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TECHNICAL REPORT OF EFSA

Meta-analysis of Dose-Effect Relationship of Cadmium for Benchmark Dose Evaluation[1]

Prepared by the Assessment Methodology Unit

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SUMMARY

A systematic review of the scientific literature was conducted on the relationship between urinary cadmium and renal or bone biomarkers of cadmium toxicity. The most frequently studied biomarker was beta2-microglobulin for which a benchmark dose evaluation was performed. The data were made of 165 matched pairs of group means of urinary cadmium and level of beta2-microglobulin from 35 different epidemiological studies. The dataset was first explored using standard linear regression techniques to screen potential covariates and model options of relevance. Then, Bayesian meta-analysis and hierarchical modelling was used to build an overall dose-effect relationship accounting for inter-study heterogeneity and for inter-individual variability of dose and effect. Subsequently, a benchmark dose was evaluated, using a hybrid approach for various cut-offs.

Key words: Meta-analysis, benchmark dose, Bayesian hierarchical models, dose-effect modelling, cadmium toxicity

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ABBREVIATIONS

α1-MG: Alpha1-Microglobulin

AAS: Atomic Absorption Spectrometry

AM: Arithmetic Mean

ANOVA: Analysis of variance

ANCOVA: Analysis of covariance

β2-MG: Beta2-Microglobulin

bALP: Alkaline phosphatase

BMD: Benchmark Dose

BMDL: Lower bound of the left-sided 95% confidence interval of the BMD

BMR: Benchmark Response

Bone MD: Bone Mineral Density

Cd: Cadmium

CSAF: Chemical-Specific Adjustment Factor

CV: Coefficient of Variation

ed50: dose at which the response is half of the maximum response

GM: Geometric Mean

GMt: Geometric Mean (recalculated)

GSD: Geometric Standard Deviation

GSDt: Geometric Standard Deviation (recalculated)

ICP-MS: Inductive Coupled Plasma – Mass Spectrometry

Log: Neperian (Natural) logarithm

LoD: Limit of Detection

MCMC: Monte Carlo Markov chain

NAG: N-acetyl-β-glucosaminidase

PLM: Piecewise Linear Model

PTH: Parathyroid Hormone

PTWI: Provisory Tolerable Weekly Intake

RBP: Urinary retinol-binding protein

SD: Arithmetic Standard Deviation

SE: Standard Error

TD: Toxicodynamic

TK : Toxicokinetic

U-Cd: Urinary Cadmium

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BACKGROUND

The CONTAM Working Group on Heavy Metals regarding cadmium in food is considering a possible revision of the current provisory tolerable weekly intake (PTWI) of cadmium in Europe. For this purpose, daily cadmium intake could be related to urinary cadmium concentrations via toxicokinetic (TK) modelling, as urinary cadmium is a classical marker of long-term exposure to cadmium. As a next step, the CONTAM Panel needs to evaluate a point of departure, meaning the choice of urinary cadmium target to be used for the derivation of the Provisory Tolerable Weekly Intake (PTWI). Such a threshold can be assessed by linking urinary cadmium to biomarkers of effects. Various publications have investigated that link and the CONTAM Panel's request is now to perform a meta-analysis of all relevant studies in order to derive a robust overall dose-response relationship to be used in the cadmium risk assessment.

A meta-analysis is a statistical procedure to review and summarize information gathered from different studies (see e.g. Berry et al., 2000). In order to evaluate the dose-response and/or dose-effect relationships between urinary cadmium and biomarkers, the body of evidence present in the scientific literature can be compiled, encompassing and quantifying the wide variation between individuals and between studies. As such, this meta-analytic approach can complement a "key study" approach, which uses less but more targeted evidence.

Two meta-analysis papers were identified regarding the relationship between urinary cadmium and renal effects biomarkers. First, Omarova et al. (2007) is a meta-analysis over the last 30 years of publication investigating the relationships between cadmium intakes and various effect or exposure biomarkers. Among the thousands publications screened, 79 met the inclusion criteria. Among these 79 papers, 12 include urinary cadmium and β2–microglobulinuria (β2-MG), which could be used for our analysis. The second meta-analysis Gamo et al. (2006) compiles 14 publications linking urinary cadmium to β2–microglobulinuria for various age groups and both genders. More than 80% of the publications are for the Japanese population. No meta-analysis was found for any biomarker of bone effect.

In order to compile in an exhaustive and systematic manner the literature data on biomarkers of toxicity of cadmium, a full systematic review process needed to be performed.

OBJECTIVES OF THE META-ANALYSIS

The final objective of this analysis is the derivation of a benchmark dose (BMD) and its 95%-confidence lower bound (BMDL) for humans using cut-off points relevant to clinical changes in the target organ.

An interim or secondary objective of the meta-analysis of biomarkers of cadmium in humans is to provide an overall relationship between urinary cadmium and biomarkers of renal and bone effects.

Once the endpoints of renal and bone effects are selected and the inclusion criteria of studies defined, the specific objectives should include:

- A review of the relevant papers selected in previous similar meta-analyses (Bellinger *et al.* used in JECFA 2004, Gamo *et al.*, Omarova *et al.*), provided the inclusion criteria can be matched
- A systematic review over at least the last decades to update the database with the latest publications
- The construction of a consolidated data base from all selected papers matching urinary cadmium to the biomarkers measurement, together with all relevant variables to be accounted for (e.g. sample size, covariates etc…)
- For each endpoint, build the "dose effect" relationship and provide an overall BMD value, for various cut-offs, independently for each endpoint
- Finally the TK model previously developed could be plugged in to the U-Cd vs. biomarkers effects, to address CONTAM Panel's investigations regarding PTWI

1. Data and methods

1.1. Choice of Biomarkers

For Renal effects, the most studied biomarkers include:

- N-acetyl-β-glucosaminidase (NAG),
- β_2– and α_1- microglobulinuria,
- Urinary retinol-binding protein (RBP)
- Proteinuria

For bone effects, the most studied biomarkers are:

- Bone mineral density (bone MD)
- Alkaline phosphatase activity (bALP),

- Serum calcium,
- Parathyroid hormone (PTH)

In very few cases, fracture risk ratios or incidence rates have also been studied as bone biomarker of effect.

The choice of endpoints is obviously crucial and has to be made as soon as possible so that the review work can start. So far, based on the current literature availability, and on internal discussions and with consulted experts, the most relevant markers may be β_2–microglobulinuria (β_2–MG) and NAG-B for renal effects, and bone mineral density for bones effects. Continuous variables are preferable as they include more information for the BMD assessment. In case papers show dichotomized measurements, this can be accounted for in statistical analysis.

1.2. Literature search and data collection

Since no global meta-analysis was available linking urinary cadmium and cadmium intake (including occupational studies and Caucasian people), and since the attempt to access datasets from previous meta-analyses of interest (e.g. from Omarova *et al.*), a full systematic review approach needed to be implemented from scratch. As far as available resources and time constraints allowed for it, the Cochrane methodology was used (see Higgins *et al.*, 2008).

Extensive literature search (from 1966 until October 2008) were performed using specific keywords related to cadmium exposure, kidney (renal: (β_2-MG, α_1-MG (or HC), NAG-total, NAG-A, NAG-B, RBP; proteinuria) and bone biomarkers (bone mineral density (Bone MD), alkaline phosphatase activity (bALP), serum calcium, parathyroid hormone (PTH) and cross-checked by two different scientists in selected databases (Web of Knowledge, Pubmed, Medline). The main keywords used were:

- Cadmium and exposure
- Cadmium and β_2-microglobulin
- Cadmium and α_1 -microglobulin
- Cadmium and NAG
- Cadmium and bone

In addition, manual searches were also performed in specialized review papers and book chapters.

More than 5000 abstracts were retrieved and each study was individually checked for its relevance with respect to urinary cadmium concentration in humans and each biomarkers of effect.

Peer-reviewed publications were selected for inclusion in a consolidated database based on the following criteria:

1. The study is published in an international peer-reviewed journal

2. The study measured urinary cadmium (in μg/g creatinine or other units that could be converted) as indicator of internal dose together with at least one biomarker of renal and/or bone effect both as continuous variables (mean, standard deviation or geometric mean and geometric standard deviation)

3. The data are not already (fully or partially) used in previous studies. In the cases where data are used in more than one study, the study providing the most complete and detailed information was chosen (e.g., the study which provides the most dose sub-groups)

Major covariates that might affect the dose-effect relationship were also collected from the original publication (e.g., body weight, age, gender, ethnicity), when reported. Additionally, important study characteristics were also collected, such as analytical methods, year of publication, type of exposure (environmental and/or occupational exposure), and presence of co-exposure to other metals.

1.3. Final database

The final numbers of studies included, classified by biomarkers are reported in Table 1 and Table 2.

Table 1: Number of studies collected by renal biomarker

Renal Biomarker	Total	β_2-MG	α_1-MG	NAG (total)	NAG a	NAG b	RBP	Proteinuria (total)
N studies Continuous data	54	35	16	27	1	2	10	11

Table 2: Number of studies collected by bone biomarker

Bone Biomarker	Total	Bone MD	Calcium serum	bALP	PTH
N studies Continuous data	9	5	5	5	4

The largest set of data was for β_2-MG. For this endpoint, 165 matched pairs of urinary cadmium and β_2-MG levels could be gathered from 35 studies, with the corresponding GSDs in most of the cases. This covers about 30,000 individuals with mostly Asians (93.5%). β_2-MG has actually been recognized as the most relevant to cadmium effects throughout the scientific literature and has been accepted as the standard biomarker in previous meta-analyses (Ikeda *et al.*, 2003, Gamo *et al.*, 2006; Omarova *et al.*, 2007) and by the JECFA and ASTDR (Bellinger *et al.*, 2004; FAO/WHO, 2004; ASTDR, 2008). Fewer studies were available for bone effects and these are regarded as very heterogeneous. Consequently, and upon advice from the CONTAM Panel, the full meta-analysis has only been carried out for β_2-MG.

The final database used for β_2-MG is reported in Annex 4 in a concise form.

Table 3: Summary of the main covariates mostly reported for β_2-MG, with the relative and absolute sample sizes of the different levels (all studies pooled)

Variable	Levels	Sample Sizes (absolute values)	Sample Sizes (percentage)
Gender	Females : Males : Mixed	21957 : 5257 : 2019	75% : 18% : 7%
Age	>=50 years : <50 years	14779 : 13964	51.5% : 48.5%
Ethnicity	Asian : Caucasian	27317 : 1916	93.5% : 6.5%
Study Type	Cross-sectional : Cohort	29095 : 138	99.5% : 0.5%
Workers status	Non-Worker : Worker	28974 : 259	99% : 1%
Co-Exposure	Yes : No	2258 : 26975	8% : 92%

Table 3 summarizes the main covariates reported; allowing for further exploration and test for effects using linear regressions (see next section). It is worth underlining that many covariates or factors thought to be important for the analysis were never or barely reported, such as body weight or the time lag between the reported exposure to cadmium and the concentration measurements. Moreover, most studies were cross-sectional and only one cohort study was recorded.

More specifically, an attempt was made to record the type and route of exposure to cadmium reported by the studies. Indeed, including occupational exposure might lead to different dose-effect relationships than for the general population with only food contamination exposure. In

general, exposure to cadmium could be environmental, occupational or from food origin. This distinction was difficult or impossible to achieve, as the three sources of exposure were often correlated to each other. For example, an environmental exposure was often associated to contaminated food. Still, those occupational studies that exclusively include exposed workers were identified ("worker status" variable).

Finally, information on analytical methods could be collected in most papers. For urinary cadmium, concentrations were measured by means of AAS (i.e. flameless AAS, Graphite furnance AAS, flame-AAS with trap, electrothermal AAS with stabilized temperature platform and Zeeman background correction) and by ICP-MS with little information regarding LODs. For β_2-MG, concentrations were assessed by immunoassay kits. Only 5 papers were recorded before the 90s (1978, 1983, 1984, 1986), and the same analytical methods were used (AAS and flameless AAS).

1.4. Data cleaning

In order to harmonize and validate the database of β_2-MG, some further checks and transformations were performed, as follows.

- Two rows with the same records of urinary cadmium and/or β_2-MG were re-checked for comparison. This enabled to further detect cases were data could have been used in different papers. In such cases, the paper chosen was that providing the most information e.g., more population or dose sub-groups.

- Abnormal or impossible values were double-checked. Abnormal values include concentrations being 10 times higher or lower than all others, coefficient of variation being 10 times higher or lower than others, geometric means greater than 10. Impossible values include geometric means below 1, mean value outside the given range [min, max], incompatible values for standard deviation, mean and range. These further checks enabled us to detect errors in the data collection and potential typographic errors: like standard errors reported instead of standard deviation and arithmetic means instead of geometric means. In particular:

    - Nogawa *et al.* 1983 claimed to report "SD" was presumed to be GSD because the magnitudes of the values reported could be very unlikely as arithmetic SD; i.e. for β_2-MG (µg/g creatinine) in female itai-itai patients aged 60-69, the geometric mean was 16391 and the reported "1 SD" was 8.6; analog for patients aged 70-79, the GM was 103098 and the reported "1 SD" was 1.2; for patients aged 80-89 the reported GM was 76863 and "1 SD" 1.3. The corresponding U-Cd values in µg/g creatinine were 17.77 (1.658), 15.41 (1.606) and 11.92 (1.234), respectively.

    - Uno *et al.* 2005 reported different units for U-Cd and β_2-MG (µg/day instead of µg/g creatinine and vice versa) which was corrected for our data collection.

- Hong *et al.* 2004 has had a shift to the left of the table-row reporting the units, so β_2-MG was reported in mg/g creatinine (which is the correct unit for urinary albumin in this publication) instead of µg/g creatinine, which was corrected for our data collection.

