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CADMIUM EXPOSURE AND INDICATORS OF KIDNEY FUNCTION

Technical Report

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Evaluation of the relationships between cadmium exposure and indicators of kidney function
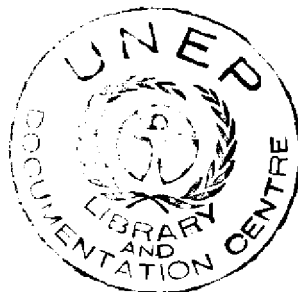
by Malcolm Hutton

A Technical Report (1983)

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SUMMARY

This report aims to establish the relationship between cadmium dose and early indications of renal dysfunction in humans and utilizes this relationship to suggest acceptable exposure levels to the metal. Data from epidemiological studies of several environmentally exposed populations in Japan and one group of occupationally exposed workers were utilized. Regression analysis was used to establish dose-effect relations, while dose-response relationships were calculated by probit analysis.

The techniques available for the estimation of cadmium dose and renal effects in exposed populations are reviewed. The relative merits and drawbacks of the methods used to estimate dose are also discussed. Increased urinary excretion of β_2-microglobulin emerges as the most widely used indicator of renal effect in epidemiological studies. For this reason, the factors which influence the usefulness of β_2-microglobulin as an index of effect are considered, as are the problems involved in interpreting the precise significance of increased β_2-microglobulin excretion.

The results of statistical analysis indicate that adherence to the provisional tolerable intake value proposed by the World Health Organization in 1972 will protect the large majority of the population from cadmium induced renal dysfunction. There are, however, indications that a small proportion of the population will show signs of renal effects at low cadmium intakes, although the errors associated with these estimates are large. The findings obtained using urinary cadmium as an index of dose are discussed in relation to recent proposals for a maximum urinary cadmium level in occupational exposure.

FOREWORD

This document is the modified version of a report submitted to the Commission of the European Communities under Contract Number ENV-475 U.K. The work on which this report is based was commissioned by the Environment, Raw Materials and Material Technologies Research Programmes, Directorate General for Science, Research and Development. The views expressed in the report do not necessarily reflect those of the Commission.

ACKNOWLEDGEMENTS

1 Background

1.1 Introduction

Increasing attention is being paid to the establishment of guide-lines for acceptable exposure levels to toxic substances in the industrial and general environment. Ideally, such guide-lines should not only be set below the estimated threshold for any adverse effect but also incorporate a reasonable safety margin. The estimation of the relationship between toxicant exposure and development of an adverse effect should serve as the toxicological basis for the setting of such guide-lines and this report is concerned with the establishment and interpretation of the relationship for the cumulative nephrotoxin, cadmium.

A decade has passed since an Expert Committee of the Food and Agriculture Organization and the World Health Organization first proposed a provisional tolerable weekly intake for dietary cadmium (WHO 1972). This estimate was not based on direct epidemiological observations of dose and effect but rather on a mathematical approach, using a simple metabolic model of cadmium. This approach was probably favoured because of the paucity of information on dose-response relationships for cadmium, associated with the inherent difficulties of quantifying dose after long-term exposure to the metal.

To date, the WHO estimate remains unrevised and has been widely adopted as the standard for safe levels of dietary exposure. In the intervening 10 years, however, several epidemiological studies have measured cadmium exposure and health effect in humans. This report therefore aims to investigate the relationship between cadmium dose and effect, utilizing some of these studies of exposed populations. The validity and accuracy of the techniques used for the estimation of dose-effect relations are also assessed, as is the choice of the dose and effects variables employed by the various studies.

1.2 The nephrotoxic action of cadmium

Excessive cadmium exposure may give rise to both acute and chronic types of poisoning. This assessment is concerned only with the initial effects arising after long-term low-level cadmium exposure. Recent reviews of cadmium toxicity (Friberg, Piscator, Nordberg and Kjellström 1974; CEC 1978; Friberg, Nordberg and Vouk 1979; Webb 1980) may be referred to for a more comprehensive treatment of the health effects of the metal.

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It is generally accepted that the kidney is the critical organ after long-term cadmium exposure. Renal damage results from the excessive accumulation of cadmium in the kidney. One of the first signs of renal dysfunction is an increased urinary excretion of proteins, caused by impaired reabsorption function of the proximal tubules. Cadmium induced proteinuria was until recently considered to be characterized by a predominance of low molecular weight proteins, particularly α_2, β_2 and γ-globulins. Examples of such proteins are β_2-microglobulin (β_2-m), retinol-binding protein (RBP), lysozyme and γ-globulin L-chains. This form of proteinuria is not specific for cadmium and may be found in hereditary forms of tubular dysfunction.

Some recent studies of cadmium toxicity (Bernard *et al.* 1979) indicate that a glomerular pattern of dysfunction may also be an early effect of cadmium exposure, resulting in an increased urinary excretion of high molecular weight proteins such as albumin and transferrin. Later effects on renal function include aminoaciduria, phosphaturia and glucosuria.

Recent investigations of cadmium induced tubular injury have invariably measured the urinary excretion of low molecular weight proteins as indicators of early effect. Of these, most use has been made of β_2-m, but some studies have also measured RBP.

1.3 Techniques used to determine the relationship between cadmium exposure and renal dysfunction

Evaluation of the relationship between cadmium exposure and renal dysfunction may be approached in two ways: by establishing either a dose-effect relationship or a dose-response relationship (Nordberg 1976). Before proceeding further, a brief explanation of these terms is given.

Exposure of populations to a toxic chemical may result in the development of an adverse effect in certain individuals. In many instances, effects are quantitative and are measured on a continuous scale. Examples of such effects are inhibition or activation of enzymes or a change in the urinary excretion of metabolites. The relationship between dose and the gradation of effect constitutes the dose-effect relationship. Other effects are qualitative, and commonly take two states, being either present or absent. Examples of such effects include death or survival and the presence or absence of a tumour. The relationship between the proportion of a population demonstrating such an effect and the dose constitutes

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the dose-response relationship. Continuous effects may be reduced to a binary form and then subjected to dose-response analysis. The transformation of continuous effects to a binary form is done by defining an upper or lower normal limit for the variable in question.

Dose-response relationships have been used for many years in estimating LD_{50} values for toxic chemicals. More recently, attention has been paid to the use of the technique in epidemiological studies (Bakir *et al.* 1973; Kjellström 1977; Piotrowski and O'Brien 1980). This approach has, however, been criticized for continuous effects, as information is lost when such an effect is transformed to a binary form (Whitehead 1980). For this reason, both dose-effect and dose-response relationships have been used in the study of cadmium induced renal dysfunction.

1.3.1   The dose variable

Dose has been defined (Nordberg 1976) as the amount or concentration of a chemical at the site of the effect. In practice, the site of effect is rarely accessible for measurement in humans and indirect estimates of dose are used. The concentration of the material in the medium associated with the major route of uptake, or the concentration in an index medium such as blood or urine is often used as an indicator of dose.

Cadmium exposure in humans occurs through inhalation, and the ingestion of food and water. The relative contribution from each route will vary according to the population examined, but dietary cadmium represents the major source of exposure in the general population. In smokers, however, cigarettes can be as important a source of cadmium.

Estimates of dietary intake have been carried out on a national basis in several countries using either the standard diet technique or the duplicate meal approach (Essing, Schaller, Szadkowski and Lehnert 1969; U.S. FDA 1977; Hubbard and Lindsay 1979; Miljøministeriet 1980). The former examines the eating habits of a typical population in order to estimate a nationally representative diet. The cadmium concentrations of the individual foodstuffs comprising this diet are then measured, after preparation and cooking. Duplicate diet studies involve the analysis of duplicate meals after preparation by typical consumers.

The standard diet technique suffers from the analytical difficulties involved in using routine equipment to measure the low cadmium concentrations present in many foodstuffs. Often these levels are less than

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the detection limit when computing the total intake. An additional problem, related to sampling, is the variability in individual consumption patterns, as well as the large variability in the cadmium content of different samples of the same food item.

One of the studies examined in this report used an estimate of dietary intake based on the cadmium content of locally grown rice from a polluted area of Japan (Kjellström 1977; Kjellström, Shiroishi and Evrin 1977a). A 'dose index' was calculated after assuming an average daily rice consumption and estimating the contribution from other food items and drinking water. This approach appears rather crude but the population examined was sedentary and almost entirely dependent on locally grown rice.

Faecal excretion of cadmium can also be used to estimate dietary intake as only about five per cent of the ingested cadmium is absorbed (McLellan, Flanagan, Chamberlain and Valberg 1978). This technique is receiving increasing attention as a method to estimate cadmium intake in the general population and has been used in Japan, Sweden and the U.S.A. (Kjellström 1979; Kowal, Johnson, Kraemer and Pahren 1979). Faecal cadmium levels are relatively high and present fewer analytical problems than those associated with standard diet studies. This may explain why estimates of cadmium intake by the standard diet approach are higher than provided by faecal analysis (Hutton 1982).

Faecal excretion of cadmium forms the basis for dose estimation in the study by Tsuchiya, Iwao, Sugita and Sakurai (1979) used in this report and also in one of the groups examined by Kjellström (1977) and Kojima et al. (1977).

Neither direct dietary studies nor estimates of faecal cadmium measure the cadmium intake from inhalation or cigarette smoking. This is generally unimportant for non-smokers, as the direct intake from ambient air in the general population is negligible, except for those individuals living close to point sources of atmospheric cadmium. In smokers, however, consumption of 20 cigarettes results in the absorption of cadmium equivalent to an additional intake of 25–30 μg dietary cadmium (Hutton 1982) and should therefore be taken into account when estimating total exposure.

A major deficiency of the intake assessments used in epidemiological studies is the reliance placed on a single intake value to estimate the accumulated exposure over long (~50 years) time periods. Indeed, a single measure of dietary intake cannot reveal the day to day variability

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in present exposure let alone past exposure regimes. Nevertheless, most dose-response studies of cadmium are compelled to use such values in order to estimate the accumulated exposure of the study group.

Inhalation of cadmium dusts and vapour represents the major route of exposure in the working environment, although contaminated food and tobacco consumed at work may also make an important contribution. Air cadmium concentrations are routinely measured in the occupational setting and several health-related studies have used the values obtained as an index of dose (Friberg *et al.* 1974; Lauwerys *et al.* 1974; Kjellström, Evrin and Rahnster 1977b). Problems do, however, exist in using air cadmium concentrations for this purpose because the accuracy of such measurements as indicators of exposure is questionable. Generally only total cadmium concentrations are measured, and factors influencing respiratory absorption such as the cadmium compounds involved and the size distribution of the collected particles are not taken into account. Also, air measurements in the past were often taken on an irregular basis using static samplers. It is generally accepted that data from static samplers are inaccurate indices of dose because they often underestimate the individual's exposure in the work-place. Thus, it is difficult to make reliable estimates of exposure which took place several decades ago, at a time when air cadmium levels were invariably greater than they are today. Personal samplers are now commonly used in the occupational setting and are considered to be a more valid indicator of workers' exposure. In practice, however, great differences in air cadmium values can be obtained when different sampler heads and filter media are used simultaneously by an operator (King 1980). Indeed, even small changes in sampler position markedly influence the air cadmium value. There is thus an obvious need for some consistency in air sampling strategies to allow air cadmium data to be used as a dose index in occupational exposure.

The measurement of cadmium in urine represents a convenient biological indicator of integrated exposure and has been used in a number of health-related studies. The precise relationship between urinary cadmium concentration, cadmium exposure and cadmium body burden has been the subject of some discussion in the last few years. It would appear that in cases of low or moderate exposure and where the burden of cadmium is below the critical level, urinary cadmium mainly reflects the renal cadmium burden (Lauwerys *et al.* 1980). When exposure has been sufficiently high to cause saturation of cadmium binding sites, urinary

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cadmium will be influenced more by recent exposure than by the body burden of cadmium.

