in concentrations in the air and to absorption through inhalation, assuming that $1 \mu\text{g Pb/m}^3$ causes Pb-concentration increase in blood of about $15 \mu\text{g/l}$. In the area of high exposure, neither a decrease of tubular reabsorption indices (i.e., RBP-U) nor disturbances in glomerular filtration (i.e., excretion of albumins) has been found. By contrast, RBP concentrations in urine were significantly lower than in the control group.

Although these investigations have not been completed, these data should be regarded as preliminary; they seem to confirm Strehlow and Barltrop's data (1981), which showed that inhabiting areas of high contamination with Cd involves no significant health effects.

4 CONCLUSIONS

(1) It seems that it will be feasible to use the proposed integrated exposure indicator, based on determinations of blood Cd concentrations and exposure duration, as a basis for programming the period of work with exposure to Cd individually for particular workers. These data should be confirmed separately in a study of selected workers having documented data on blood Cd concentrations over a longer period of time. The problem of properly programming the period of employment under conditions of exposure to Cd is very important, because of the need to avoid the irreversible renal dysfunction manifested by decreased tubular reabsorption of low molecular proteins.

(2) The results obtained with the help of biological monitoring methods confirm Bennett's (1981) and Strehlow and Barltrop's (1981) data indicating that food coming from rural areas of low contamination plays an important role in Cd body burden for general populations. Cd quantities absorbed through lungs in the areas of high contamination as well as participation of the locally produced food in the diet do not seem to create, unlike lead, a serious problem.

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