- The following adjustments were made to display records in consistent units:
  - U-Cd values reported in nmol/mmol were transformed into µg/g by applying the factor of 0.99375 (= Molar Mass(Cd)/ Molar Mass (creatinine)) applied to Hotz *et al.* 1999
  - β_2-MG values reported in nmol/mmol were transformed into µg/g by applying the factor of 104.3158 (= Molar Mass(β_2-MG)/ Molar Mass (creatinine)) applied to Hotz *et al.* 1999
  - All other units in other weight-based units like mg/g were also recalculated into µg/g

- The following data transformations were made:
  - In cases where no geometric standard deviation (GSD) was reported, but the geometric mean (GM), Sample Size (n) and Range (Minimum (min) and Maximum (max)), the *GSDt* (geometric standard deviation transformed) was calculated by:

$$GSDt = \exp\left(\max\left[\log\left(\frac{GM}{\min}\right), \log\left(\frac{\max}{GM}\right)\right] \Big/ \varphi^{-1}(1 - 1/2n)\right)$$

    where φ stands for the cumulative density function of the normalized Gaussian distribution. This formula was applied for values reported in Aoshima *et al.* 2003; Bernard *et al.* 1995; Cikrt *et al.* 1992; Hong *et al.* 2004; Jin *et al.* 2004; Karakaya *et al.* 1993; Kim *et al.* 2008; Roels *et al.* 1991; Tohyama *et al.*1986; Uno *et al.* 2005; Yamagami et.al.2008

  - In cases where no GSD was reported, but GM and arithmetic SD, the *GSDt* was calculated by:

$$GSDt = \exp\left(\sqrt{\log\left(0.5\left(1+\sqrt{1+4(SD/GM)^2}\right)\right)}\right)$$

    This formula was not applied in any of the final publications used for β_2-MG data.

  - In cases where no GSD and GM were reported, but the arithmetic mean (AM) and the arithmetic SD, the *GMt* and *GSDt* were calculated by:

$$GMt = AM \Big/ \sqrt{\left(1+(SD/AM)^2\right)}$$

$$GSDt = \exp\left[\sqrt{\log\left(1+(SD/AM)^2\right)}\right]$$

The formulas were applied for values reported in Hotz *et al.* 1999; Nogawa *et al.*1984a; Nogawa *et al.*1984b; Piscator 1978, Satarug et.al.2004a, Satarug et.al. 2004b, Teeyakasem et.al.2007.

- In the case were the standard error (*SE*) was reported the arithmetic standard deviation (*SD*) was calculated by:

$$SD = SE\sqrt{n}$$

It was applied once for values reported in Nogawa *et al.*1984a.

The validity of the formula above can be illustrated via Monte Carlo simulations. Using a typical level of β_2-MG drown from a lognormal distribution, with parameters 4 and 1, one could simulate data for various study sample sizes and compute the proposed approximations for GSDt and GMt. Those approximations are then compared with the true GSD and GM and the relative error occurred is illustrated by Figure 1 and Figure 2. The conclusion of these simulations is that the use of the proposed transformations is likely to provide estimates of missing GM with less than 10% error and of GSDs with less than 2% error.

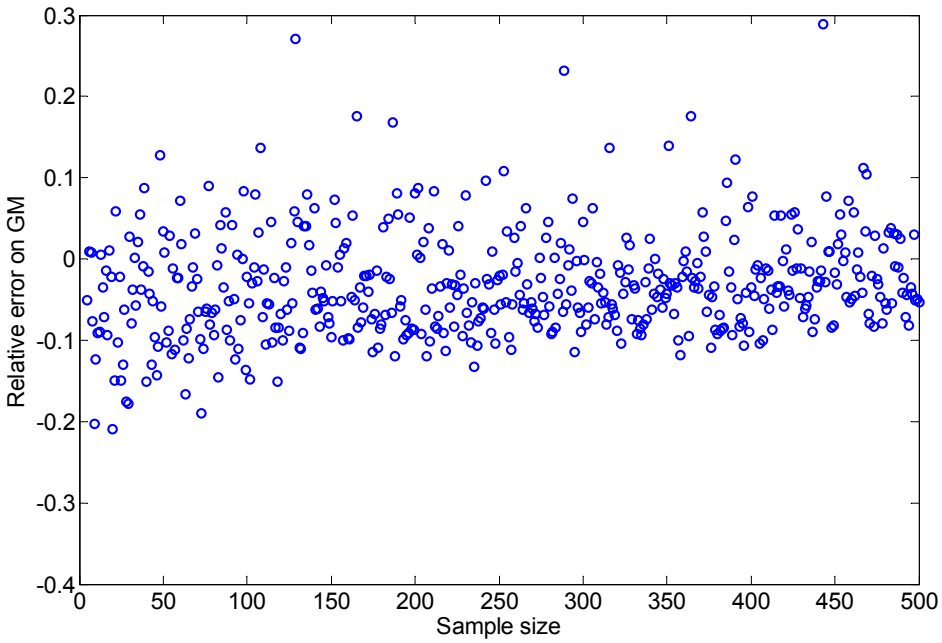

Figure 1: Relative error of GMt with respect to the true value GM for various sample sizes, assuming the data are lognormally distributed, with parameters 4 and 1.

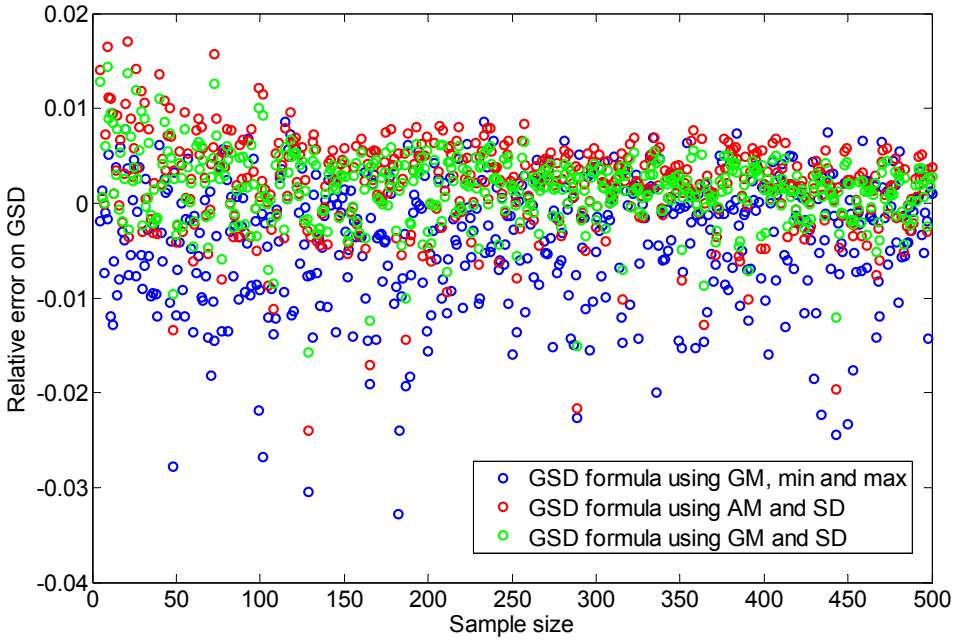

Figure 2: Relative error of the three GSDt formula with respect to the true GSD for various sample sizes, assuming the data are lognormally distributed, with parameters 4 and 1.

1.5. Data exploration methods

The final set of selected studies will then be screened to extract all relevant variables (covariates) needed for the analysis. The selection of those variables was carefully assessed in agreement with experts. Among them, the most frequently reported were:

- Gender: described by a 2-level indicator (=0 for males and =1 for females). In the few cases where it was missing, an even mix of males and females was assumed (indicator set to 0.5)
- Age (sub-group means)
- Ethnicity (Caucasian vs. Asians): described by a 2-level indicator. Preliminary analysis showed that Asian (Chinese, Thaïlandese, Korean) and Japanese were not showing any significant differences, hence could be grouped together into "Asians".
- Type of study (cross-sectional vs. cohort study): described by a 2-level indicator.
- "Workers" status ("workers only" vs. "non exclusively workers"): subgroups were marked as "sub-group of workers only" when it was explicitly reported that such a sub-group is exclusively made of exposed workers in occupational studies. Obviously, the "non-exclusively workers" subgroups mostly correspond the general population and are likely to include some workers with occupational exposure.

- Co-exposure: described by a 2-level indicator set to 1 (yes) in the cases where co-exposure to other heavy metals (e.g. mercury, lead) was explicitly reported, and set to 0 else.

The factors above were specifically analysed at the data exploration stage with ANCOVA techniques using SAS version 9.1. All factors were treated as class variables except "age" and "gender". ANOVAs and/or ANCOVAs where made on the dependent variable "concentration of β_2-MG" after log-transformation. Urinary cadmium was also included as a fixed effect in ANCOVAs without log-transformation to enhance the linearity relationship. In a first step, no interaction term was included in the model. When sample size allowed for it, the "study" variable was treated as a random effect. Proc MIXED was used when study random effect was included, proc GLM was used elsewhere. In order to account for various sample sizes, the WEIGHT option in the SAS procedures was used to weight independent variables according to the sample sizes of each dose groups. Finally, in order to evaluate co-linearity problems, multiple regressions were also performed on urinary cadmium against all possible confounders of exposure (e.g. age, co-exposure etc…). For the variables shown to be significantly co-linear with urinary cadmium, interactions factors were added into the regression for further analysis.

In order to visualize all data, graphical display of them were made using MATLAB Release 14.

1.6. Statistical methodology for the meta-analysis

Once the data-base is constructed and consolidated, the meta-analysis can be carried out and the relationship between urinary cadmium and biomarkers quantitatively assessed and adjusted to the influencing variables. In particular, more weight should be given on larger studies or those with higher data/design quality, following standard meta-analysis procedures. Differences between some population sub-groups may also be statistically evaluated as needed (e.g. difference between genders, between age groups, time trends) if the required data are available.

The meta-analysis was then performed to determine the overall relationship between urinary cadmium and β2-microglobulin for subjects over 50 years of age (with purely occupational studies excluded) and for the whole population with all studies. Especially in those cases of large inter-study variability due to many uncontrolled factors, heterogeneity can not be assumed and variation between studies should be accounted for (see e.g. Sutton *et al.*, 2008). This is usually made using a random study effect in the statistical model (see Berry *et al.*, 2001, Morales and Ryan, 2005). Therefore, a mixed-effect model was fitted to geometric means and geometric standard deviations of both β_2-MG and U-Cd concentration, under log-normality assumption. The statistical model allows accounting for inter-study and inter-individual variability and to weight studies according to their sample sizes. If $Y_i^{(k)}$ stands for a

measurement of e.g., β_2-MG for individual (i) in the study (k), then empirical means and variances ($S^{(k)2}$) of $\log(Y_i^{(k)})$ (as derived from the recorded geometric means and standard deviations in the data) are assumed to follow the following statistical distributions:

$$\sum_i \frac{\log(Y_i^{(k)})}{n^{(k)}} \sim N\left(\mu^{(k)}, \frac{\sigma^2}{n^{(k)}}\right)$$

$$S^{(k)2} \sim \frac{\sigma^2}{n^{(k)}} \chi^2(n^{(k)} - 1)$$

Where $n^{(k)}$ is the sample size of study k, $\mu^{(k)}$ is the population sub-group mean effect (in log scale) and σ^2 is the inter-individual variance of the effect at a given dose.

The model was fitted via Bayesian inference as it particularly suited to hierarchical models fitting. Bayesian set-up also offers an integrated and robust framework to derive BMDs and BMDLs by Monte Carlo simulations using posterior samples. The Bayesian evaluation was made using WinBUGS (version 1.4, see Lunn *et al.*, 2000). For each fitted model, three Monte Carlo Markov chains were simultaneously run until convergence. Convergence was assessed using the Gelman-Rubin test available in the WinBUGS software, and by visual inspection of the chains. Posterior means were reported as statistical estimates of model parameters. Prior distributions were chosen as "non-informative" i.e. flat normal distributions for mean parameters and flat gamma distributions for precision parameters.

1.7. Dose-effect models

The dose-effect model is the mathematical relationship linking the mean dose of each population subgroup to the mean response. Approaching dose-effect curve estimation in the context of meta-analysis where only aggregated data from the literature are available can be made under additional assumptions (here lognormality of population distributions). This should also be accounted for in the statistical modelling and in the use of adjustment factors for any BMD evaluation. Examples and discussion of analysis of aggregated epidemiological data for dose-response analysis can be found in Ryan, 2008. There is generally not a unique model that can be chosen to meet the purpose. Therefore, it is necessary to assess the sensitivity of results with respect to the modelling assumption. This can be achieved e.g. by comparing results from different possible models or using model averaging techniques (see Wheeler *et al.*, 2007). In our case, one reason to test more than one model is also that the biological mechanisms underlying such a relationship are different for low and high urinary cadmium levels. Therefore, it would be important complementary information to evaluate a BMD with a more precise focus on the low urinary cadmium levels.

From the data exploration (see Results section), it appeared that an S-shaped curve is an adequate dose-effect model to describe the relation between urinary cadmium and β_2-MG, in the log-log scale. Common S-shaped models that are widely used, including for BMD calculations, are exponential models (e.g. Gompertz model) and Hill models (see Sand *et al.*,

2008 and Sand *et al.*, 2005 for a detailed discussion). The two model options are expected to lead to very similar results, as they both describe an S-shape with 4 parameters in that log-log scale. Here, the Hill model was chosen, especially because it uses more interpretable parameters than the exponential one. The model equation is given by:

$$\text{Effect}(d) = \text{background} + \text{amplitude} \times (d^{\eta} / (d^{\eta} + \text{ed}_{50}^{\eta}))$$

Where *d* stands for the dose i.e. urinary cadmium (log scale), 'amplitude' corresponds to the difference between the 2 plateaux of the S-shape, ed_{50} corresponds to the dose where 50% of the maximal effect is achieved, and eta (η) corresponds to the shape parameter defining the steepness of the S curve. The Hill curve is illustrated by Figure 3.

As a complementary model for sensitivity analysis and to allow a finer description of the low dose part of the dose-effect curve, it is proposed to also investigate a piecewise linear model. Such a model could then model the low dose area by a linear relationship and then the larger dose area by another linear relationship. The breakpoint dose is also a parameter of the model to be estimated, hence avoiding fixing it to an arbitrary value.