Evidence for the dependence of urinary cadmium on the cadmium body burden in situations of low exposure is provided by recent studies of the general population in Sweden and the U.S.A. (Elinder, Kjellström, Linnman and Pershagen 1978; Kowal *et al.* 1979). It was observed in both these groups that urinary cadmium excretion, like the body burden of cadmium, increases with age.

1.3.2 The effect variable

In the last five years, measurement of urinary β_2-m has become widely adopted as the favoured technique for detection of cadmium induced renal dysfunction. Some workers in Japan have also used RBP excretion as an index of disruption to tubular function. This protein has a molecular weight of about 21,000 daltons and is responsible for the transport of vitamin A in the plasma. RBP has a single binding site for one molecule of retinol. The complex formed is too large to pass through the glomerulus and thus only uncomplexed RBP will be eliminated by glomerular filtration and be available for tubular reabsorption. Until recently, RBP has been generally measured by relatively insensitive methods, using a single immuno-diffusion assay (e.g. see Nogawa, Ishizaki and Kawano 1978). In the last few years, however, a more sensitive, latex immunoassay method has been developed and this offers a good possibility of detecting moderate increases of urinary RBP excretion.

β_2-m is a low molecular weight protein of 11,800 daltons located on the plasma membranes of lymphocytes, macrophages, endothelial cells, some epithelial cells and in serum. Synthesis of β_2-m occurs in lymphocytes and many other cell types as a result of continuous regeneration of membrane proteins. The function of β_2-m is unknown at present but its structural similarity to immunoglobulins suggests a receptor role within the immune system, possibly related to cell recognition (Cooper and Plesner 1980). In normal individuals, β_2-m passes freely through the glomerular membrane of the kidney nephron followed by almost complete reabsorption (~99.9 per cent) in the proximal tubules (Wibell 1974).

Tubular damage can result in decreased absorption and increased urinary excretion of β_2-m. This effect is not specific for cadmium and may be caused by other agents or diseases which produce tubular

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damage. One such condition, considered to be of importance because of its frequency in the general population, is infection of the upper urinary tract. Individuals with this disease have been shown to excrete abnormally large amounts of β_2-m (Schardijn *et al.* 1979). Another disease state which requires consideration is glomerular dysfunction, a condition which results in elevated serum levels of β_2-m (Wibell 1974). Wibell concluded that serum levels in excess of 4.5 mg ℓ^{-1} saturate the reabsorption capacity of the kidney and this also results in an increased excretion of urinary β_2-m as shown in Figure 1. Thus, only below this threshold will elevated urinary β_2-m be diagnostic of tubular impairment.
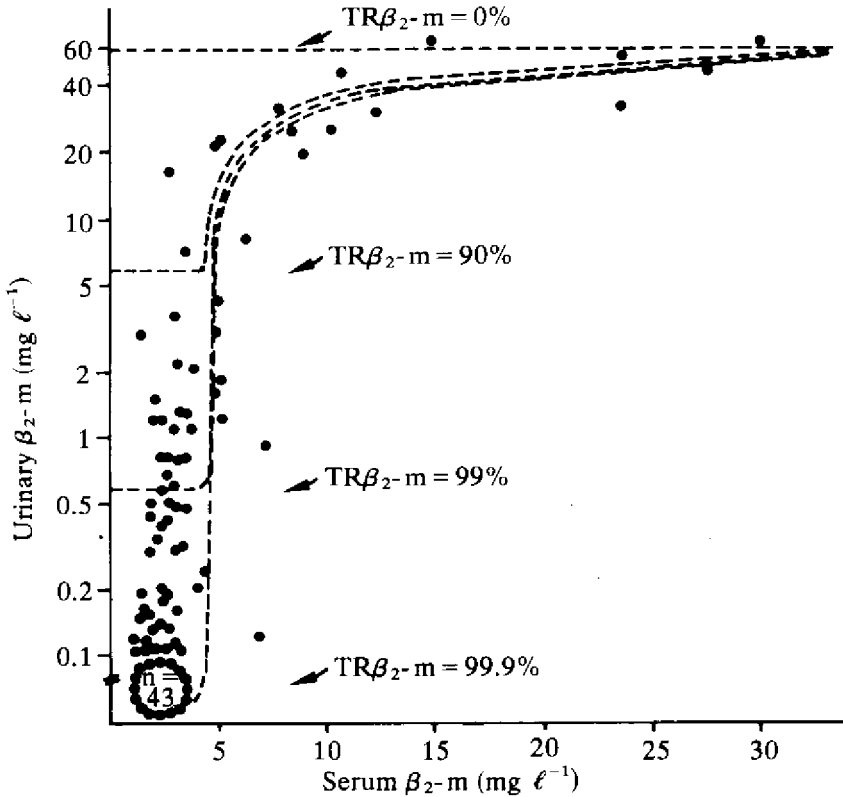

Figure 1 The relationship between serum and urinary β_2-microglobulin concentration in patients with renal disorders. The dotted lines represent the expected values of urinary β_2-m at various tubular reabsorption efficiencies, assuming a serum threshold of 4.5 mg ℓ^{-1}
(Adapted from Wibell 1974)

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Increased urinary β_2-m excretion may therefore occur for reasons other than excessive cadmium exposure. The frequency of elevated β_2-m values unrelated to cadmium exposure thus requires estimation in any study group, if β_2-m is to be a valid indicator of cadmium induced effect. This can be done by determining the frequency distribution of β_2-m levels in a control group taken from the general population. Table 1 shows the urinary β_2-m values obtained from reference groups for this purpose. In each case, values were determined by a commercial kit, the Phadebas β_2-Microtest, which utilizes a radio-immunoassay technique (Evrin, Peterson, Wide and Berggård 1971; Kjellström and Piscator 1977). Several of these studies have analysed the frequency distribution of this variable and in each case found the data to show a log.normal distribution (Kjellstöm et al. 1977a; Stewart and Hughes 1981; Kowal and Kraemer 1982). It is thus inappropriate to use the arithmetic mean to summarize the distribution of urinary β_2-m and the geometric mean has been used wherever possible.

Table 1 shows that the average values obtained in reference groups from four countries are generally similar. There are, however, some exceptions which require consideration. First, the contrasting values reported in two studies from the U.K. are somewhat surprising as the figure given by Strehlow and Barltrop (1981) is an arithmetic mean, but this is actually lower than the geometric mean quoted by Stewart and Hughes (1981). Differences also exist in the values given in two Swedish studies, and these are also difficult to explain. Evrin and Wibell (1972) expressed β_2-m on an arithmetic mean basis as the quantity excreted over a 24-hour period. Assuming that a 24-hour urinary excretion volume of 1.4 ℓ results in a β_2-m concentration of about 55 μg ℓ^{-1}, this value is lower than the geometric mean of 84 μg ℓ^{-1} obtained by Kjellström et al. (1977b).

The upper 2.5 percentile, or in some cases 5 percentile, is generally used as the criterion for defining the upper normal limit of urinary β_2-m excretion. It can be seen from Table 1 that the upper normal limit varies markedly between some of the reference groups; the values found in the U.S.A. are, however, similar to the figure reported by Stewart and Hughes (1981) for the U.K. At present, it is not clear why there should be such a pronounced variation between the upper normal limits of these reference populations, but several factors may influence the values obtained. Possibly geographic differences exist in the incidence of renal dysfunction, or in the consumption patterns of nephrotoxic chemicals

Table 1 Urinary β_2-microglobulin levels in individuals from the general population

Country	Age	Sex	n	urinary β_2-m ($\mu g\,\ell^{-1}$) Average[1]	urinary β_2-m ($\mu g\,\ell^{-1}$) Upper normal limit[2]	Reference
Sweden	17–76	male	58	$75 \pm 52^{a*}$	177	Evrin and Wibell (1972)
	17–76	female	61	$78 \pm 59^{a*}$	194	
Sweden	22–67	male	87	84 *adj.*	290[b]	Kjellström *et al.* (1977b)
U.K.	18–55	male	203	76 *adj.*	617	Stewart and Hughes (1981)
U.K.	—	male & female	542	$57 \pm 55^{a*}$	165	Strehlow and Barltrop (1981)
France	25–67	male & female	165	60	230[b]	Jeandot *et al.* (1980)
U.S.A.	20–74	male	484	89.4 *adj.*	770	Kowal and Kraemer (1982)
	20–74	female	493	71.2 *adj.*	613	

[1] Geometric mean, except those marked *a*, where the arithmetic mean ±SD is given.
[2] 97.5 percentile calculated as either GM (SD[1.96]) or $\bar{x} + 1.96$ SD.
Values marked *b* are the 95 percentile.
* $\mu g\,24\,hr^{-1}$.
adj., values adjusted to the average urinary specific gravity of the population.

such as analgesics and certain antibiotics. It is also conceivable that differences in the experimental protocol adopted or variability between different batches of the Phadebas kit could be involved. Storage conditions of urine samples vary in different studies and this may also influence the values obtained. This factor, together with the effect of urinary pH on β_2-m stability, is considered in more detail later.

The age composition of individuals comprising the control group may also influence the frequency distribution of β_2-m values. Table 2 depicts the urinary β_2-m data of the recent U.S.A. study (Kowal and Kraemer 1982) divided into six age groups. Statistical analysis of the average values indicated an "almost significant" relationship with age. There would, however, appear to be a marked change in the distribution of this variable with age, as Table 2 shows a pronounced increase in the upper normal limit with age. This finding suggests that the reference population should be matched for age with the study group to remove this effect.

Other studies, however, have reported no increase in urinary β_2-m with age (Kjellström and Piscator 1977; Stewart 1980). Stewart (1980) performed a linear regression on these variables in the U.K. reference population shown in Table 1. The correlation coefficient ($r = 0.11$, $n = 203$), although non-significant, was not far removed from the five per cent level of significance ($r = 0.138$). It should also be noted that only five out of the 203 individuals in Stewart's reference group were more than 50 years old. The control group examined by Kjellström and Piscator (1977) contained a wider age range but the majority of individuals older than 70 years had acidic urines (pH <6). Visual inspec-

Table 2   Urinary β_2-microglobulin levels ($\mu g\, \ell^{-1}$) in individuals from the general population of the U.S.A. subdivided according to age

Age	n	Geometric mean (standard deviation)	Upper normal limit
20–29	221	80.6 (2.3)	413
30–39	141	71.5 (2.7)	490
40–49	142	68.6 (2.7)	472
50–59	117	75.5 (3.0)	635
60–69	268	84.2 (3.7)	1,099
70–74	88	107.1 (3.8)	1,451

Source: Kowal and Kraemer (1982)

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tion of the data after removal of these points gives the impression of an increasing excretion of β_2-m above the age of 60.

It is well established that β_2-m undergoes degradation when the urinary pH is less than 6 (Berggård and Bearn 1968; Evrin and Wibell 1972; Schardijn et al. 1979). Whether this results from the instability of β_2-m itself, or whether proteolytic enzymes with acidic pH optima are responsible, is not clear at present. Whatever the cause, the degradation is rapid at body temperature, indicating that breakdown will occur in the bladder. Standardized procedures should therefore be adopted where possible, to prevent both the *in vivo* and *in vitro* degradation of β_2-m. Administration of sodium bicarbonate to subjects prior to urine collection is one method which has been used to prevent *in vivo* degradation (Shardijn et al. 1979). It should, however, be noted that despite this precaution, the pH of a minority of the samples collected by Schardijn et al. was still below 6. Thus, sodium bicarbonate administration will not always prevent the development of acidic urine. Urinary pH should therefore be measured, discarding specimens with pH less than 6.

The *in vitro* degradation of urinary β_2-m may be minimized by the addition of phosphate buffer. Lauwerys (1980) recommends the dilution of 9 mℓ urine with 1 mℓ phosphate buffer. Different workers have stored the diluted, buffered samples at either $+4°C$ or $-20°C$, but there have been no published investigations to determine which temperature is the most effective at preventing β_2-m degradation.

Undiluted urine specimens have usually been stored frozen; neither Evrin and Wibell (1972) nor Kjellström and Piscator (1977) reported losses of β_2-m in frozen samples. However, Stewart and Hughes (1981) observed a decline in the β_2-m values of a group of samples which were frozen and thawed twice before analysis. Obviously more work is needed to determine the optimum storage conditions of urine specimens required for β_2-m analysis. Such an investigation should employ specimens with both elevated and normal β_2-m concentrations, as these may exhibit different storage behaviour. In this way, it may be possible to propose guide-lines for the standardized storage of urinary specimens.

The variability in dilution between urine samples will also influence the concentration of β_2-m. This requires correction and can be done either by expressing the data on the basis of the urinary creatinine (Cr) concentration or by adjusting to the average specific gravity of the group. Unfortunately not all studies have carried out this procedure, as shown in Table 1. The choice of technique for correction of urinary dilution

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results in two possible ways of expressing β_2-m concentration—either as $\mu g\,\ell^{-1}$ or $\mu g\,g^{-1}$ Cr. It is therefore suggested that agreement be reached on the most appropriate way of expressing β_2-m concentration.

The precise significance of an increased excretion of urinary β_2-m is still unclear. Figure 1, for example, shows that markedly elevated β_2-m levels correspond to relatively small reductions in the tubular reabsorption efficiency. Thus, a reabsorption efficiency of 90 per cent is predicted to cause a urinary excretion of over 5,000 $\mu g\,\ell^{-1}$ β_2-m. Furthermore, it is often not appreciated that the kidneys play an important role in the catabolism of low molecular weight proteins like β_2-m and RBP. There is no significant transport of low molecular weight proteins back to the blood stream after tubular uptake; instead, these proteins are actually broken down in the kidney (Mogielnicki, Waldmann and Strober 1971; Cooper and Plesner 1980). This would suggest that the overall effect of tubular dysfunction on the metabolic pathway of low molecular weight proteins is simply a shift from tubular uptake and subsequent catabolism to one of urinary excretion. Apparently, therefore, the significance of this tubular lesion is the loss of those amino acids which in a normal individual are salvaged from the catabolism of these proteins. It should, however, be borne in mind that increased urinary excretion of β_2-m is indicative of an impairment of tubular function and, as such, is considered by some workers to constitute a health effect. In this respect, it is of interest to note that some recent investigations in Japan (Nogawa, Kawano and Nishi 1981) report that low molecular weight proteinuria is associated with increased mortality in cadmium polluted areas.

1.3.3 Analysis of data

Data were extracted from published epidemiological studies and from unpublished results supplied by workers in the field. The first step in data analysis was to establish the upper and normal limit for the effect variable. This was usually defined as the 97.5 percentile value obtained from the control population in each study. If no control group had been examined, the upper normal limit from another population of similar age to the exposed group was used.

Once the upper normal limit was set, the dose range was stratified into a number of intervals and the average dose in each interval computed. The total number of data points and the number of points above the upper normal limit were then calculated for each of these groups. The data were then in a form convenient for dose-response analysis.

Dose-response relationships were established by the technique of probit analysis (Finney 1977), using the Biomedical Statistics Package, BMD 035 (Biological Assay: Probit Analysis). This programme obtains maximum likelihood estimates of curve parameters by an iterative technique. Data input consists of the average dose level for each group, the sample size and the number of individuals showing a positive response. The programme transforms dose to a log.form and allows a background response to be set by the operator. In addition to estimates of the slope and intercept of the probit equation, the output also gives the co-variance matrix of these parameters, enabling calculation of the fiducial limits of the probit line.

2 Analysis of cadmium exposed populations

2.1 Introduction

This section is concerned with the extraction and analysis of data generated by several important epidemiological studies of cadmium exposure and health effects. The methods involved in these studies are critically evaluated, as are the original procedures used for any dose-response analysis.

All the published investigations concerned with exposure in the general population originate from Japan. In this respect, it should be noted that certain nutritional and metabolic factors may influence the dose-response relationship. One population of occupationally exposed workers is also examined.

2.2 Populations examined by Kjellström and co-workers

2.2.1 Background

In the mid-1970s, Kjellström, together with co-workers from the Karolinska Institute and personnel from several Japanese institutes, carried out three epidemiological studies of cadmium exposure in Japan (Kjellström 1977; Kjellström et al. 1977a; Kojima et al. 1977). The dose-response data generated were used by Kjellström (Kjellström 1977; 1978; 1980) to estimate the daily cadmium intake sufficient after 50 years exposure to cause tubular damage in different proportions of the general population. These estimates recently formed the basis of a controversial statement from the Umweltbundesamt concerning the

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numbers of individuals in the Federal Republic of Germany suffering from cadmium induced renal dysfunction (Umweltbundesamt 1980).

2.2.2 Population characteristics

The three studies were carried out on farming communities from the Toyama and Akita Prefectures of Japan. Past mining and smelting activities upstream of these areas have caused persistent water-borne cadmium contamination, producing elevated cadmium levels in locally grown rice. River water used directly for drinking and cooking purposes is an additional source of exposure.

The three target populations were matched for age with a reference group from an adjacent area considered to be unpolluted with cadmium. Dose-response analysis was restricted to certain females in each population as described below. Table 3 shows the age distributions and sample sizes of these groups. In Akita only those consuming their own rice were used, while in Toyama selection was based on those individuals with no direct river water exposure who had lived in one place during their residence in the area. This selection procedure resulted in a large reduction in the size of the target groups and the numbers examined in the 50 to 59-year-old group from Toyama are particularly small for a study of this nature.

The upper normal limits of urinary β_2-m levels in the reference populations are also given in Table 3. Comparison of these limits with those found in the corresponding age range of the U.S.A. population (Table 2) indicates that the 40 to 44-year-old Toyama group have

Table 3 **Characteristics of the three female populations from Japan used by Kjellström for dose-response analysis**

Location	Age	n	Reference group Upper normal limit*	n	Dose index	Reference
Toyama	40–44	47	640 µg g^{-1} Cr	37	rice Cd	Kjellström (1977)
Toyama	50–59	35	700 µg g^{-1} Cr	40	rice Cd	Kjellström *et al.* (1977a)
Akita	50–69	89	700 µg ℓ$^{-1}$	92	faecal Cd	Kojima *et al.* (1977)

* 95 percentile of β_2-microglobulin excretion

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anomalously high β_2-m values. Possibly this reference group is also exposed to sufficient cadmium to cause tubular damage.

In Toyama, the weighted average rice cadmium content of each household formed the basis for estimating dose. A 'contamination index' was calculated for each person in the target group using the formula:

$$(RT_E \times \bar{r}_E) + (RT_C \times \bar{r}_C)$$

where RT_E is the residence time in the exposed area; $\bar{r}_E$, the weighted average rice cadmium content for each household; RT_C, residence time outside the exposed area; $\bar{r}_C$ average rice cadmium content from Toyama reference area $(0.13~\mu g~g^{-1}$ wet wt). Note that this latter value does not refer to the actual areas where individuals may have resided in the past.

The contamination index was used to estimate total dose for each individual, termed a 'dose index', by taking into account other dietary sources of cadmium. This was done by making some assumptions, based on average patterns of food consumption in the general population of Japan. Thus, Kjellström (1977) assumed that daily rice consumption would amount to 350 g in the target group and that half the daily cadmium intake was derived from rice. Additionally, it was assumed that polished rice would be consumed by Toyama residents and that this contained 85 per cent of the cadmium content present in unpolished rice.

The dose index $(\mu g~d^{-1}~y)$ was therefore given by:

$$\text{Contamination index} \times 2 \times 350 \times 0.85$$

In Akita, faecal cadmium content formed the basis for dose estimation. In contrast to Toyama, the geometric mean values of the target and reference groups were used, rather than individual values.

The dose index $(\mu g~d^{-1}~y)$ was given by:

$$(RT_E \times \bar{f}_E) + (RT_C \times \bar{f}_C)$$

where $\bar{f}_E$ is the geometric mean of the daily faecal cadmium excretion in the exposed area $(160~\mu g~d^{-1})$ and $\bar{f}_C$ the corresponding value in the reference or control area $(41~\mu g~d^{-1})$.

Kjellström (1977) stratified each of the three target populations into a number of subgroups according to residence time in the exposed areas.

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Table 4 Characteristics of the three target groups examined by Kjellström, subdivided according to dose index, with corresponding prevalence rates of increased β_2-microglobulin excretion

Location	Age	Dose index $(\mu g\,d^{-1}\,y)$	n	Increased β_2-m excretion	
				$n+$	Prevalence %
Toyama	40–44	1,835	10	0	—
		3,212	13	1	7.7
		7,135	12	1	8.3
		14,428	12	3	25
Toyama	50–59	2,964	12	1	8.3
		7,064	12	3	25
		14,920	11	6	54.6
Akita	50–59	4,200	15	1	6.7
		7,200	59	9	15
		9,600	15	3	20

$n+$ Number of individuals showing increased β_2-m excretion.

He then calculated the average dose index and prevalence of excessive β_2-m excretion for each subgroup; these data formed the basis for a dose-response analysis by Kjellström (1977).

2.2.3   Dose-response analysis

The data base used for dose-response analysis by the present report, as well as by Kjellström (1977), is shown in Table 4. These values were estimated or extracted from tables and figures in several papers by Kjellström. The average dose indices of the subgroups in the two Toyama populations were estimated from *Figure 9* in Kjellström (1977), while the same report provided the actual values for the Akita subgroups. The prevalence of elevated β_2-m excretion in the 10 subgroups was also estimated from *Figure 9* in Kjellström (1977). The accuracy of the estimates for the Akita population was confirmed by examination of *Table 2* in Kojima *et al.* (1977).