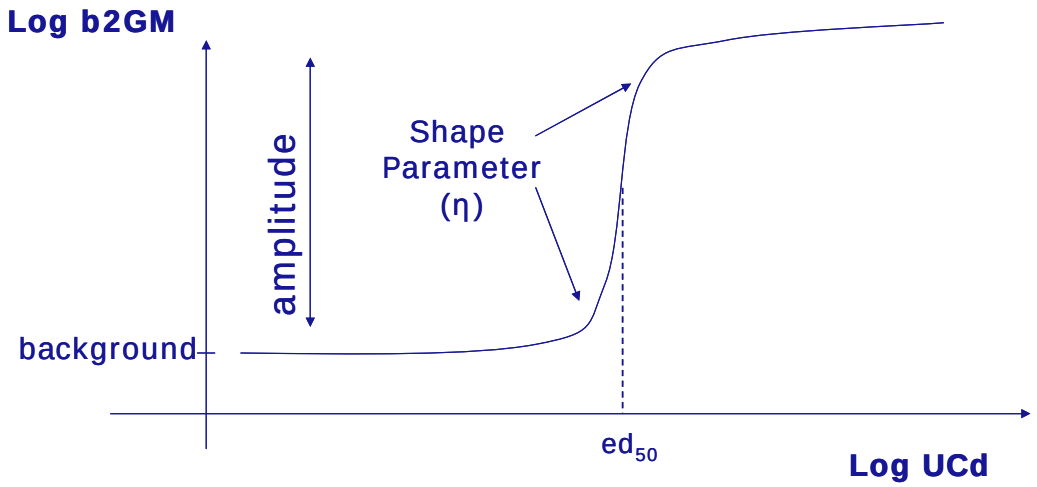

Figure 3: the Hill model is an S-shaped model with 4 parameters

The model equation is as follow:

$$\text{Effect}(d) = \begin{cases} \text{Background} + \text{Slope1} \times (d - \text{dose at background}) & \text{if } d < \text{breakpoint} \\ \text{Effect at breakpoint} + \text{Slope2} \times (d - \text{breakpoint}) & \text{if } d > \text{breakpoint} \end{cases}$$

The curve is illustrated in Figure 4 below, with log(U-Cd) as internal dose.

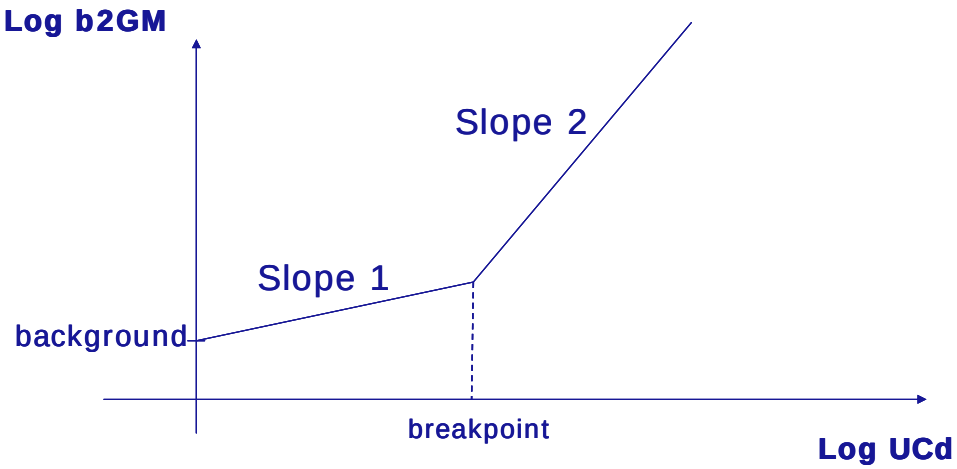

Figure 4: a piecewise linear model needs also 4 parameters

In both models, the background level was defined as the effect at the lowest dose group reported. Considering the very small slope at low dose, this definition is seen as relatively robust.

Finally, a variance model is needed to describe possible influence of urinary cadmium levels on the population variability. Based on the data exploration and for the sake of simplicity, a constant variance model was used.

1.8. Benchmark dose approach

Subsequently to the choice and calibration of a dose-effect model, the benchmark dose (BMD) methodology was implemented (see e.g., Crump, 1984, Budtz-Jørgensen, 2001 and Sand, 2008 for a recent overview of the state-of-the-art). The BMD is defined as the dose needed to achieve an excess risk of a given adverse effect compared to the background exposure, usually defined as level of a biomarker (here β_2-MG concentration in urine), above a given threshold. If P(d) denotes the probability for an individual to reach the defined threshold for adverse effect, when exposed to dose d, then the BMD can be defined in 2 different ways:

- as the dose leading to "Additional risk" of X% (often called benchmark response (BMR)):

$$P(BMD) = P(Background) + X\%$$

where P(Background) stands for the probability of adverse effect at background exposure, and X% (BMR) for the additional prevalence.

- as the dose leading to "Extra risk" of X%:

$$P(BMD) = P(Background) + X\%(1- P(Background))$$

where P(Background) stands for the probability of adverse effect at background exposure, and X% for the probability to observe adverse effect at BMD given it was not observed at background exposure.

The benchmark dose approach used was the so-called hybrid approach (see e.g., Crump, 2002, Suwazono *et al.*, 2006 and Sand *et al.*, 2008), which allows for calculation of risks without dichotomizing the outcome, hence using all information available from continuous data. Risks or prevalence can then be derived with respect to any given biologically relevant threshold. This approach is valid under the assumption that β_2-MG levels are log-normally distributed over the population at a given dose of urinary cadmium. The main idea of this hybrid approach is to model the population variability around the mean dose-effect curve using a statistical (log-normal) distribution at each given dose, as illustrated by Figure 5. Then, for any cut-off, one could derive the corresponding prevalence and dose-response curve.

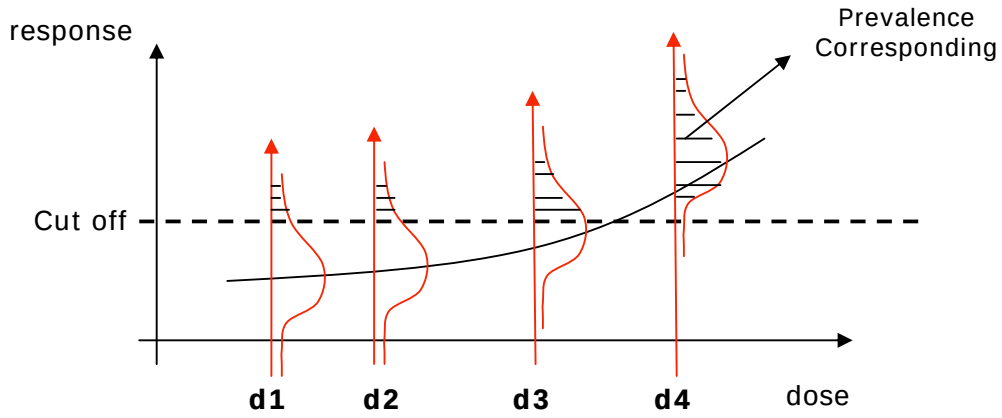

Figure 5: Hybrid approach used for benchmark dose evaluation

Three different cut-off values were chosen to derive the BMD and BMDL: 95th percentile of background exposure (statistical cut-off 1), 300 and 1000 µg/g creatinine. In cases where adjustment for ethnicity was made, a fourth cut-off was added, corresponding to the cut-off covering the same proportion of Caucasians than 300 µg/g creatinine would cover of the Asian population (statistical cut-off 2).

The BMD evaluation was performed for the following data sets:

- For the whole population from all studies, without any adjustment for covariates
- For the whole population from all studies, with an adjustment for significant covariates (e.g. ethnicity)

- For a focus population made of subgroups with age mean greater than 50 years, with purely occupational studies excluded (i.e. excluding population subgroups made of purely exposed workers), adjusted for ethnicity

No further data exclusion was made e.g. on the basis of whether the control groups reported from the studies were at "sufficiently low" levels. Indeed, this would reduce the number of included studies too drastically, and the cut-off for inclusion would be arbitrary. As a matter of fact, exposure levels found from the data collected were on average about 1.8 μg/g creatinine, mostly ranging from 0.38 to 4-5 μg/g creatinine.

The benchmark doses were all estimated as any other statistical parameters, consistently through the Bayesian inference machinery. BMDs were therefore defined as the posterior median benchmark dose, and BMDL as the 5th percentile of the posterior distribution on BMDs (i.e. the lower bound of the 95% left-sided credibility interval). In practice, a posterior sample (size of at least 10,000) was drawn from the MCMC simulations of the parameter vectors. Then, Monte Carlo simulations were implemented in Matlab for the calculation of BMDs, using the following formula:

$$BMD = \exp\left(\mu^{-1}\left(\log(cutoff) - \sigma\varphi^{-1}(p)\right)\right)$$

With:

$$p = (1 - BMR)\,\varphi\left(\frac{\log(cutoff) - background}{\sigma}\right) \quad \text{for extra risk}$$

$$p = \varphi\left(\frac{\log(cutoff) - background}{\sigma}\right) - BMR \quad \text{for additional risk}$$

Where σ stands for the population log(GSD) of effect, BMR for the benchmark response, φ for the cumulative distribution function of the standardized normal distribution, μ for the dose-effect function.

In each case, the BMD and BMDL were derived for excess risks (BMR) of 5% and 10%, for each cut-off.

1.9. Model validation and sensitivity analysis

Although two models have been fitted in parallel for increased robustness, some validation steps need to be carried out. Model checking consisted in plotting mean predictions versus observed entries for the complete dataset. Visual inspection of the fit and diagnostic plots should confirm that residual errors are small compared to the variability explained by the model, and no strong deviation from the diagonal should be observed. The use of Chi-square test statistics is not very suitable in the context of hierarchical models and Bayesian set-up. In spite of that, since it is a commonly reported test, the related p-value was computed.

2. Results

2.1. Data exploration

2.1.1. Data visualization

We first report elementary scatter plots showing the data from various angles. Figure 6 shows the raw data in the log-log scale, with all entries pooled. It suggests that in this scale, the dose-effect curve may be described by an S-shaped function.

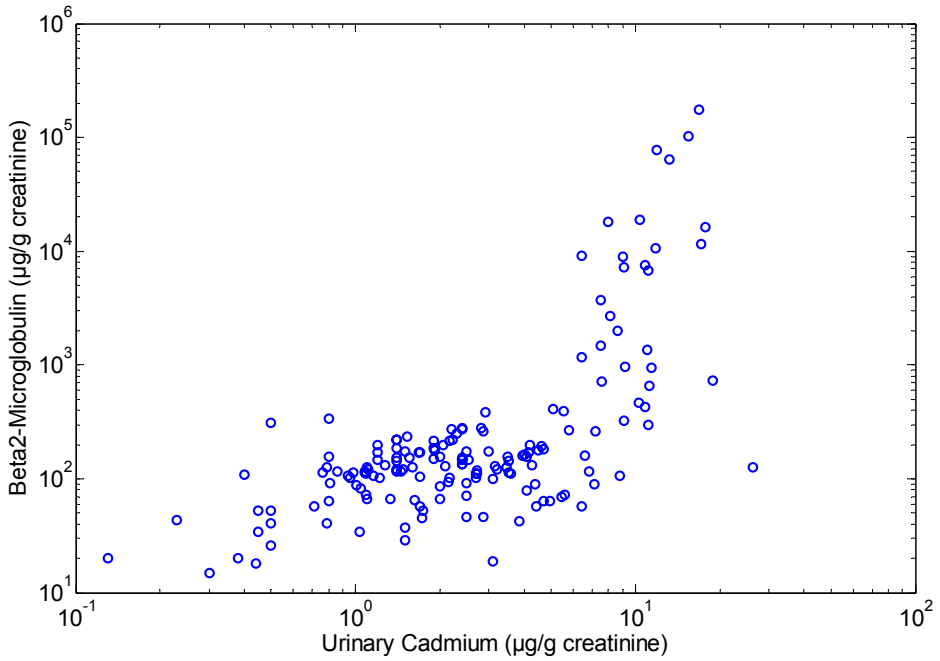

Figure 6: Scatter plot of dose groups from all studies linking urinary cadmium to β_2-microglobulinuria in the log-log scale. Each study contributes to one or more dose groups.

Figure 7 shows the same data as above but on the semi-log scale (urinary cadmium kept untransformed). This representation seems more adequate for exploration with ANOVAs and linear regressions, and was therefore used for ANCOVAs. Finally, Figure 8 shows again the same data on the log-log scale with now one colour by study. This illustrates the inter-study variability and how not accounting for it may bias estimates.

Figure 10 scatters the GSDs data for β2-microglobulin in urine versus urinary cadmium.