Some anomalies exist for the published prevalence rates in the two Toyama populations. *Figure 9* in Kjellström (1977) indicates that a total of 10 individuals in the 50 to 59-year-old population had elevated β_2-m values, but it was stated in the text that only nine showed such an effect. However, examination of *Figure 10* in Kjellström *et al.* (1977a) reveals

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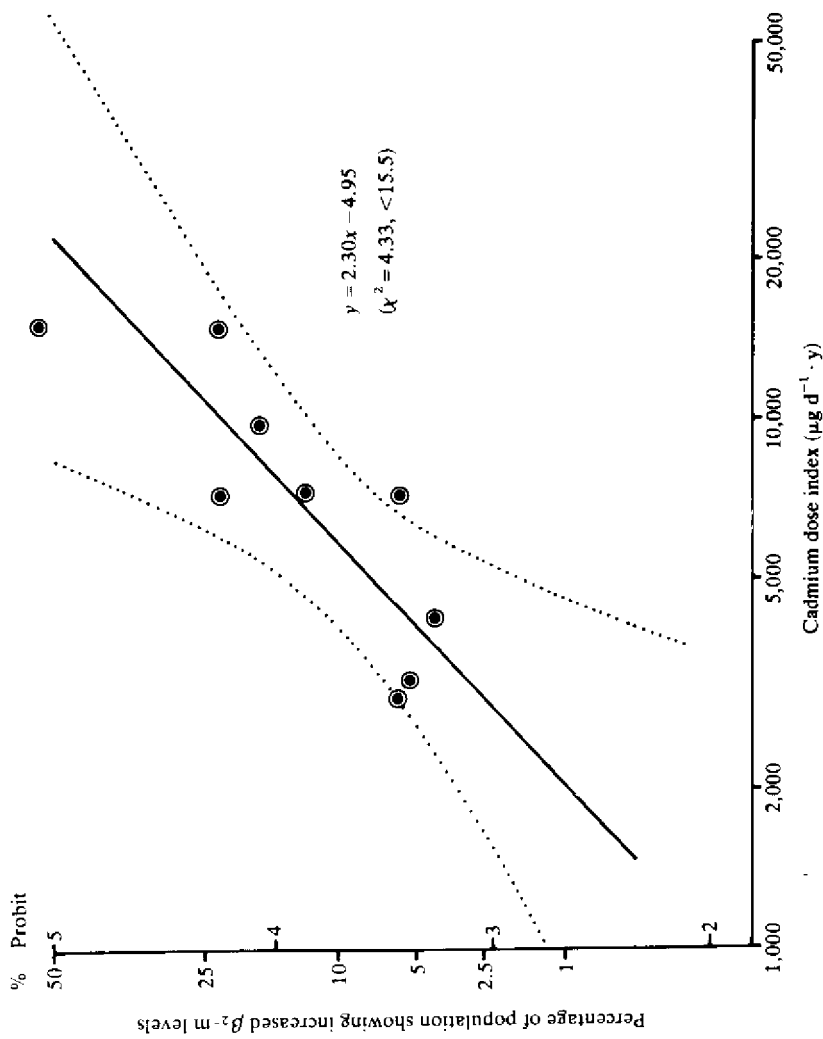
Figure 2 Dose-response relationship for cadmium induced increase in urinary β_2-micro-globulin levels, with upper and lower 95 per cent fiducial limits. Original data taken from Kjellström's studies.

% Probit

50 5

25

10

5

2.5 3

1

2

Percentage of population showing increased β_2-m levels

1,000   2,000   5,000   10,000   20,000   50,000

Cadmium dose index ($\mu g\, d^{-1}\cdot y$)

$y = 2.30x - 4.95$
$(\chi^2 = 4.33,\ <15.5)$

that 10 individuals exceed the upper normal limit of β_2-m excretion. In the 40 to 44-year-old population, the subgroup with the lowest dose index appears to have been assigned the incorrect prevalence rate of about one per cent in *Figure 9* by Kjellström (1977). This subgroup consists of 10 individuals and even one positive responder would produce an unadjusted prevalence rate of 10 per cent. This apparent mistake is maintained in *Figure 11* of Kjellström (1977) where a prevalence of about 0.1 per cent is assigned to this subgroup. It is unclear why this should be, especially as Kjellström (1977) stated that only five individuals in this population showed elevated β_2-m values and these can be accounted for by the two higher dose groups. In view of the above, it was decided to assume in the present report that no individuals in this subgroup showed elevated β_2-m levels.

Figure 2 shows the dose-response relationship for log.cadmium dose versus prevalence of excess β_2-m excretion calculated by probit analysis. Note that the response rate for each subgroup has been adjusted to take account of the assumed background (2.5 per cent) response unrelated to cadmium exposure. The probit line equation has been used to calculate the dose associated with elevated β_2-m levels in varying proportions of the population after 50 years' exposure. These estimates are shown in Table 5 for both Japanese and European adults. The European figures take into account the larger body weight of this group. Error estimation was carried out on the European data and the 95 per cent fiducial limits are also shown in Table 5.

Table 5   Estimated daily cadmium intakes ($\mu g\,d^{-1}$) sufficient to cause excessive β_2-microglobulin excretion after 50 years' exposure in varying proportions of the population

	Percentage of the population with elevated β_2-m levels						
	0.1%	1%	2.5%	5%	10%	20%	50%
Japanese (53 kg)	19	41	60	82	118	183	426
European (70 kg)	(7)	(25)	(44)	(70)	(109)	(152)	(220)
	25	55	79	108	156	242	562
	(91)	(123)	(142)	(167)	(223)	(386)	(1,439)

Estimates for the European population have the lower and upper 95 per cent fiducial limits attached.

Values based on data taken from Kjellström's studies.

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It is apparent from Table 5 that there are large errors associated with those estimates for less than 2.5 per cent and more than 10 per cent of the population. The minimum error is associated with the 10 per cent estimate and thus most reliance should be placed on this value. The actual figure obtained for Europeans at this response rate was $156 \mu g d^{-1}$, a value equivalent to about twice the WHO provisional tolerable weekly intake for dietary cadmium.

The values shown in Table 5 are not dissimilar to the estimates of these parameters made by Kjellström (1980), yet he apparently used the anomalously low prevalence rate in the 40 to 44-year-old Toyama group. The reason for this coincidental agreement may be because Kjellström apparently did not carry out probit analysis by the maximum likelihood method (Finney 1977). Instead, he simply appears to have used a least squares regression of log.dose versus probit of response. If this technique is used with the anomalous prevalence rate, the intake values associated with various proportions of the population are very similar to those given by Kjellström (1980).

2.2.4 Dose-effect analysis

This section examines the relationship between dose index and urinary β_2-m concentrations which have not been converted to a binary form. The individual data pairs were extracted from the relevant figures described earlier. Unfortunately this could not be done for the 40 to 44-year-old Toyama population as the data have never been fully published.

In the Akita population, *Figure 5* in Kojima *et al.* (1977) has only the individual residence times in the exposed area for two subgroups, the 50 to 59 and 60 to 69-year-olds. To convert this to a contamination index, it was assumed in this report that the age of each individual was either 55 or 65 years depending on the subgroup, unless the residence time was greater than 55 or 65 years, when it was assumed that the individual was either 59 or 69 years old. The assumed age was subtracted from the residence time to give an estimate of the years spent away from the exposed area. These residence time values were then applied to the formula using the average faecal cadmium contents of the two areas to calculate the dose index.

In the 50 to 59-year-old Toyama population, individual contamination indices were given directly in *Figure 10* of Kjellström *et al.* (1977a).

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These were converted to a dose index by the procedures described earlier.

Linear regression of log.dose index versus urinary β_2-m concentration was carried out for the two populations. The correlation coefficients obtained for both Akita ($r = 0.003$) and Toyama ($r = 0.03$) were very low and indicate that the two variables are not related in a linear manner.

2.3 Populations examined by Tsuchiya and co-workers

2.3.1 Background

An epidemiological study of cadmium exposure has recently been carried out by Tsuchiya and his co-workers in Japan. The data obtained have been summarized (Tsuchiya *et al.* 1979) but have never been subject to dose-response analysis. For this reason a request for the raw data was made to Professor K. Tsuchiya who kindly sent the information for statistical analysis.

2.3.2 Population characteristics

The individuals examined consisted entirely of males whose age ranged from 50 to 70 years. The target populations came from three areas in Japan known to be polluted with cadmium. As in Kjellström's studies, reference groups were also selected in these areas from localities considered to be unpolluted. Table 6 shows the location of the groups examined, as well as the average daily faecal excretion and urinary concentration of cadmium obtained. The faecal cadmium values shown in Table 6 indicate that these populations were subject to considerable cadmium exposure and even the control groups from Ishikawa and Akita displayed elevated levels. For this reason, data from the Ishikawa controls as well as from the exposed groups were considered as one population for statistical analysis. The Akita controls could not be used as no β_2-m data were available for this group.

The Nagasaki control population was used to define the upper normal limit of this variable. The 97.5 percentile of this population was calculated to be 520 $\mu g\ g^{-1}$ Cr. Values above this limit in the exposed populations were therefore classified as positive responders.

Two indicators of dose were employed in this study, daily faecal cadmium excretion and urinary cadmium concentration. Faecal cadmium was converted to a dose index by multiplying daily excretion by the subject's age. In both cases, individual data from the exposed population

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Table 6 Characteristics of the populations from three cadmium polluted areas in Japan studied by Tsuchiya

| Location | n | Dose indices* | | Urinary β_2-m§ ($\mu g\,g^{-1}$ Cr) |
		Faecal Cd ($\mu g\,d^{-1}$)	Urinary Cd ($\mu g\,\ell^{-1}$)	
Nagasaki				
Polluted	30	255	7.4	300
Control	30	42.3	4.8	110
Ishikawa				
Polluted	40	149	10.8	560
Control	40	102	7.0	270
Akita				
Polluted	37	158	6.1	160
Control	37	60	—	—

* Arithmetic means.
§ Geometric mean of β_2-microglobulin.   Source: Tsuchiya *et al.* (1979).

Table 7 Characteristics of the cadmium exposed population examined by Tsuchiya, subdivided according to dose index, with corresponding prevalence of increased β_2-microglobulin excretion

| Dose index | n | Increased β_2-m excretion | |
		n +	%
Faecal Cd × age ($\mu g\,d^{-1}$ y)			
2,263	36	6	16.7
5,377	33	11	33.3
8,331	23	8	34.8
11,608	14	6	42.9
14,046	17	4	23.5
22,372	17	9	52.9
Urinary Cd ($\mu g\,g^{-1}$ Cr)			
1.59	24	4	16.7
4.43	42	14	33.3
7.42	29	9	31
10.1	18	5	27.8
12.8	14	5	35.7
17.3	17	8	47

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were grouped together and then stratified according to dose range. Table 7 depicts the characteristics of these groups which formed the basis of dose-response analysis.

2.3.3 Dose-response analysis

Figure 3 shows the dose-response relationship for log.dose (faecal cadmium $\times$ age) versus prevalence of excessive β_2-m excretion calculated by probit analysis. The same relationship employing urinary cadmium as the dose index is depicted in Figure 4. The associated regression equations were used to calculate the dose associated with elevated β_2-m levels in varying proportions of the population. These estimates are shown in Tables 8 and 9. Once again, the errors associated with these estimates have also been given.

Estimates of the daily cadmium intake associated with a positive response in 0.1 to 20 per cent of the population are unrealistically low. This results from the presence of elevated β_2-m levels in a large proportion of the low dosage subgroups (see Table 7). This finding suggests that dose has been underestimated in the exposed population. Indeed, the large errors associated with the estimates in Table 8 indicate that a single measure of daily faecal cadmium excretion is not an accurate indicator of dose. The alternative approach of using the average faecal excretion value for each òf the four groups comprising the exposed population may yield more meaningful results.

The results of dose-response analysis utilizing urinary cadmium are also unrealistic. The estimates shown in Table 9 indicate that 20 per cent of the population exhibit excessive β_2-m excretion at a urinary cadmium concentration of just $2 \mu g\, g^{-1}$ Cr. This concentration is far too low and is only a little higher than is found in the general population. To place these estimates in perspective, Lauwerys, Bernard, Buchet and Roels (1981) concluded that renal dysfunction is associated with a urinary cadmium concentration of 10 to $15 \mu g\, g^{-1}$ Cr.

Apparently the population studied by Tsuchiya et al. (1979) exhibited elevated β_2-m levels at relatively low levels of urinary cadmium, but the reason for this situation is not clear.

2.3.4 Dose-effect analysis

Before proceeding with dose-effect analysis, it was decided to investigate the possible confounding influence of age on β_2-m excretion, as there
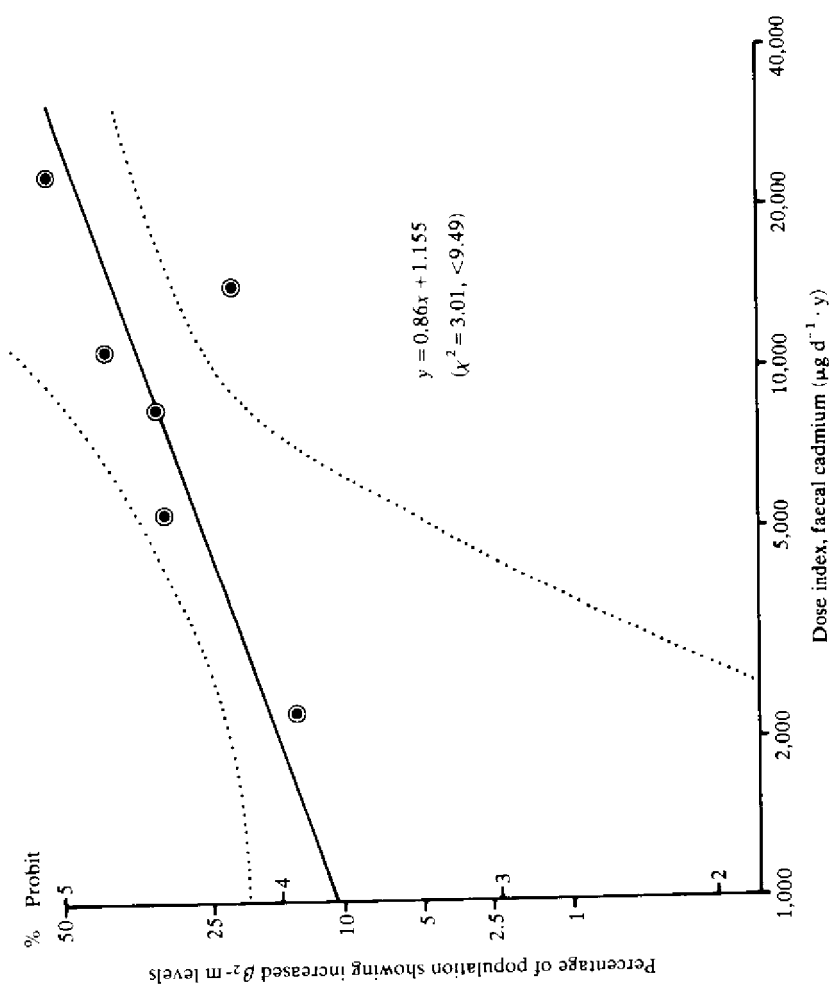
22

Figure 3 Dose-response relationship for cadmium induced increase in urinary β_2-microglobulin levels, with upper and lower 95 per cent fiducial limits. Raw data supplied by K. Tsuchiya
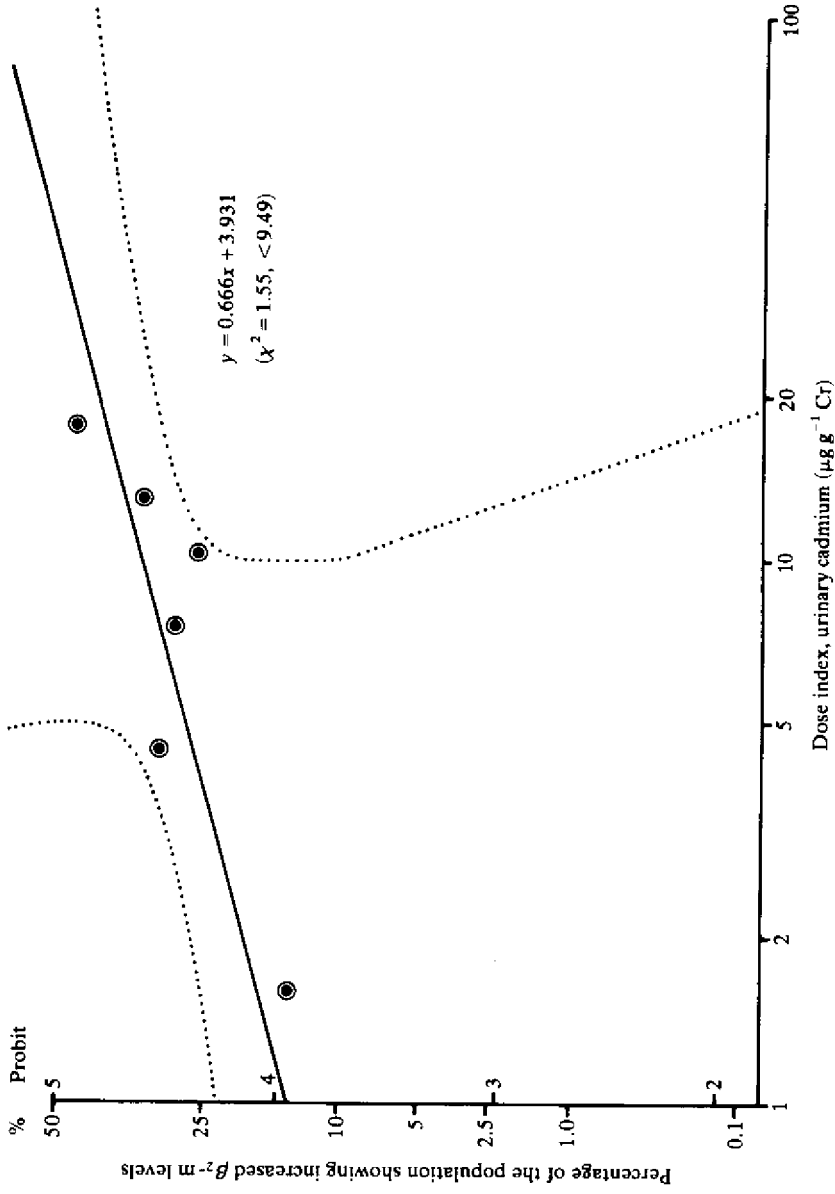
$y = 0.86x + 1.155$
$(x^2 = 3.01, <9.49)$

Dose index, faecal cadmium ($\mu g\ d^{-1}$, y)

Percentage of population showing increased β_2-m levels

% Probit

Figure 4 Dose-response relationship for cadmium induced increase in urinary β_2-micro-globulin, with upper and lower 95 per cent fiducial limits. Urinary cadmium concentration employed as dose index. Raw data supplied by K. Tsuchiya

Probit axis labels: 5, 4, 3, 2

% axis labels: 50, 25, 10, 5, 2.5, 1.0, 0.1

Percentage of the population showing increased β_2-m levels

Dose index, urinary cadmium ($\mu g\,g^{-1}$ Cr)

$y = 0.666x + 3.931$
$(x^2 = 1.55, <9.49)$

Table 8 Estimated daily cadmium intake (μg d^{-1}) sufficient to cause excessive β_2-microglobulin excretion after 50 years' exposure in varying proportions of the population

	Percentage of the population with elevated β_2-m levels						
	0.1%	1%	2.5%	5%	10%	20%	50%
Japanese	0.2	1.2	3	7	19	62	592
European	—	—	—	—	(4)	(29)	(231)
	0.2	1.5	4	9.5	25	82	780
	(72)	(99)	(118)	(142)	(171)	(223)	(2,632)

Estimates for the European population include the lower and upper 95 per cent fiducial limits.
Daily cadmium intake is assumed to equal faecal excretion.
Values based on data supplied by Tsuchiya.

Table 9 Estimated urinary cadmium concentrations (μg g^{-1} Cr) associated with excessive β_2-microglobulin levels in varying proportions of the population

	Percentage of the population with elevated β_2-m levels		
	10%	20%	50%
Urinary cadmium	—	(0.5)	(5)
(μg g^{-1} Cr)	0.5	2.2	40
	(10)	(9.9)	(326)

Estimates include the lower and upper 95 per cent fiducial limits.
Values based on data supplied by Tsuchiya.

is some evidence for an age related increase in urinary β_2-m (see Table 2). For this reason, a linear regression of age versus β_2-m was carried out on the Nagasaki control group. Analysis of variance indicated a significant ($p < 0.01$) regression and the size of the correlation coefficient ($r = 0.37$ $p < 0.05$) confirmed a positive relationship between the two variables. For the purposes of this study, it was assumed that the exposed population displayed the same relationship between age and urinary β_2-m. The regression equation (β_2-m $= (0.006 \times \text{age}) - 0.244$) was then used to adjust the β_2-m levels for age in the exposed population, thus eliminating the effect of this variable. In this way, the relationship

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between cadmium exposure and urinary β_2-m could be examined more closely.

Dose-effect analysis could now be carried out on the data using the β_2-m values corrected for age and two indicators of dose, daily faecal cadmium $\times$ age and urinary cadmium.

The regression equation of log. (daily faecal cadmium $\times$ age) versus β_2-m was $y = 0.163x - 1.003$. The correlation coefficient ($r = 0.21$ $p <$ 0.01) indicates a positive relationship between the two variables. The confidence limits are smaller than the error estimates associated with dose-response analysis, allowing more reliance to be placed on the relationship. The regression equation was used to calculate the predicted β_2-m levels associated with daily cadmium intakes after 50 years' exposure; the derived values are shown in Table 10. For the purposes of this study, it was assumed that daily cadmium intake was equivalent to faecal excretion.

The value of 53 $\mu g\,d^{-1}$ was chosen as this is equivalent to the WHO provisional tolerable intake value for the Japanese when expressed on a daily basis (i.e. 1 $\mu g\,kg^{-1}\,d^{-1}$) and assuming a body weight of 53 kg (Kjellström 1978). The predicted β_2-m levels associated with 53 $\mu g\,d^{-1}$ indicate that this intake will not produce excessive β_2-m levels (defined as $\geqslant 520\,\mu g\,g^{-1}$ Cr) in at least 95 per cent of the population after 50 years' exposure. This finding therefore gives support to the safety of the WHO guide-line.

A dietary intake of 100 $\mu g\,d^{-1}$ is estimated to result in elevated urinary β_2-m levels in 5 per cent of the Japanese population after 50 years' exposure. It is of interest to note that this value is quite similar to the intake of 82 $\mu g\,d^{-1}$ associated with 5 per cent prevalence, estimated by dose-response analysis of Kjellström's data (Table 5).

Table 10 Daily cadmium intakes and associated urinary β_2-microglobulin levels derived from dose-effect analysis of Japanese males examined by Tsuchiya

Daily Cd intake ($\mu g\,d^{-1}$)	Predicted β_2-m level ($\mu g\,g^{-1}$ Cr)	95% Confidence limits	
		lower	upper
53	283	114	452
100	386	253	520
227	520	366	673

Values refer to individuals after 50 years' exposure.

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The value of 227 μg d^{-1} in Table 10 is the intake estimated to give rise to an average urinary β_2-m level of 520 μg g^{-1} Cr after 50 years' exposure in the population studied. In other words, this is the 50-year critical dietary intake value for this population. For comparison, it was implied by the Joint FAO/WHO Expert Committee on Food Additives (WHO 1972) that a daily cadmium intake of 4 μg kg^{-1} (body wt) gives rise to a cadmium concentration in the renal cortex of 200 μg g^{-1}. This is equivalent to an intake of 214 μg d^{-1} for the Japanese, a value similar to the critical intake value estimated by this study. In Europeans, the equivalent critical intake value would be 300 μg d^{-1}.

Urinary cadmium concentration was also used as an index of dose in this investigation. The correlation between log.urinary cadmium versus β_2-m was, however, non-significant ($r = 0.14$ $p > 0.05$).

2.4 Populations examined by Nogawa

2.4.1 Background

Nogawa and his colleagues from the Kanazawa Medical University, Ishikawa-ken, Japan, have carried out several health-related studies in cadmium polluted areas of Japan. The results of two of these investigations (Nogawa, Ishizaki and Kawano 1978; Nogawa, Kobayashi and Honda 1979) form the data base used in this report to examine the dose-response relationship for cadmium. The data in the above papers were presented in a way that prevented a complementary dose-effect analysis.