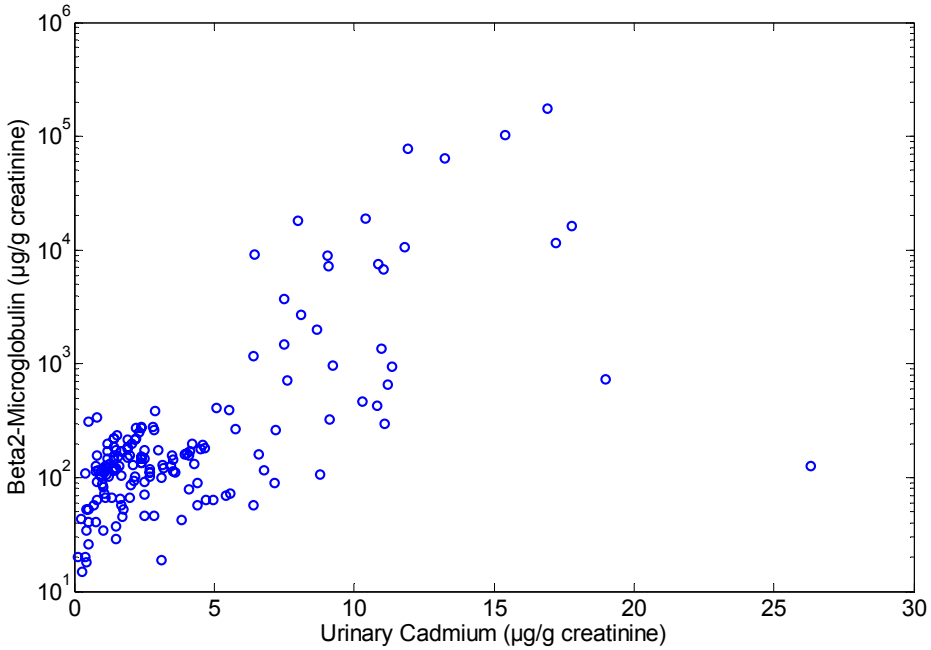

Figure 7: Scatter plot of data from all studies linking urinary cadmium to β_2-microglobulinuria, in the semi-log scale

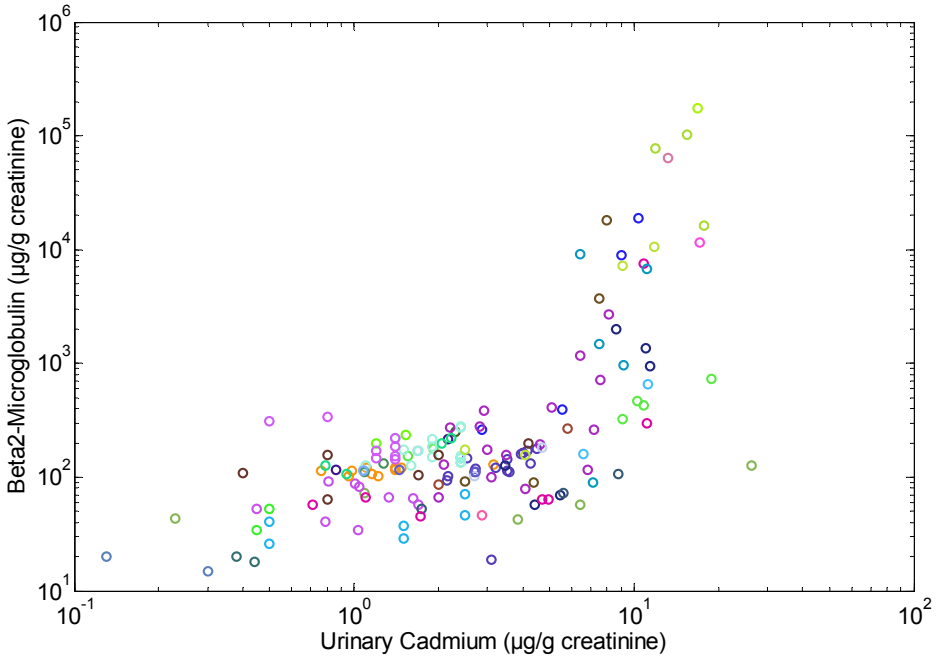

Figure 8: Scatter plot of data from all studies linking urinary cadmium to β_2-microglobulinuria, using a different colour for each study

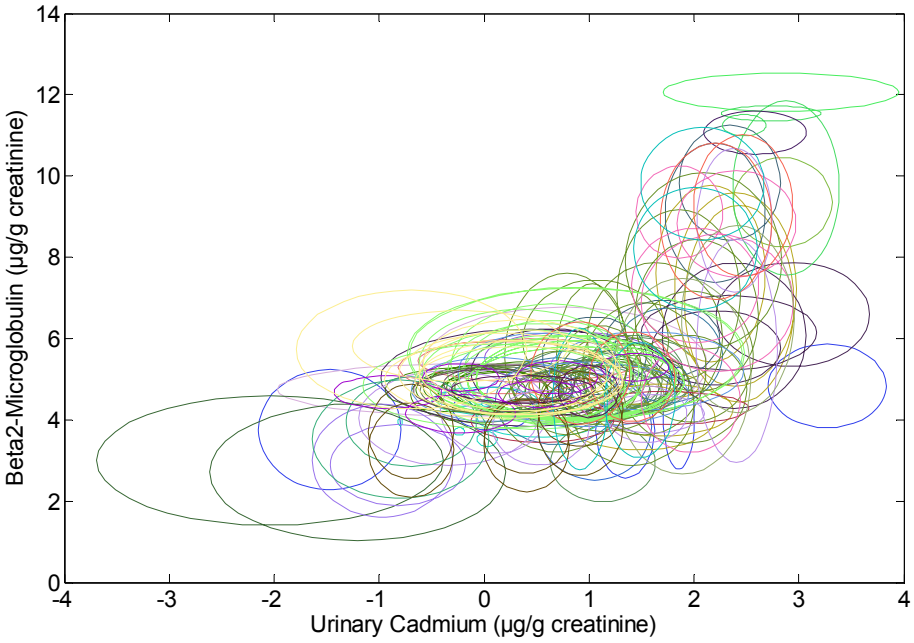

Figure 9: Scatter plot of data from all studies linking urinary cadmium to β_2-microglobulinuria, using a different colour for each study and illustrating within group variability. Each dose group is represented by an ellipse on the log scale, with log(GSD) as radium.

Figure 10 scatters the GSDs data for β2-microglobulin in urine versus urinary cadmium. It shows no clear visible pattern.

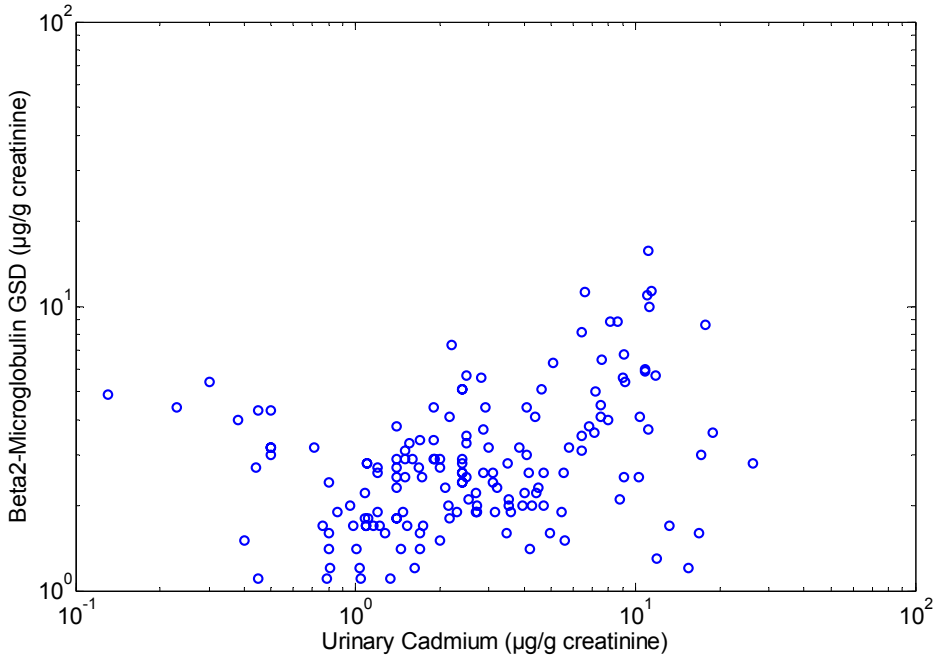

Figure 10: Scatter plot of data from all studies displaying urinary cadmium to β_2-microglobulinuria GSDs

2.1.2. Influence of covariates

A primary objective of the data exploration is also to detect which are the main covariates available in the dataset that should be adjusted for in the dose-effect modelling. For this purpose, we first display the main plots with various covariates of potential biological relevance. Then we discuss results from statistical tests for effect.

Figure 11, Figure 12, Figure 13, and Figure 14 display the complete data, highlighting differences between respectively: "exclusively workers" versus mixed population sub-groups, older than 50 years old sub-groups versus younger than 50 years old, males versus females, and Asians versus Caucasians.

The visual inspection of those plots suggests that:

- Workers status may show a shift to the right of the dose-effect relationship
- Older population sub-groups follow, the same S-shape curve, but at a raised level as expected for higher levels of both dose and biomarker of effect,
- No obvious difference between males and females
- Caucasians may show a systematically lower level of β2-microglobulin as compared to Asians

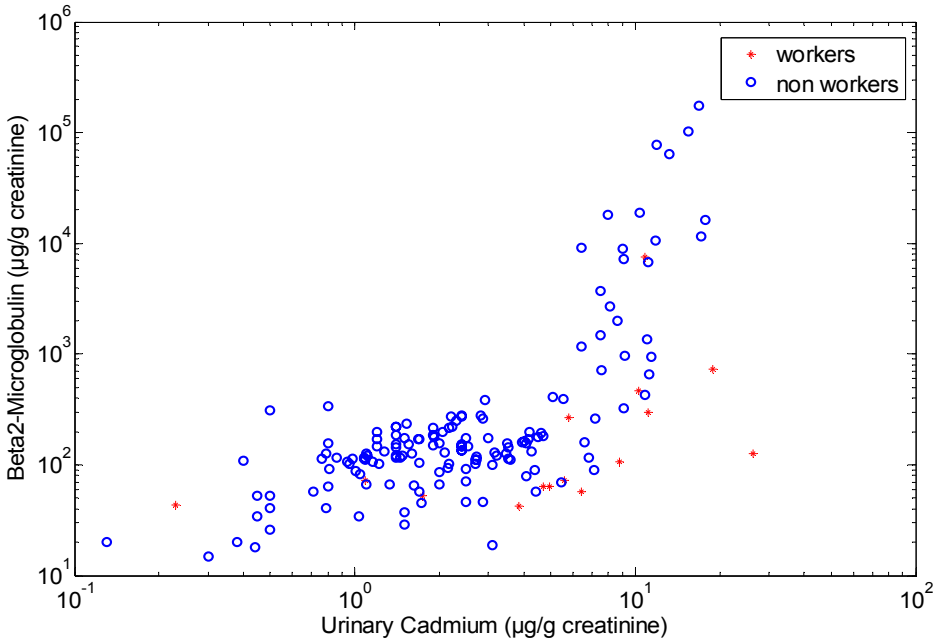

Figure 11: Scatter plot of data highlighting entries reporting "exclusively workers" (red stars) versus those from with mixed population (blue circles)

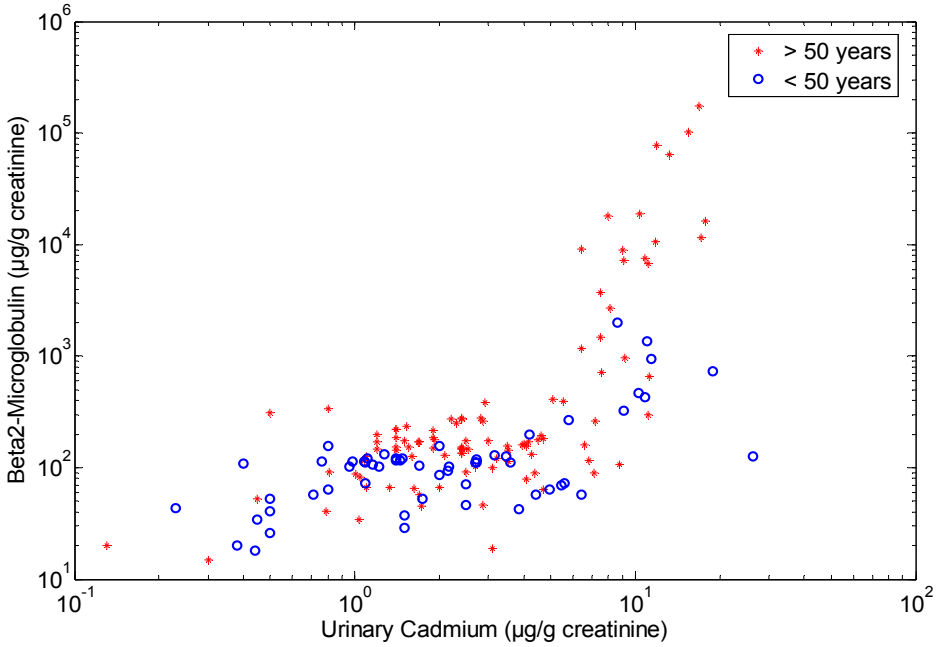

Figure 12: Scatter plot of data showing sub-groups with age mean greater than 50 years (red stars) versus subg-groups with age mean lower than 50 years (blue circles)

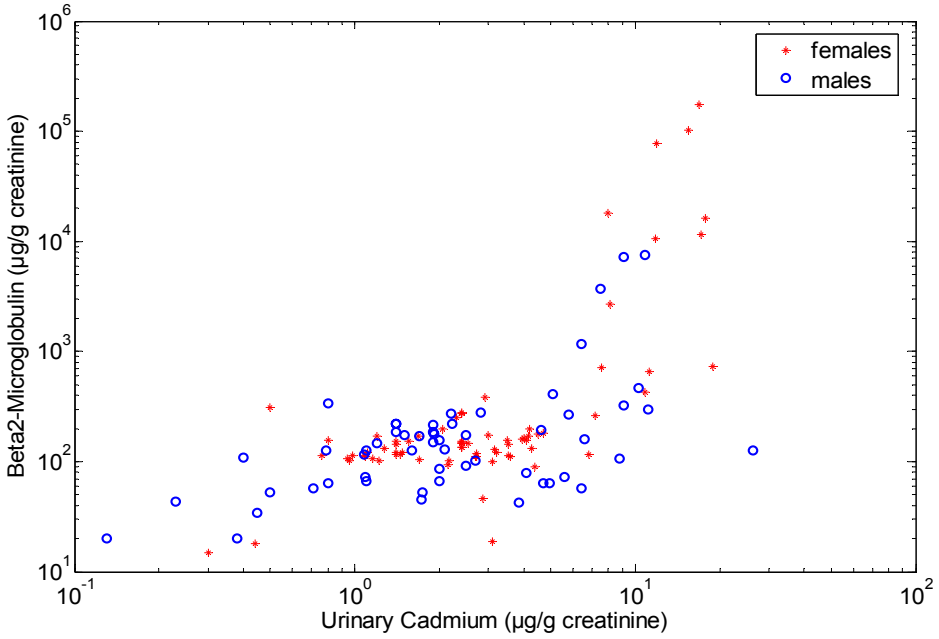

Figure 13: Scatter plot of data showing sub-groups with females (red stars) versus sub-groups males (blue circles)