2.4.2 Population characteristics

Nogawa *et al.* (1978) examined villages in the Ishikawa Prefecture, an area where water-borne contamination has caused elevated cadmium levels in local soils and rice. The target population consisted of inhabitants of both sexes from 22 villages in the area who were more than 50 years of age.

Originally, the village average rice cadmium content (wet wt) was used by Nogawa *et al.* (1978) as the index of dose. The 22 villages were stratified into five groups according to this average value. In order to allow comparisons with other studies, it was decided to convert rice cadmium content to an estimated daily intake. The assumptions used by Kjellström (1977), already described, formed the basis of this conversion.

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Urinary excretion of RBP was used as the effect variable in this study. RBP was measured by an insensitive technique, using a single immunodiffusion method. RBP levels were measured in a reference group but the values obtained were not used to estimate an upper normal limit in the manner described earlier by other studies. Instead, Nogawa *et al.* (1978) simply defined an excessive RBP value as one greater than 4 mg ℓ^{-1}.

Nogawa *et al.* (1978) concluded that there was an increasing prevalence of proteinuria (i.e. elevated RBP levels) with increasing rice cadmium content. However, the data were not subjected to any dose-response analysis. The estimated dietary intakes and associated prevalences in the groups making up the target population are shown in Table 11. The values in Table 11 served as the basis for dose-response analysis.

The second epidemiological survey (Nogawa *et al.* 1979) was carried out on inhabitants of the Jinzu River basin, an area heavily polluted with cadmium and where Itai-itai disease has been reported in the past. In contrast to the previous study (Nogawa *et al.* 1978), individuals were not preselected for sampling. Instead, all inhabitants over 20 years of age from nine hamlets were examined. Additionally, males and females were considered separately for the purposes of dose-response analysis.

Urinary cadmium concentration was the index of dose in this study as no data on dietary or faecal cadmium values are published. The exposed population was stratified into a number of subgroups according to urinary cadmium content. The median value for each group formed the dose index; these values are shown for the eight female and seven male groups in Table 12.

Table 11   **Characteristics of the cadmium exposed population from Ishikawa examined by Nogawa, classified according to dose index with corresponding prevalence of increased retinol-binding protein excretion**

Dose index (μg d^{-1})	n	Increased RBP excretion	
		$n+$	%
143	1,122	51	4.6
226	413	29	7
268	616	36	5.8
333	353	46	13
387	140	42	30

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Table 12 Characteristics of the cadmium exposed population from the Jinzu River basin examined by Nogawa, classified according to urinary cadmium concentration with corresponding prevalences of increased β_2-microglobulin retinol-binding protein excretion

Dose index Urinary Cd ($\mu g\, g^{-1}$ Cr)	n	Increased β_2-m excretion		Increased RBP excretion	
		$n+$	%	$n+$	%
Females					
3.6	26	1	3.9	0	—
7.6	36	1	2.8	0	—
12.5	36	8	22.2	3	8.3
17.2	37	10	27	4	10.8
23.1	45	23	51.1	15	33.3
27.3	30	21	70	14	46.7
34.2	39	31	79.5	21	53.8
47.8	47	40	85.1	33	70.2
Males					
4.0	29	0	—	0	—
7.7	48	8	16.7	4	8.3
12.6	45	21	46.7	9	20
17.0	25	19	76	9	36
22.7	49	34	69.4	25	51
27.7	21	20	95.2	15	71.4
41.1	29	27	93.1	24	82.8

Several indices of renal dysfunction were measured by Nogawa *et al.* (1979) but only urinary β_2-m and RBP are used in this report. In contrast to the investigations by Kjellström and Tsuchiya, β_2-m was not analysed by the Phadebas radio-immunoassay technique. Instead, an insensitive immunodiffusion method was employed, with a detection limit of about 5 mg ℓ^{-1}. This may be compared with the detection limit of about 1 μg ℓ^{-1} using the Phadebas Microtest (Kjellström and Piscator 1977). As in the previous study, Nogawa *et al.* (1979) did not use a control population to define the upper normal limits of urinary β_2-m and RBP, but simply used the detection limits of these proteins. The prevalence rates of elevated β_2-m and RBP levels defined in this way are shown in Table 12 in conjunction with the median urinary cadmium levels of each subgroup.
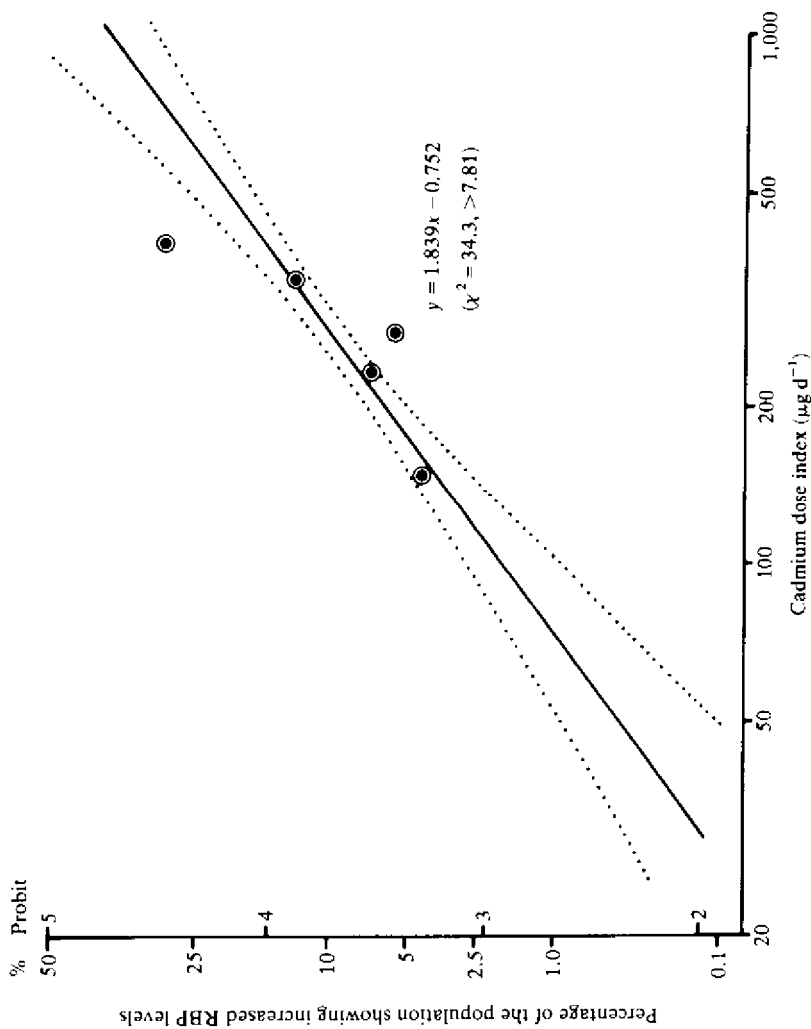
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Figure 5 Dose-response relationship for cadmium induced increase in urinary retinol-binding protein levels, with upper and lower 95 per cent fiducial limits. Data extracted from Nogawa et al. (1978)

$y = 1.839x - 0.752$
$(x^2 = 34.3, >7.81)$

Cadmium dose index (μg d^{-1})

Percentage of the population showing increased RBP levels

% Probit

2.4.3 Dose-response analysis

Figure 5 shows the probit analysis of log. cadmium dose versus prevalence of elevated RBP, based on data taken from Nogawa *et al.* (1978). In contrast to previous probit analyses carried out in this report, the response rates for each subgroup were not adjusted for a background response unrelated to cadmium exposure. This may be justified when one considers the insensitivity of the method used for RBP measurement.

The equation of the probit line was used to calculate dietary intakes of cadmium causing elevated RBP levels in varying proportions of the Japanese and European populations; these values are shown in Table 13. Note that these estimates refer to individuals over 50 years of age, as this was the age group of the population examined by Nogawa *et al.* (1978).

The "critical intake values" for 1 to 10 per cent of Nogawa's population are about twice those estimated from Kjellström's data for β_2-m (Table 5). It is not known whether this difference results from β_2-m being a more sensitive indicator of cadmium induced renal dysfunction or from differences in the methodologies of the two studies.

Probit analysis of log.urinary cadmium versus prevalence of elevated β_2-m for the male and female populations examined by Nogawa *et al.* (1979) is shown in Figure 6; the corresponding analysis for RBP is depicted in Figure 7. Once again, there was no adjustment for a background response in these analyses. Table 14 presents the estimated

Table 13 Estimated daily cadmium intakes (μg d^{-1}) sufficient to cause excessive retinol-binding protein excretion after 50 years or more exposure in varying proportions of the population

	Percentage of the population with elevated RBP levels						
	0.1%	1%	2.5%	5%	10%	20%	50%
Japanese	28	73	115	171	269	468	1,340
European	(21)	(69)	(121)	(197)	(324)	(516)	(1,143)
	37	96	152	226	355	618	1,767
	(166)	(134)	(191)	(260)	(390)	(740)	(2,732)

Estimates based on data from Nogawa *et al.* (1978).
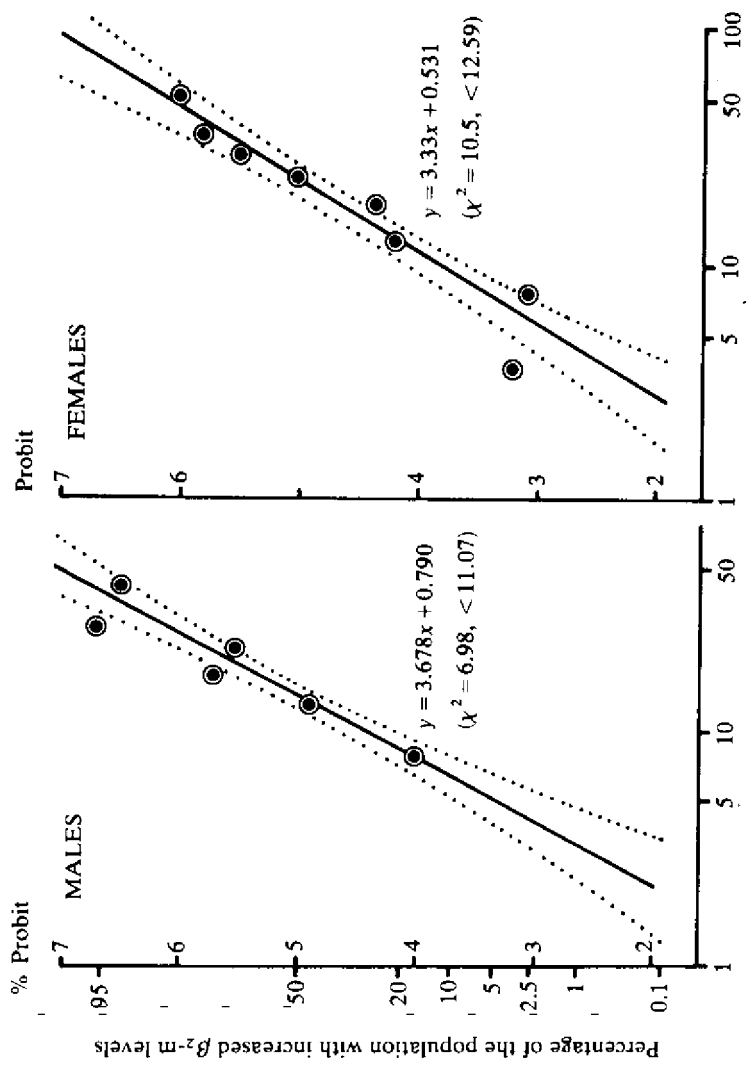Estimates for the European population include the lower and upper 95 per cent fiducial limits.