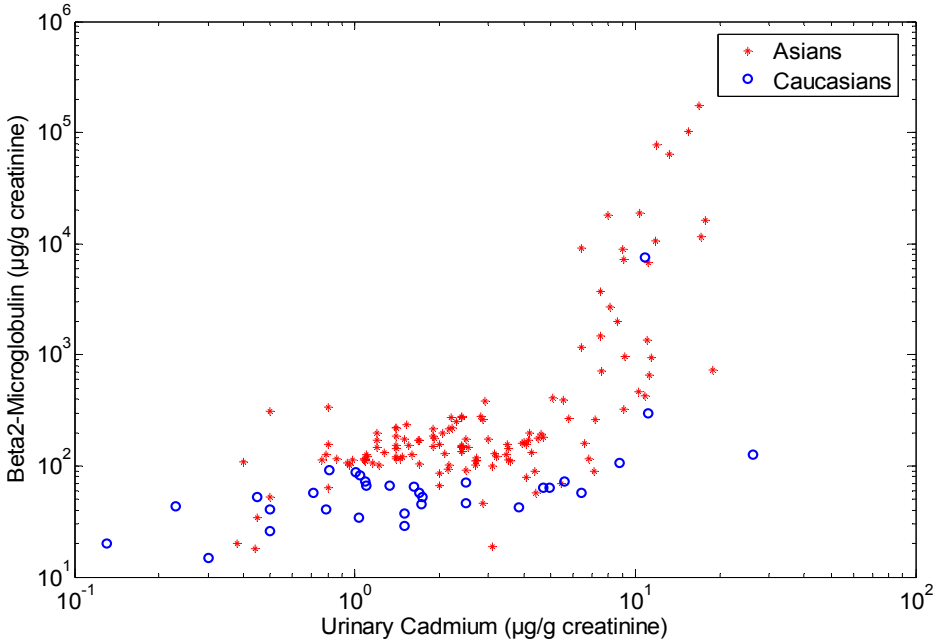

Figure 14: Scatter plot of data Asian sub-groups (red stars) versus Caucasian sub-groups (blue circles)

Of course, it is important to underline that the visual inspection above are only suggestive of potential effects. They are rather weak measure of effect as :

- they do not allow visualisation of small but significant effects,
- they do not address confounding issues, which are very likely here
- they do not address the possible interactions between covariates

Therefore, more refined statistical assessment is needed to drive to dose-effect model building.

First, the full weighted multiple regression models with all variables included and without interaction terms are reported, with and without accounting a "study" random effect.

All SAS outputs for ANCOVAs are reported in Annex 1. When convergence problems were specified in the SAS log files, a mention of them was added in the caption of the table. They indicate that less reliability should be given to the corresponding results due to too small sample size or too high model complexity.

From the full model analysis (all variables included, Table 21 and Table 22), it appears that effect shown to have a significant effect on β_2-MG levels are primarily urinary cadmium ($p<0.0001$) and age ($p<0.0001$), and then ethnicity ($p=0.0093$) and gender ($p=0.0217$).

From the co-linearity analysis based on a multiple regression on urinary cadmium (Table 23), it appears that highly significant co-linearity is found between urinary cadmium and age ($p<0.0001$), and between urinary cadmium and workers status ($p=0.0003$). Moderate significance was found for co-linearity between urinary cadmium and ethnicity ($p=0.035$), between urinary cadmium and gender ($p=0.055$), and between urinary cadmium and type of study ($p=0.059$).

As a consequence, all significant effects are to some extent co-linear with urinary cadmium and should therefore be included in the ANCOVA analysis with interactions with urinary cadmium. In addition, the "worker status" variable should also be included as it is also strongly co-linear with urinary cadmium.

From the ANCOVA with interaction terms (urinary cadmium against age, gender, worker status and ethnicity) as reported in Table 24 and Table 25, it appears that only age has both an effect as such and in interaction with urinary cadmium. Gender remains a significant effect (moderately significant, $p=0.03$) only in interaction with urinary cadmium, suggesting different metabolism of cadmium between males and females. Some estrongenicity effect might be associated to such differences. This may also be due to a confounding effect (e.g. body weight) which could not be investigated here. Furthermore, no significant effect was left for the "workers status" variable. This may be due to the strong co-linearity in particular with urinary cadmium. Finally, ethnicity appears to be still significant as such ($p=0.02$) but not significant in interaction with urinary cadmium. This suggests that difference between ethnic groups may be due to physiological differences which lead to different levels of β_2-MG (lower for Caucasians) independently from urinary cadmium levels. Alternatively, this may also be due to the poor overlap of exposure levels between Caucasians and Asians, leading to a limited statistical power to detect any interaction effect. One possible confounder could have been the difference in urine pH between Asians and Caucasians, if the studies did not adjust for it. However, all studies reporting exclusively Caucasian data did adjust for pH. More specifically:

- in Bernard *et al.*, 1995: pH was buffered at pH=7
- in Cikrt *et al.*, 1992: pH was buffered at pH=7.4 with phosphate buffer (cont. 1% NaN3)
- in Hotz *et al.*, 1999: alkalinised urine samples
- in Piscator *et al.*, 1978: NaN3 was added (mainly for sterilization, but has also an alkalinisation effect)
- in Roels *et al.*, 1991: Phosphate buffer at pH=7.6 (cont.0.2%NaN3)
- in Trzcinka-Ochocka *et al.*, 2004: Phosphate buffer at pH = 7.4 (cont. 1% NaN3)

As a conclusion, pH adjustment was made directly after sampling (not only before measurement) for all "Caucasian" studies listed. For the study Piscator 1978, where only NaN3 was added, obviously for preservative reasons and not for pH-adjustment reasons, a "pH-raising effect" by the NaN3-addition is likely to occur (e.g. NaN3 + H2O → HN3 +NaOH).

The main conclusions from the data exploration are:

- The data provide strong evidence for a significant age effect on the level of β_2-MG, both as such and in interaction with urinary cadmium
- The data provide strong evidence of significant ethnicity effect on the level of β_2-MG, independently from the levels of urinary cadmium
- The data provide evidence of gender effect in interaction with urinary cadmium, suggesting significant difference in cadmium metabolism between males and females
- The data do not allow to conclude on the workers status effect due to co-linearity issues (and probably also to data quality)
- The data provide no evidence of any "co-exposure" effect or "type of study" effect
- S-shaped Hill model and piecewise linear model are both reasonable choices for dose-effect model on the log scale
- To adjust for covariates such as gender, ethnicity and workers status, a constant term could be added to the model equation (linear adjustment on the log scale)

2.2. Results for the dose-response modelling

2.2.1. Results for all population without adjustment to covariates

We first report the statistical outputs for the Hill model fitted to the complete dataset, without any adjustment for covariates. The statistical estimates of the model parameters are reported in Table 4. Background, amplitude and ed_{50} parameters have been exponentiated to ease their interpretability.

Table 4: Parameter estimates for the Hill model fitted to the complete dataset, without covariates

Parameter	Estimate (95%-confidence interval)
Background level (µg/g crea)	81 (62 - 110)
Amplitude ratio (max level / min level)	3344 (557 - 8412)
ed_{50} (dose at 50% max response in (µg/g crea))	10.3 (8.8-12.9)
Shape (steepness) parameter	15.2 (12.4-18.7)

Figure 15 illustrates the fitted curve, using posterior mean estimates (note that it does not correspond to the mean curve because the model is non-linear).

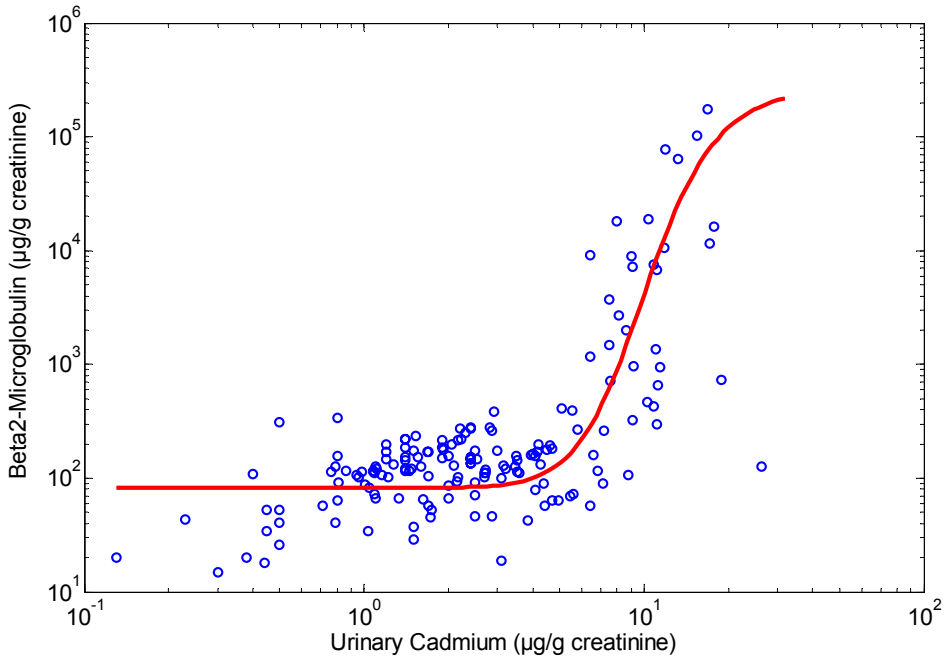

Figure 15: Hill model fitted to the complete dataset, using posterior mean estimates for the curve parameters

Figure 16 visualizes the goodness-of-fit by plotting posterior mean predictions of effect levels against the levels observed on the log-log scale. The fit looks reasonably good. The goodness-of-fit test could not reject equal distribution of predicted versus observed ($p>0.5$) with number of bins=50.

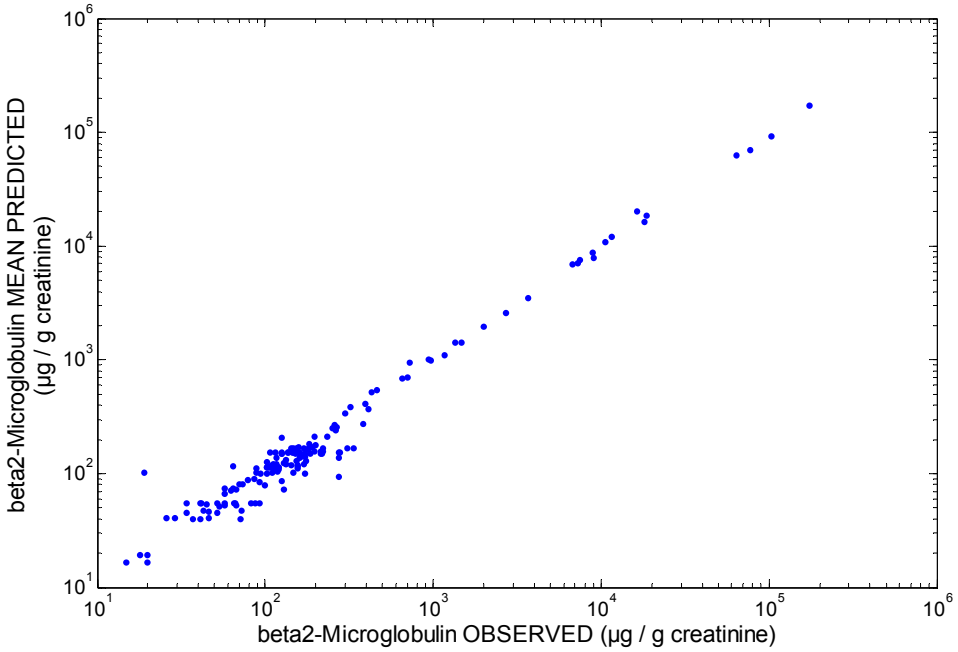

Figure 16: Predicted vs. observed scatter plot for the Hill model fitted to the complete data set

Then, we report the statistical outputs for the Piecewise Linear model fitted to the complete dataset, without any adjustment for covariates. The statistical estimates are reported in Table 5. Background and breakpoint dose parameters have been exponentiated to ease its interpretability.

Table 5: Parameter estimates for the Piecewise linear model fitted to the complete dataset, without covariates

Model parameter	Estimate (95%-confidence interval)
Background level (µg/g crea)	57 (44-75)
Breakpoint U-Cd concentration (µg/g crea)	5.54 (5.24 – 5.82)
Slope of first line	0.16 (0.12-0.20)
Slope of second line	6.17 (5.38-7.08)

The corresponding fitted curve and predicted-versus-observed plot are displayed by Figure 17 and Figure 18 below. The fit was seen reasonable and comparable to the Hill model's one. The chi-square goodness-of-fit was similarly conclusive.