Figure 6 Dose-response relationships for cadmium induced increase in urinary β_2-micro-globulin levels of males and females, with upper and lower 95 per cent fiducial limits. Data extracted from Nogawa et al. (1979)
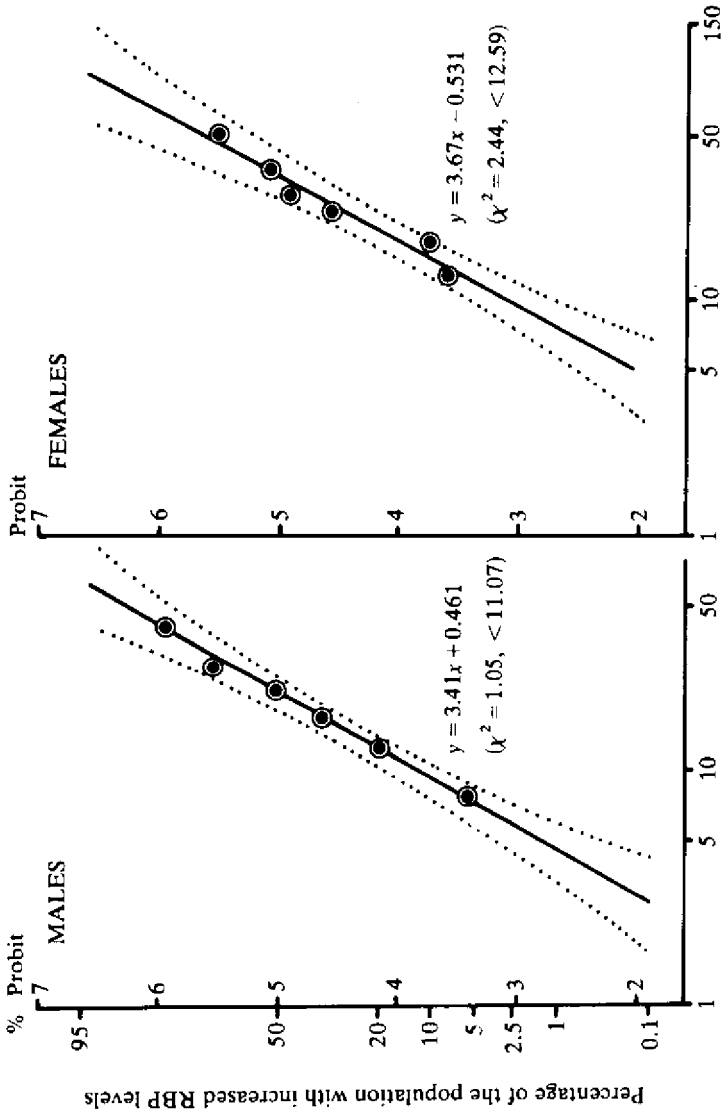
Figure 7 Dose-response relationships for cadmium induced increase in urinary retinol-binding protein levels of males and females, with upper and lower 95 per cent fiducial limits. Data extracted from Nogawa et al. (1979)

Table 14 Estimated urinary cadmium concentrations (μg g^{-1} Cr) associated with elevated β_2-microglobulin levels in varying proportions of the population from the Jinzu River basin

Protein	sex	Prevalence of abnormal urinary findings*		
		1%	10%	50%
β_2-m	males	(2.3)	(5)	(12.4)
		3.3	6.3	14
		(4.7)	(7.9)	(15.8)
	females	(3.2)	(7.5)	(19)
		4.4	9.1	22
		(6.0)	(10.9)	(25.4)
RBP	males	(3.2)	(7.5)	(18.2)
		4.5	9.0	21.4
		(6.3)	(10.8)	(25.2)
	females	(5.8)	(12.2)	(25.5)
		7.5	14.4	32.2
		(9.7)	(17)	(40.5)

Estimates based on data from Nogawa *et al.* (1979).

* Estimates include the lower and upper 95 per cent fiducial limits.

urinary cadmium values associated with elevated β_2-m and RBP levels in selected proportions of the male and female populations. It is apparent from Table 14 that elevated β_2-m levels are associated with lower urinary cadmium concentrations than are elevated levels of RBP, suggesting that β_2-m is a more sensitive indicator of renal effect. This difference in sensitivity might have been even more marked had β_2-m been measured by a method with a lower detection limit. Table 14 also indicates that elevated β_2-m and RBP values in males are associated with lower urinary cadmium concentrations than in females; whether this difference is significant is not known at present. Despite differences in sensitivity between both β_2-m and RBP and between males and females, it would appear from Table 14 that urinary cadmium concentrations of 5–15 μg g^{-1} Cr are associated with both signs of renal dysfunction in at least 10 per cent of the population.

2.5   Examination of a group occupationally exposed to cadmium

2.5.1   Background

In many countries, the health of workers occupationally exposed to cadmium is monitored on a regular basis. Data generated as a result of such a programme were obtained from a factory manufacturing nickel-cadmium batteries in order to carry out a dose-response analysis in the industrial setting. The information obtained included the number of years the worker was employed in the factory, the department in which he worked and the recent urinary concentrations of cadmium and β_2-m. β_2-m levels were measured by an independent laboratory, using the Phadebas Microtest method.

2.5.2   Group characteristics

A total of 118 individuals were used in the analysis, with the number of years employed ranging from 0.5 to 44 years (mean 7.8 years). Most individuals worked or had worked in the platemaking department where ambient cadmium concentrations are highest. The remaining subjects worked in the assembly or repair workshops.

Individuals with signs of clinical proteinuria were excluded from the analysis, as were those workers who had been removed from cadmium exposure prior to β_2-m monitoring.

Urinary cadmium concentrations of the work-force are measured by two independent laboratories, designated Laboratory A and Laboratory B in this study. Inspection of the recorded values revealed that Laboratory A generally assigns higher concentrations than Laboratory B, respective means being 12.7 $\mu g \, \ell^{-1}$ and 8.53 $\mu g \, \ell^{-1}$*. This difference is highly significant ($p < 0.001$). In view of this disparity, it was decided to carry out a duplicate statistical analysis using the separate results of the two laboratories.

In addition to the interlaboratory variability, there were in some instances large fluctuations in the reported urinary cadmium values over relatively short time periods. It is not clear whether this was due to any analytical error or simply a reflection of changes in the exposure regimes.

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* These group averages were calculated from the arithmetic mean values of each worker, which in turn were computed from the urinary concentrations reported over the last few years.

Table 15 Stratification of an occupationally exposed population according to urinary cadmium concentrations, with corresponding prevalence of increased β_2-microglobulin excretion

Dose index Average urinary Cd ($\mu g\ \ell^{-1}$)	Increased β_2-m excretion		
	n	$n+$	%
Laboratory A			
2.6	24	0	—
5.1	22	0	—
8.3	20	2	10
12.6	18	2	11.1
17.1	13	2	15.4
34.1	21	10	47.6
Laboratory B			
3.0	27	0	—
4.5	28	2	7.1
6.6	21	1	4.8
10.1	22	2	9.1
14.6	11	5	45.5
29.0	9	6	66.7

Whatever the reason, it was decided to compute the arithmetic mean of the values reported in the recent past for each individual concerned and use this for statistical analysis.

The upper normal limit of urinary β_2-m excretion was set at 617 $\mu g\ \ell^{-1}$, the 97.5 percentile of a reference population from the United Kingdom examined by Stewart and Hughes (1981) and shown in Table 1. The response rates of elevated β_2-m excretion together with the corresponding average urinary cadmium levels for the laboratories are shown in Table 15.

2.5.3 Dose-response analysis

Figure 8 shows the probit regression lines of log.urinary cadmium versus prevalence of elevated β_2-m in the occupationally exposed population for the two laboratory data sets. The response rate was adjusted, assuming a background response of 2.5 per cent unrelated to cadmium exposure. The urinary cadmium concentrations associated with elevated
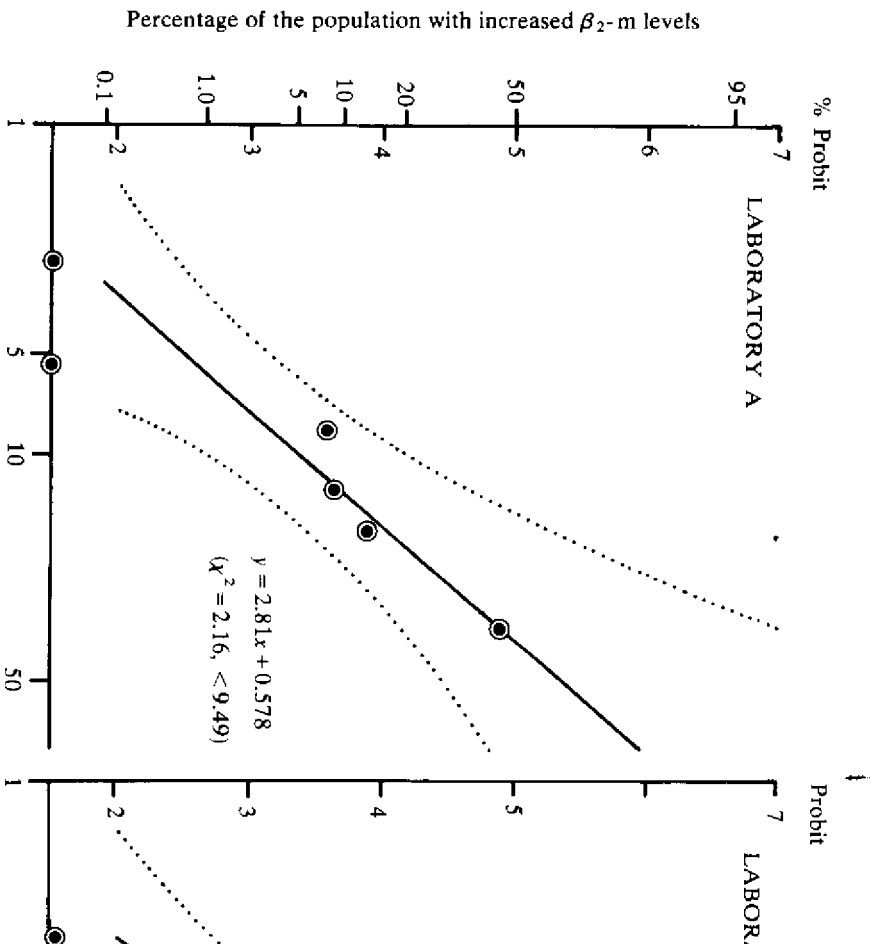
36

Percentage of the population with increased β_2-m levels

% Probit

LABORATORY A

$y = 2.81x + 0.578$
$(x^2 = 2.16, < 9.49)$

Probit

LABOR

Figure 8 Dose–response relationships for cadmium induced increase in elevated β_2-m
workers, with upper and lower 95 per cent fiducia

Dose index, urinary cadmium ($\mu g\ \ell^{-1}$

Table 16 Estimated urinary cadmium concentrations associated with elevated β_2-microglobulin values in varying proportions of the occupationally exposed population: results from the two laboratories

Urinary cadmium ($\mu g \, \ell^{-1}$)	Percentage of the population with elevated β_2-m values		
	10%	20%	50%
Laboratory A	(7.5)	(9.8)	(14.8)
	13.1	18.8	37.3
	(23.0)	(36.0)	(93.9)
Laboratory B	(6.0)	(7.6)	(10.7)
	9.1	12.1	20.8
	(13.7)	(19.2)	(40.9)

Estimates include the lower and upper 95% fiducial limits.

β_2-m levels in varying proportions of the population are presented in Table 16.