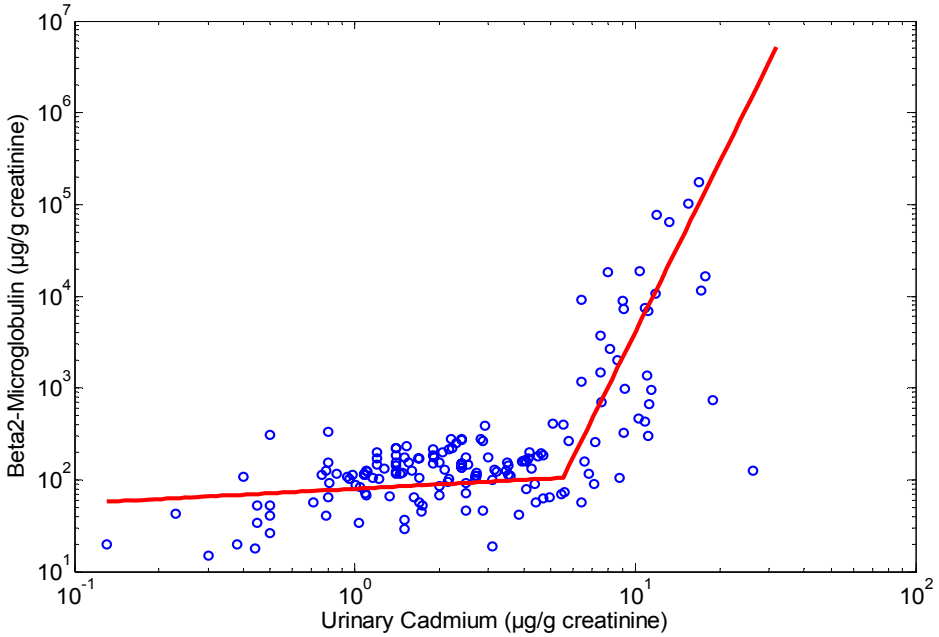

Figure 17: Piecewise linear model fitted to the complete dataset, using posterior mean estimates for the curve parameters

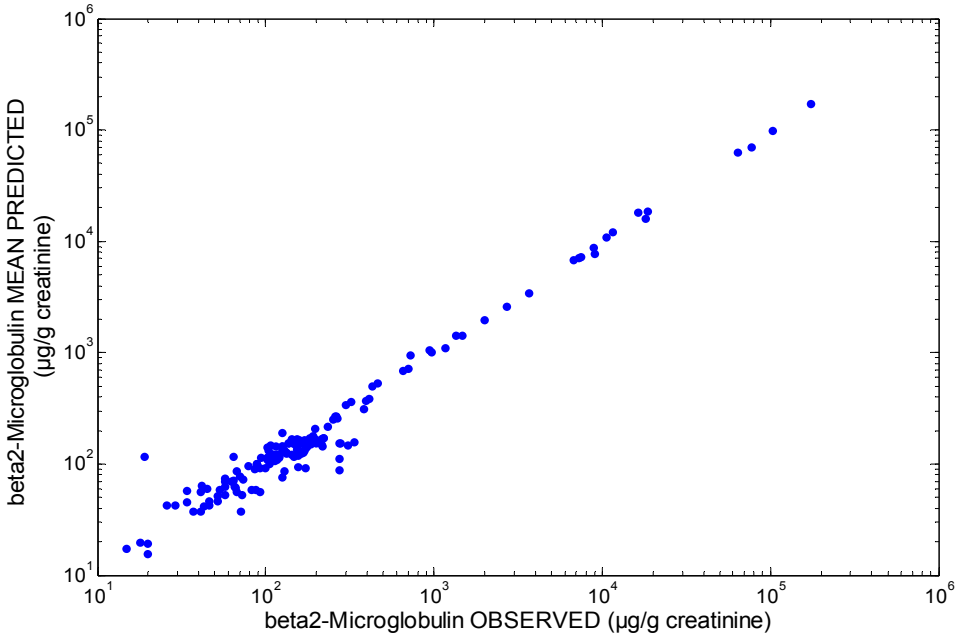

Figure 18: Predicted vs. observed scatter plot for the Piecewise linear model fitted to the complete data set

Finally, variance parameters are displayed and compared between the two models in Table 6. Population variance components are the same for both models and inter-study CV is slightly greater for the Hill model.

Table 6: Variance parameter estimates for the Hill and the Piecewise linear model fitted to the complete dataset, without covariates

Variance parameter	Estimate (95%-confidence interval)	
	Hill Model	PLM Model
CV for inter-individual variability of β_2-MG at a given dose	132% (130-133)	131% (130-133)
CV for inter-individual variability of urinary cadmium within dose groups	87% (86-88)	87% (86-88)
Inter-study CV	91% (65-134)	83% (59-120)

2.2.2. Results for all population with adjustment to covariates

An attempt to adjust for the most significant covariates was made based on the outcome from the data exploration and expert judgement. The three main covariates considered were: ethnicity (Caucasians vs. Asians), workers status (exclusively workers subgroups vs. mixed population sub-groups) and gender (males vs. females). As concluded from the data exploration, adjustment for ethnicity and gender was made by assuming a constant proportional factor between mean effect levels of respectively Asians and Caucasians; and males and females. In order to accelerate convergence speed, initial values for Markov chains were set as final states of MCMCs used for the same model without covariates.

Convergence issues were seen for the "workers effect" parameter and the background parameter. It is illustrated by Figure 19 showing non-mixing Markov chains used for the statistical estimation of parameters. The figure shows that even after more than 500,000 iterations, the three Markov chains do not mix to sample a same common distribution. This inference issue is likely due to non-identifiability problems, i.e. over-parameterization with respect to the data available. As a consequence, no adjustment for "workers status" was performed on the complete dataset.

Without the "workers" effect parameter, no convergence problems were encountered anymore.

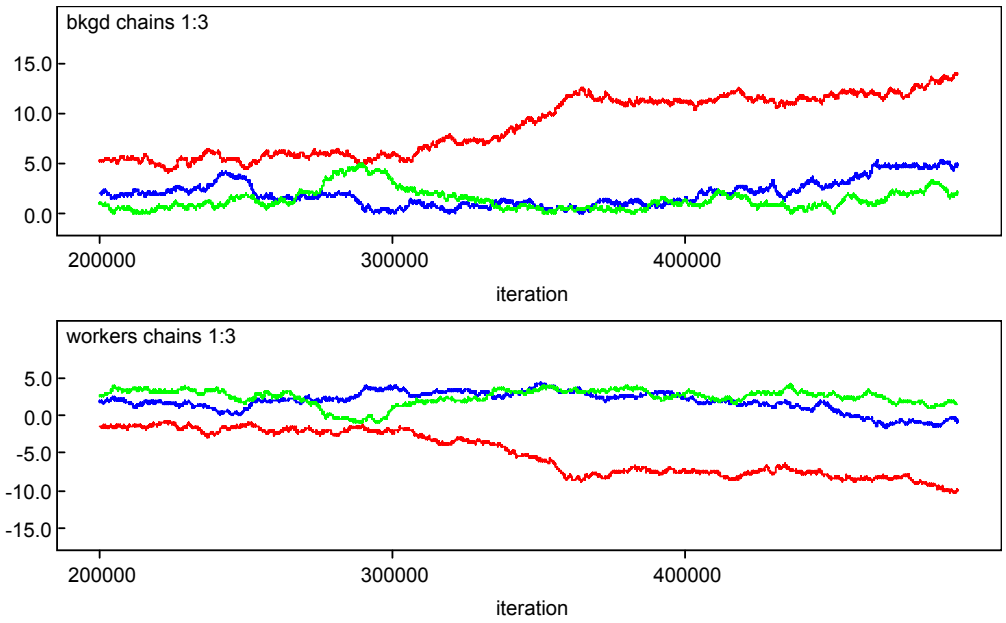

Figure 19: Three Monte Carlo Markov chains simulated with WinBUGS software for statistical inference: after 500,000 iterations, the three chains for the "background" parameter and the "workers" effect parameter are still not converging towards the same distribution due to identifiability problems.

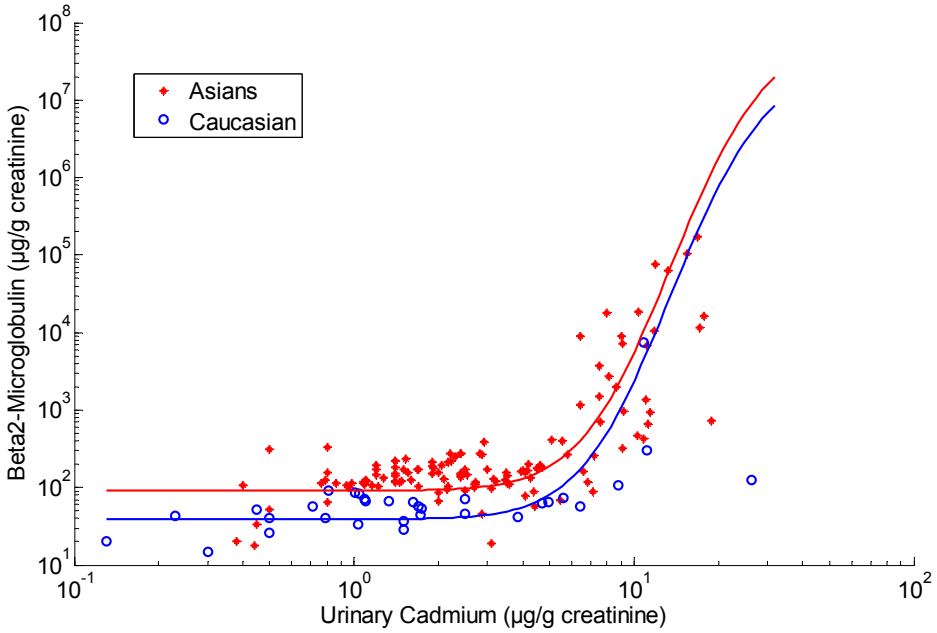

Figure 20: Hill model fitted to the Caucasians data (blue curve) versus to the Asians data (red curve), using the complete dataset

Table 7: Parameter estimates for the Hill model fitted to the complete dataset, with covariates (ethnicity and gender) included

Parameter	Estimate (95%-confidence interval)
Background level (µg/g crea)	42 (24 - 74)
Amplitude ratio (max level / min level)	>100000
ed_{50} (dose at 50% max response in (µg/g crea))	14.4 (9.8-34.4)
Shape (steepness) parameter	11.6 (9.2-14.7)
Ratio men to women	0.87 (0.83 – 0.91)
Ratio Asians to Caucasians	2.3 (1.2-4.1)

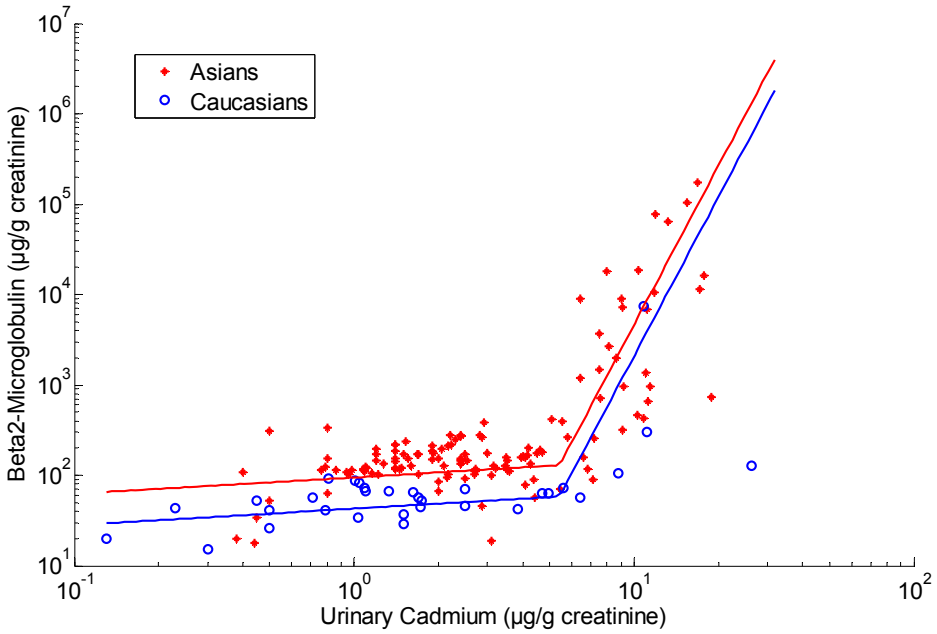

Figure 21: Piecewise linear model fitted to the Caucasians data (blue curve) versus to the Asians data (red curve), using the complete dataset

Table 8: Parameter estimates for the Piecewise linear model fitted to the complete dataset, with adjustment for ethnicity and gender

Parameter	Estimate (95%-confidence interval)
Background level (µg/g crea)	32 (18-53)
Breakpoint U-Cd concentration (µg/g crea)	5.42 (5.09-5.74)
Slope of first line	0.18 (0.14-0.22)
Slope of second line	5.82 (5.08-6.7)
Ratio men to women	0.86 (0.90-0.94)
Ratio Asians to Caucasians	2.21 (1.22-4.08)

The corresponding estimates of the variance components are reported on Table 6. Inter-individual variability components are sensibly the same as for the model fitted without covariates. However, inter-study variance is now lower than without the adjustment for covariates. This is to be expected as part of the inter-study variance could be explained by those covariates.

Table 9: Variance parameter estimates for the Hill and the Piecewise linear model fitted to the complete dataset, with covariates included

Variance parameter	Estimate (95%-confidence interval)	
	Hill Model	PLM Model
CV for inter-individual variability of β_2-MG at a given dose	131% (130-133)	132% (130-133)
CV for inter-individual variability of urinary cadmium within dose groups	87% (86-88)	87% (86-88)
Inter-study CV	77% (54-113)	72% (51-104)

2.2.3. Results for non-workers elderly (>50 years old) with adjustment for ethnicity

Finally, analyses were performed for the focus population made of sub-groups with age mean greater than 50 years old, and with excluding the purely workers sub-groups In the case of missing information on age or workers status, studies were also removed.. After this selection, 99 entries were left out of the 165 in total dataset. A first attempt was made to adjust for both gender and ethnicity like for the complete dataset, but it turned out to be unsuccessful (convergence issues of fitting algorithms). Given the time constrains and the expected weak impact of gender adjustment for risk assessment purposes (see Discussion section for details), no further attempts or refinements were performed, and only adjustment for ethnicity was made on that focus population.

The contrast between the focus population and the other sub-groups left out is illustrated by the scatter plot of Figure 22. However, it is important to note that, as illustrated by Figure 23, the dose range on which Caucasians and Asians records overlap is minimal. Therefore, the model will extrapolate the dose-effect curve for Caucasians for larger urinary cadmium levels, based on the shape of the curve seen for Asians. The assumption that Caucasians and Asians have similar (but shifted) dose—effect shapes is motivated by the previous analysis on the total population, and on the fact that age does not seem to affect the shape of the curve.

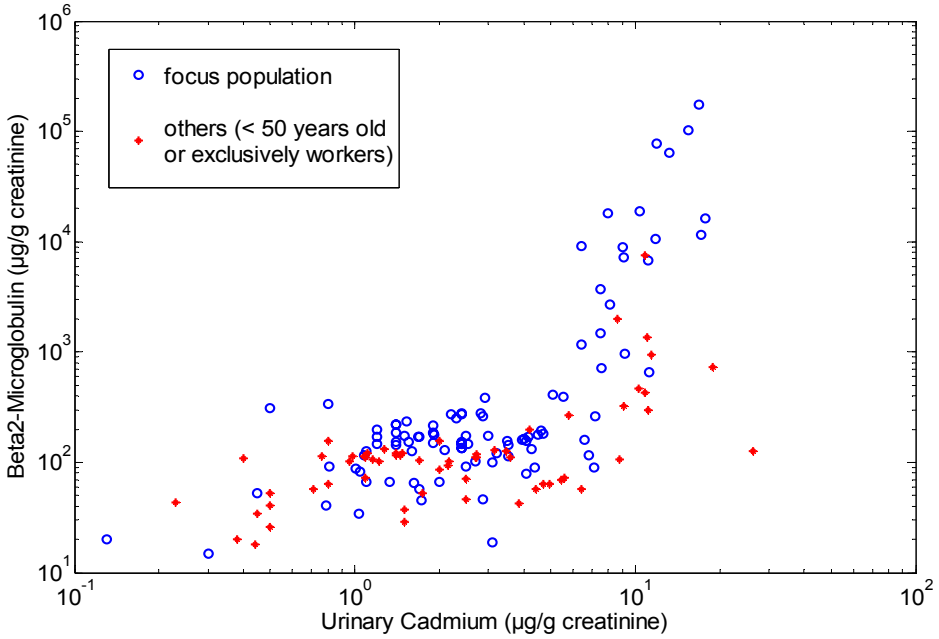

Figure 22: Scatter plot of the complete data showing the focus population made of sub-groups with age mean greater than 50 years old and with workers sub-groups excluded (blue circles) versus other sub-groups (red stars)

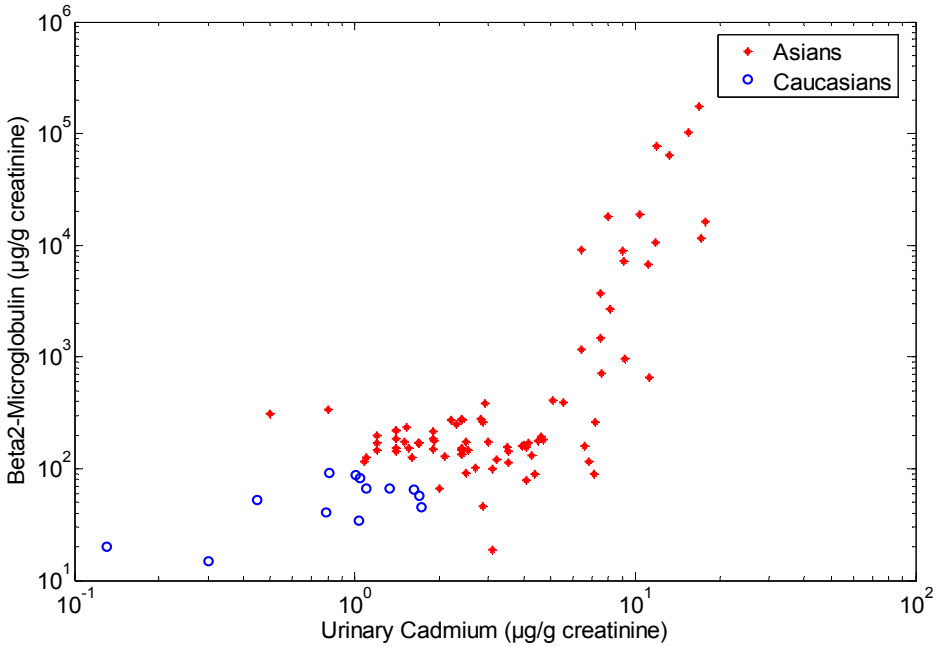

Figure 23: Scatter plot of the focus population data showing Caucasian sub-groups (blue circles) versus Asian ones (red stars)

The fitted curves for both models are simultaneously plotted on Figure 24, with the adjustment for ethnicity. The models were seen close to each other, except for the low (lower

than 0.5µg/g creatinine) and very large (greater than 10 µg/g creatinine) urinary cadmium concentrations.

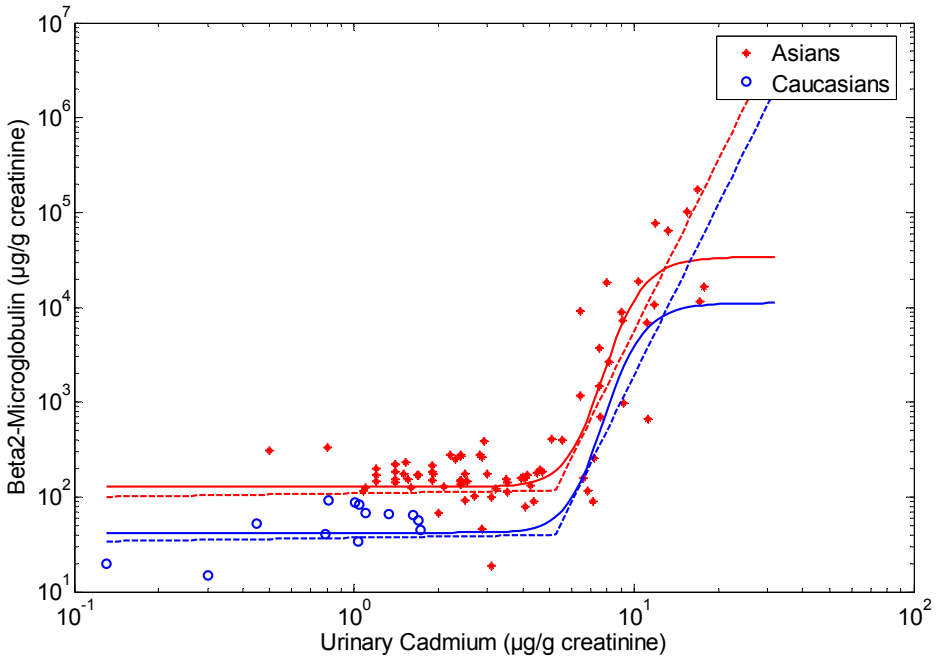

Figure 24: Hill model (plain lines) and piecewise linear model (dotted lines) fitted to the Caucasians data (blue curves) versus to the Asians data (red curves), using only the focus population data (more than 50 years old, workers data excluded)

The full set of parameter estimates is given in Table 10, Table 11 and Table 12. As expected, confidence intervals are larger than when using the complete datasets. Interestingly, parameters such as ratio Asians to Caucasians, or background level for Caucasians show similar best estimate, suggesting that age or workers status are unlikely to impact on those parameters.

Table 10: Parameter estimates for the Hill model fitted to the complete dataset, for the focus population, with adjustment for ethnicity

Parameter	Estimate (95%-confidence interval)
Background level (µg/g crea)	42 (13 - 119)
Amplitude ratio (max level / min level)	2645 (2-11402)
ed_{50} (dose at 50% max response in (µg/g crea))	7.9 (7.1-9.1)
Shape (steepness) parameter	25.3 (17.6-38.8)
Ratio Asians to Caucasians	3.1 (1.0-11.7)

Table 11: Parameter estimates for the Piecewise Linear model fitted to the complete dataset, for the focus population, with adjustment for ethnicity

Parameter	Estimate (95%-confidence interval)
Background level (µg/g crea)	34 (12-82)

Breakpoint U-Cd concentration (µg/g crea)	5.24 (4.94-5.57)
Slope of first line	0.04 (0.0013-0.13)
Slope of second line	5.99 (5.17-6.99)
Ratio Asians to Caucasians	2.9 (1.2-8.2)

Table 12: Variance parameter estimates for the Hill and the Piecewise linear model fitted to the focus population dataset, with adjustment for ethnicity

Variance parameter	Estimate (95%-confidence interval)	
	Hill Model	PLM Model
CV for inter-individual variability of β_2-MG at a given dose	192% (187-196)	192% (187-196)
CV for inter-individual variability of urinary cadmium within dose groups	100% (98-101)	100% (98-101)
Inter-study CV	127% (70-244)	95% (55-172)

2.3. Results for the benchmark doses evaluation

The BMDs and BMDLs derived for the complete dataset are displayed in Table 13 and Table 14 for both extra and additional risks of 5% and 10%, for the three cut-offs investigated.

We note that extra risk and additional risks are very close to each other in all cases. BMDs derived from the two models are comparable except in the case of simultaneously low cut-off and low excess risk, e.g. for BMD5 at cut-off=300 µg/g creatinine. In those cases, the PL model suddenly becomes instable and provides much lower BMDs. This is due to the very specific model structure which gets very constrained and biologically unrealistic for low doses. This modelling artefact is further explained in the discussion.

Note that the mean estimate for the statistical cut-off was 424 µg/g creatinine for the Hill model and about 300 µg/g creatinine for the PL model.

Table 13: BMD and BMDL estimates for the Hill and Piecewise Linear (PL) model at various cut-offs leading to extra risks of 5% and 10% in the total population present in the data

EXTRA RISK		Cut-off (µg/g crea)		
		Statistical cut-off 1	300	1000
BMD5 (BMDL5)	Hill model	4.52 (4.18)	4.09 (3.68)	5.83 (5.39)
	PL model	1.13 (0.78)	1.17 (0.69)	6.14 (5.84)
BMD10	Hill model	5.17 (4.85)	4.72 (4.32)	6.40 (5.99)

(BMDL10)	PL model	4.93 (2.68)	5 .18 (2.38)	6.51 (6.20)

Table 14: BMD and BMDL estimates for the Hill and Piecewise Linear (PL) model at various cut-offs leading to additional risks of 5% and 10% in the total population present in the data

ADDITIONAL RISK		Cut-off (µg/g crea)		
		Statistical cut-off 1	300	1000
BMD5 (BMDL5)	Hill model	4.57 (4.23)	4.18 (3.80)	5.83 (5.40)
	PL model	1.23 (0.84)	1.28 (0.77)	6.14 (5.84)
BMD10 (BMDL10)	Hill model	5.21 (4.90)	4.82 (4.45)	6.40 (5.99)
	PL model	5.36 (3.00)	5.42 (2.78)	6.51 (6.20)

Similarly BMDs were derived when adjusting for ethnicity and gender. The results displayed in Table 15 and Table 16 were calculated for an evenly mixed population of Caucasian males and females. BMDs are overall slightly larger than those for the pooled population without adjustment.

Table 15: BMD and BMDL estimates for the Hill and Piecewise Linear (PL) model at various cut-offs leading to extra risks of 5% and 10% in the Caucasian population (adjusted for gender) based on the total population

EXTRA RISK		Cut-off (µg/g crea)			
		Statistical cut-off 1	Statistical cut-off 2	300	1000
BMD5 (BMDL5)	Hill model	3.98 (3.62)	3.38 (2.96)	4.65 (3.84)	6.80 (5.95)
	PL model	0.89 (0.64)	0.69 (0.47)	5.47 (2.03)	6.68 (6.22)
BMD10 (BMDL10)	Hill model	4.67 (4.36)	4.03 (3.63)	5.32 (4.53)	7.31 (6.51)
	PL model	3.29 (1.92)	2.31 (1.29)	5.83 (5.43)	7.10 (6.62)

Table 16: BMD and BMDL estimates for the Hill and Piecewise Linear (PL) model at various cut-offs leading to additional risks of 5% and 10% in the Caucasian population (adjusted for gender) based on the total population

ADDITIONAL RISK		Cut-off (µg/g crea)			
		Statistical cut-off 1	Statistical cut-off 2	300	1000
BMD5 (BMDL5)	Hill model	4.04 (3.68)	3.50 (3.10)	4.67 (3.89)	6.80 (5.95)
	PL model	0.96 (0.69)	0.76 (0.52)	5.47 (2.12)	6.68 (6.22)
BMD10 (BMDL10)	Hill model	4.72 (4.42)	4.17 (3.80)	5.34 (4.60)	7.31 (6.52)
	PL model	3.71 (2.13)	2.68 (1.52)	5.84 (5.44)	7.10 (6.62)

Note that the new mean estimate (adjusted for gender) for Caucasians are displayed on Table 17 below.

Table 17: Estimates for the two statistically-based cut-offs for Caucasians, using the Hill and the Piecewise Linear (PL) models, based on the total population

In µg/g creatinine	**Statistical cut-off 1**	**Statistical cut-off 2**
Hill model	211	135
PL model	154	138

Finally, results for the focus population are reported below.

Table 18 and Table 19 report the various BMDs and BMDLs for the 2 models, the 4 cut-offs, and for excess risks of 5% (BMD5 and BMDL5) and 10% (BMD10 and BMDL10), using either the "extra risk" or the "additional risk" definition.

Table 18: BMD and BMDL estimates for the Hill and Piecewise Linear (PL) model at various cut-offs leading to extra risks of 5% and 10% in the Caucasian population, based on the focus population

EXTRA RISK		Cut-off (µg/g crea)			
		Statistical cut-off 1	Statistical cut-off 2	300	1000
BMD5 (BMDL5)	Hill model	5.28 (4.89)	4.46 (3.97)	5.25 (4.45)	6.33 (5.46)
	PL model	5.49 (5.10)	5.19 (0.83)	5.57 (5.09)	6.43 (5.78)
BMD10 (BMDL10)	Hill model	5.77 (5.46)	4.93 (4.49)	5.73 (4.97)	6.77 (5.94)
	PL model	5.78 (5.49)	5.40 (4.24)	5.86 (5.41)	6.92 (6.17)

Table 19: BMD and BMDL estimates for the Hill and Piecewise Linear (PL) model at various cut-offs leading to additional risks of 5% and 10% in the Caucasian population, based on the focus population

ADDITIONAL RISK		Cut-off (µg/g crea)			
		Statistical cut-off 1	Statistical cut-off 2	300	1000
BMD5 (BMDL5)	Hill model	5.31 (4.93)	4.65 (4.18)	5.30 (4.57)	6.33 (5.49)
	PL model	5.51 (5.16)	5.26 (1.18)	5.59 (5.16)	6.44 (5.78)
BMD10 (BMDL10)	Hill model	5.81 (5.50)	5.14 (4.75)	5.79 (5.12)	6.77 (5.96)
	PL model	5.80 (5.51)	5.47 (5.16)	5.89 (5.45)	6.92 (6.18)

The difference between the two models was relatively small and consistent, except for the case of BMDL5 at the statistical cut-off 2 for the Piecewise Linear model. This translates a technical artefact due to the instable estimation of confidence intervals (see Discussion for details) and the value should then be disregarded.