As expected, Laboratory A data gave results in which higher urinary cadmium values were associated with a particular prevalence rate. Thus, depending on the laboratory selected, a prevalence of 50 per cent is associated with a urinary cadmium level of about 20 or 40 $\mu g \, \ell^{-1}$. Less difference exists in the two data sets at lower prevalence rates; at 10 per cent, for example, the corresponding urinary cadmium levels for the two laboratories range from 9 to 13 $\mu g \, \ell^{-1}$.

2.5.4   Dose-effect analysis

The regressions of β_2-m versus urinary cadmium were calculated for the occupationally exposed population using the urinary cadmium data of the two laboratories. In the case of Laboratory A, the correlation coefficient ($r = 0.45$ $p < 0.001$) was high, indicating a strong positive relationship between the two variables. The corresponding regression equation ($y = 151.1x - 1258.2$) was used to calculate the urinary cadmium associated with a β_2-m of 617 $\mu g \, \ell^{-1}$; this was 12.4 $\mu g \, \ell^{-1}$. In the case of Laboratory B, the correlation coefficient ($r = 0.12$ $p < 0.05$) was low, indicating the absence of a relationship between the two variables. It is not clear whether these contrasting findings are a reflection of the analytical accuracy of the two laboratories.

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2.6   Evaluation of results

The major objective of this study was to establish the dose-response and dose-effect relationships for early indicators of cadmium induced renal dysfunction. In carrying out this exercise, it became apparent that great difficulties exist in making accurate estimates of the total cadmium dose by the use of the external indices, faecal excretion or daily intake. Nevertheless, the findings obtained in this study are considered to be of importance in terms of assessing the significance of cadmium exposure in the general population. Furthermore, the results may be used to validate the provisional guide-lines proposed by the WHO some 10 years ago.

2.6.1   Relationships obtained using external indices of dose

Data from the epidemiological studies of Kjellström (1977) and Nogawa *et al.* (1978) were used to estimate the 50-year dietary intakes of cadmium associated with proteinuria in various proportions of the general population. These values are shown in Tables 5 and 13 and summarized in Figure 9. Examination of these tables reveals that larger errors are associated with the estimates derived from Kjellström's data. In both instances, however, errors were at a minimum for estimates of the 10 per cent response value. The 10 per cent critical value derived from Nogawa's data was 355 μg d^{-1} and therefore about twice that estimated from Kjellström's work. These intake estimates may be compared with the WHO tolerable intake value of about 70 μg d^{-1} for Europeans. It is of interest to note that this intake was associated with an estimated prevalence of tubular dysfunction in less than one per cent of the population, according to Nogawa's data. Indeed, even the lower 95 per cent fiducial limit of the one per cent estimate is similar to the WHO tolerable intake value. Kjellström's data indicate that a larger proportion of the population, about 2.5 per cent, would show signs of renal dysfunction exposed to a cadmium intake equal to the WHO value. However, it must be stressed that there are large errors associated with the estimates derived from Kjellström's data, particularly at these lower prevalence rates. It should also be borne in mind that 2.5 per cent prevalence was the assumed background response unrelated to cadmium exposure used in this study.

Some of the differences in the dose-response relationships derived from Kjellström's and Nogawa's data may be related to the choice of
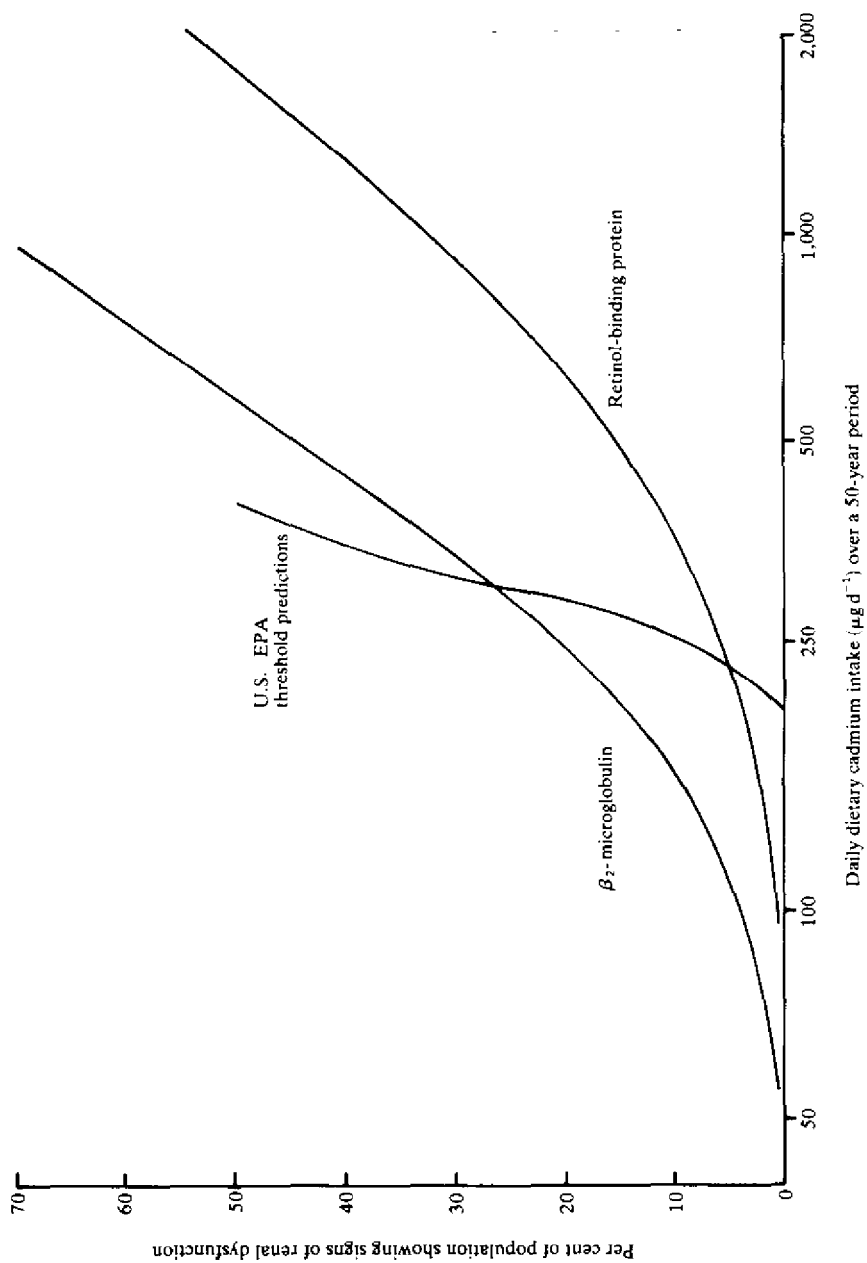
39

Figure 9 Dose-response curves for dietary cadmium exposure calculated in this report for β_2-microglobulin and retinol-binding protein for a European adult, together with the U.S. EPA estimate for proteinuria

the effect variable. It will be recalled that Nogawa employed the urinary excretion of RBP as the index of effect and this may be a less sensitive indicator of renal dysfunction than β_2-m. Additionally, the low sensitivity of the analytical method used by Nogawa may also contribute to the differences in the dose-response curves.

Ideally, the dose-response relationships and attendant error estimates obtained in this study should be compared with the findings of other investigations of this nature. Unfortunately, there appears to be no publication dealing directly with this subject. The only paper of relevance briefly described the current views of the U.S. EPA on the dose-response relationship for cadmium (Grant and Galke 1980). It was considered that a clear threshold of exposure exists for the induction of proteinuria and this threshold was stated to be $200 \, \mu g \, d^{-1}$. Below this threshold, renal dysfunction does not occur, but above $200 \, \mu g \, d^{-1}$ proteinuria will be induced in the more sensitive sectors of the population. The dose-response curve proposed by the EPA and depicted in Figure 9 is obviously much steeper than those obtained in this study, as Grant and Galke (1980) go on to state that the prevalence rate will rise to 50 per cent with a daily cadmium intake of $440 \, \mu g$. Unfortunately, the basis of the EPA work, whether it be empirical observation or modelling, is not stated.

Dose-effect analysis of Tsuchiya's data (Tsuchiya *et al.* 1979) also provides support for the safety of the WHO tolerable intake value. Thus, the regression obtained from Tsuchiya's data base indicates that the WHO intake value would not cause elevated β_2-m levels in at least 95 per cent of the population after 50 years' exposure. Furthermore, the 50-year critical intake value derived from this study is more than four times greater than the WHO provisional guide-line.

In conclusion, it would appear from analysis of the available epidemiological data that adherence to the WHO provisional tolerable intake value will protect the large majority of any population from cadmium induced renal dysfunction. What requires clarification, however, is the validity of findings which link low cadmium intakes with prevalence rates of 5 per cent or less in the population.

2.6.2 Relationships obtained using urinary cadmium as an index of dose

Data from two populations were used to investigate the relationships between urinary cadmium and signs of renal dysfunction. In the case of the environmentally exposed population studied by Nogawa *et al.* (1979),

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an insensitive analytical method for β_2-m measurement was used, with a detection limit about a thousandfold higher than the Phadebas Microtest method. It is therefore somewhat surprising that the urinary cadmium concentrations associated with particular prevalence rates were about half those found in the occupationally exposed population. One possible explanation for this finding is that the elderly were a significant component of the population studied by Nogawa, and in the case of the male group about 25 per cent of the total were 60 years old or more.

In the case of the occupationally exposed population there were differences in the relationships obtained using data from the two analytical laboratories. Nevertheless, the results indicate that a 10 per cent prevalence of elevated β_2-m excretion is associated with a urinary cadmium level of about 10 μg ℓ^{-1}. This finding is in agreement with that of Lauwerys *et al.* (1980) who observed that the prevalence of signs of renal dysfunction starts increasing in those workers excreting 10–20 μg g^{-1} Cr urinary cadmium. Lauwerys *et al.* (1980) further proposed that 15 μg g^{-1} Cr urinary cadmium is the threshold for renal effects and that individuals should not develop kidney lesions if cadmium in urine is not allowed to exceed this value. It must, however, be emphasized that the upper normal limit of β_2-m excretion used by Lauwerys *et al.* (1980) was 200 μg g^{-1} Cr, a value three times lower than that employed in the present study for an occupationally exposed work-force. Nevertheless, the results of dose-response analysis for the occupationally exposed population in this investigation indicate that adoption of the threshold proposed by Lauwerys *et al.* (1980) as a health based limit would indeed protect the majority of the population. In this respect, it is of interest to note that linear regression analysis using data from Laboratory A indicated that the upper normal limit value for urinary β_2-m is associated with a urinary cadmium of 12 μg ℓ^{-1}.

The hypothetical limit of 15 μg g^{-1} Cr (or 15 μg ℓ^{-1}) discussed above may be compared with the more stringent "Health Based Biological Limit" for cadmium in urine (Cd–U) recently recommended by the WHO (WHO 1980). The WHO document concluded that "the individual Cd–U concentration should not be allowed to reach 10 μg g^{-1} creatinine, since above this concentration there is some risk of renal dysfunction". The WHO study group went on to recommend a biological limit of 5 μg g^{-1} Cr urinary cadmium, a value derived in an arbitrary manner by halving the assumed threshold concentration. Dose-response analysis of the occupationally exposed work-force reported in this study suggests

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that a urinary cadmium of 5 µg ℓ^{-1} is associated with a prevalence rate of about one per cent or less in the population. Thus the relationships obtained from this population indicate that the biological limit recommended by the WHO study group may be unneccessarily stringent. It must, however, be stressed that this is only a tentative suggestion and as such requires confirmation from studies involving larger populations of occupationally exposed workers.

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