The statistically-based cut-offs were slightly dependent on the model used, their estimates are reported in Table 20 below. It can be noticed that the cut-off defined as the 95th percentile of the background exposure is sensibly close to 300 µg/g creatinine.

Table 20: Estimates for the two statistically-based cut-offs, using the Hill and the Piecewise Linear (PL) models, based on the focus population

In µg/g creatinine	Statistical cut-off 1	Statistical cut-off 2
Hill model	374	117
PL model	289	115

3. Discussion

3.1. Limitations of the analyses

Like every model-based analysis, the scope and limitations of the method needs to be summarized for proper use of the results.

A first limitation comes from the data, which are obviously not selected from any controlled nor random process. Moreover, publication bias is likely to occur especially in the Japanese studies, or from environmental studies focusing on polluted areas. More specifically, gender which showed statistically significant effect on effect was not adjusted for in the focus population. However, this should not have much impact in terms of risk assessment, because:

- Effect of gender was seen to be relatively small
- Women are mosty represented in the focus population, this corresponds to both the most vulnerable population sub-group for risk assessment, and to the population group on which the TK modelling has been previously performed (see Amzal *et al.*, 2009)

It is also important to recall two main potential limitations:

- The modelling assumptions and the sensitivity of results with respect to them
- The fact that inter-individual variability within all dose groups has been evaluated but should also be accounted for in the final BMD determination. There is a need for an adjustment factor with that respect

These two issues are discussed in the two following sub-sections.

3.2. Sensitivity to the model assumptions

First of all, one can investigate further the instability issue showed for BMDs using the PL model in the case of simultaneously low excess risk (below 5%) and low cut-offs (below 300-500 µg/g creatinine). For a better illustration of the issue, one could display the relationship between BMDs and the defined cut-offs (see Figure 25).

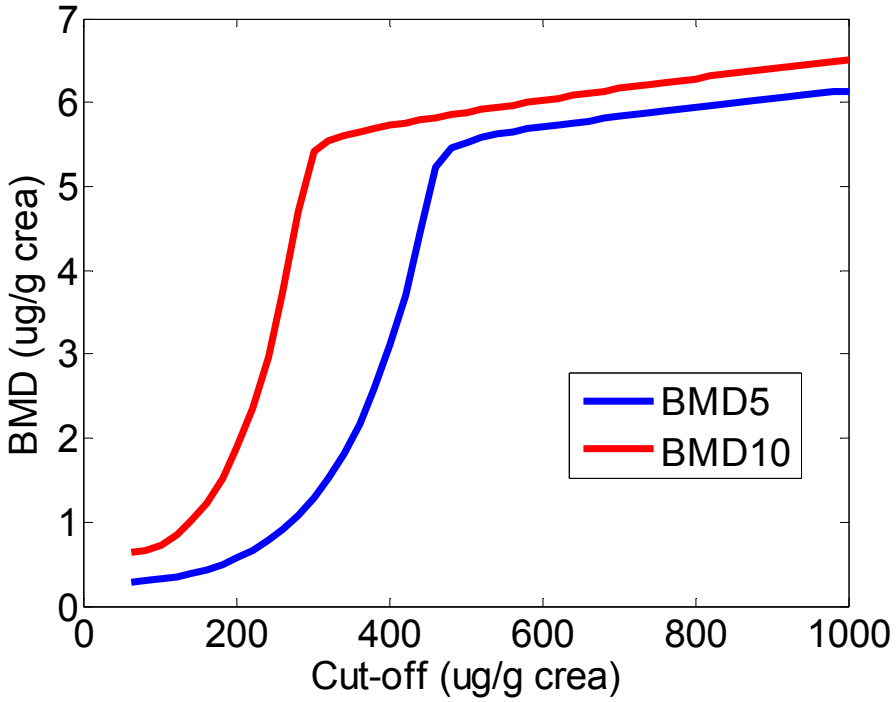

Figure 25: BMD5 and BMD10 as a function of cut-off for Piecewise Linear model, showing instability for low cut-offs

The plots show that for cut-offs below 500 µg/g creatinine for BMD5 and below 300 µg/g creatinine for BMD10, a very steep slope is observed on the curve, leading to very instable estimates around this area. This modelling artefact is engendered by the combination of two things. First, the existence of a breakpoint in the dose-effect curve is "propagated" into the BMD formula, which is then also described by a "broken" curve (discontinuity of the first derivative at the breakpoint). Second and most important, the fact that the dose-effect curve is assumed to be linear in the log scale, even for very small urinary cadmium levels, makes the BMDs very sensitive even at low doses. In contrast the Hill model curve is much flatter in the low urinary cadmium part (e.g. below 1 µg/g creatinine), leading then to more robust BMD estimates.

Note that preliminary attempts to use Bayesian model averaging techniques (see e.g. Wheeler *et al.*, 2007) showed a higher weight for the Hill model than for the PL model. However, this criterion can not be used for model selection our case, as it is based on the overall model fit

and does not account e.g. the use of the model for BMD assessment, the biological relevance of each model, or the possible instabilities that may occur in estimation. Therefore, a refined and more specific analysis should be carried out to determine the best model(s) to use according to a sound statistical and biological criterion which suits to the BMD evaluation purposes. This was outside the scope of this work..

On the same line, one could prefer analyzing only the Caucasian popluation data from the focus population data, hence restricting to the linear part of Figure 23 (Caucasian sub-groups with urinary cadmium below 2 μg/g creatinine). This would then restrict the analysis to only 13 entries from only three studies. The interest of a large scale screen analysis would then be lost, and the "key study" approach highly preferable in that case. Note that an attempt to fit a linear model to this much smaller data was made but successful only when the inter-study variance component was removed.

Another aspect that could be questioned is the relevance for the choice of background levels. With the chosen definition (model mean prediction at the lowest dose level), it is theoretically dependent both on the data and the model. In practice however, and since the curve of the dose-effect is very flat for low U-Cd levels, this background definition and estimation was quite robust with respect to the data observed. It was also robust with respect to the choice of model, as shown by the estimated background levels which are very similar between the two models.

3.3. Use of an adjustment factor

Traditionally, tolerable daily intake are are usually determined by dividing a surrogate for the threshold of toxicity such as the benchmark dose determined from chronic/subchronic studies using the most sensitive animal species (usually mouse, rat, rabbit or dog), by an uncertainty factor of a 100-fold corresponding allowing for interspecies differences and human variability). Historically, further refinement of these uncertainty factors have included the subdivision of both the interspecies and human component into toxicokinetics and toxicodynamics to allow the inclusion of chemical specific data and the use of chemical-specific adjustment factors. In humans such uncertainty or adjustment factors for toxicokinetics and toxicodynamics correspond each to $10^{0.5}$ (3.16) (WHO, 2005; Dorne, 2007).

In the case of cadmium, the extrapolation between animals and humans is not required so that the interspecies uncertainty factor is not relevant anymore. The toxicokinetic model has also covered the human variability aspects so that the use of an uncertainty factor for toxicokinetics is not relevant either. However the meta-analysis of biomarkers for renal effects in humans has enabled to model the data and derive a "group-based" benchmark dose using the hill model. In order to protect a large proportion of the European population, the variability and uncertainty sources should all be accounted for, especially those caused by the fact that the benchmark dose modelling has been performed on aggregated data by dose groups.

There are three main sources of variability described by the models:

- The inter-study variability
- The population variability of the effect given a urinary cadmium level
- The population variability of urinary cadmium within each dose group

In addition there are two main sources of uncertainty in this analysis:

- The uncertainty around the statistical estimates, given a model
- The uncertainty on the model (and the underlying assumptions)

The inter-study variability has been removed using the random effect model. The population variability of effect is addressed by the BMD approach itself as it uses this variability to determine the dose leading to a given excess risk. The uncertainty around statistical estimates is addressed by considering BMDL instead of BMD. Finally, the uncertainty on the modelling assumptions is, at least partially, addressed by comparing two models and performing some sensitivity analyses.

The remaining source of uncertainty in this analysis is due to the fact that group means with associated ranges of U-Cd levels were used, and not data points from individual subjects. Therefore the estimated BMDs are likely to be greater than when calculated with individual data. This problem can be overcome by applying an adjustment factor based on the estimated coefficient of variation of inter-individual variability in U-Cd within all recorded study populations. This factor can be considered to be a chemical-specific adjustment factor (CSAF) as recommended by the WHO and can be defined as the ratio of a 95th population percentile to the median BMD (WHO, 2005):

$$CSAF = \text{95th Percentile (BMD)/Median (BMD)}$$

Since concentrations have been assumed to be lognormal, this CSAF can be computed using the standard formula for lognormal percentiles, namely:

$$CS\,AF = \exp\left(1.64\sqrt{\ln\left(1+CV^2\right)}\right)$$

Using the CV estimate for the focus population (CV= 100%), the proposed CSAF to adjust BMD values based on the focus population is CSAF=3.9. It is important to underline that this value is specific to this meta-analysis. In particular, it may not apply if the focus would be given e.g. on the sub-population with only low urinary cadmium levels. Such an approach is recommended by the WHO (WHO, 2005).

Note that this factor is valid only for median BMDs (not for BMDLs) but differences between BMDLs and BMDs are small enough to expect that the same factor could also be applied for BMDLs as a good first-order approximation. Moreover, it is valid under the conservative assumption that the observed variability of β_2-MG between individuals is mainly due to true response differences between individuals and not to differences in actual exposure. In the case of β_2-MG (highly variable) at such a large epidemiological scale, the intra-group U-Cd variability can be expected to have a minor contribution to the observed β_2-MG variability. This is confirmed by the fact that no obvious or clear correlation is observed between variances of U-Cd and β_2-MG.

CONCLUSIONS

This work shows how an extensive systematic review and meta-analysis of dose-effect data can serve as a tool for benchmark dose evaluation for risk assessment. It allows the quantification of the main sources of variability, and a robust BMD calculation. In the case of cadmium, it shows that an internal dose of 1ug/g creatinine of urinary cadmium would preserve at least 95% of European population under cut-offs from the range 100-300 ug/g creatinine of β2-MG in urine. As a comparison, BMDs based on β2-microglobulin biomarker range from 0.5 µg/g creatinine up to 10-12 µg/g creatinine (Ikeda *et al.*, 2003, Gamo *et al.*, 2006; ASTDR, 2008), but with various cut-offs, various definitions of BMDs and various population (mostly Japanese).

Such a global approach should be compared to a key-study approach selected to be the most representative of the European population considered to be the most vulnerable, in order to verify the proposed BMD values. Meta-analysis and "key-study" approach are two complementary steps for robust BMD evaluation in risk assessment set-up.

Finally, further work is needed to analyze all the other biomarkers collected is a harmonized set-up, and to refine the statistical methodology to handle group-based data for benchmark dose evaluation.

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ANNEX 1: OUTPUTS FROM ANCOVA TESTS FOR EFFECTS

Table 21: weighted ANCOVA with study random effect (proc MIXED) with all factors/regressors, without interactions (convergence criteria met but final Hessian not positive definite)

effect	p-value
Urinary cadmium	<0.0001
Gender	0.0217
Ethnicity	0.0093
Age	<0.0001
Workers status	0.4861
Co-exposure	0.7634
Type of study	0.4507

Table 22: weighted ANCOVA without study random effect (proc GLM) with all factors/regressors, without interactions

effect	p-value
Urinary cadmium	<0.0001
Gender	0.0002
Ethnicity	<0.0001
Age	0.0009
Workers status	0.2695
Co-exposure	0.1405
Type of study	0.3918

Table 23: weighted ANCOVA on urinary cadmium (proc GLM) without study random effect with all factors/regressors, without interactions

effect	p-value
Gender	0.055
Ethnicity	0.035
Age	<0.0001
Workers status	0.0003
Co-exposure	0.8936
Type of study	0.059

Table 24: weighted ANCOVA with study random effect (proc MIXED) with all significant and/or collinear factors/regressors, with their interactions with urinary cadmium (convergence criteria met but final Hessian not positive definite)

effect	p-value (type III tests)
Urinary cadmium	<0.0001
Gender	0.5132
Ethnicity	0.021
Age	0.0352
Workers status	0.8471
Gender * Urinary Cadmium	0.0290
Ethnicity * Urinary Cadmium	0.9202
Age * Urinary Cadmium	<0.0001
Workers status * Urinary Cadmium	0.8970

Table 25: weighted ANCOVA without study random effect (proc GLM) with all significant and/or collinear factors/regressors, with their interactions with urinary cadmium

effect	p-value (type III tests)
Urinary cadmium	<0.0001
Gender	0.0988
Ethnicity	<0.0001
Age	0.0143
Workers status	0.4727
Gender * Urinary Cadmium	0.0041
Ethnicity * Urinary Cadmium	0.1860
Age * Urinary Cadmium	<0.0001
Workers status * Urinary Cadmium	0.2376

ANNEX 2: LIST OF VARIABLES EXTRACTED FROM THE LITERATURE

Variable label	Defintion
study_type	cohort=0, crosssectional=1
Gender	Males=0, females=1, both/unknown=0.5
Sample size	Number of subjects of the study
Exposure_status	unexposed=0, exposed=1, both/unknown=0.5
Ethnicity	Asian=1, Caucasian=2, Hispanic=3
Exposure at Work	Workers=1, non-workers=0
Cd-Exposure_Scenario	Short description of Scenario
Source_of_exposure	Environmental / Food / Work
Smoking_Status	Smoker=1, non-smoker=0
Co-exposure_	yes=1, no=0
Kind of Co-exposure	Pb, As, Zn, Hg etc.
Disease or Menopause	Kind of Disease or pre/post-Menopause
Age	Years of age
Age SD	Standard-deviation
AM/GM	Arittmetic or geometric mean
Age-sample_size	Number of subjects for calculation of mean values for age
Age-range	Minimum –Maximum in years
Age-Categorisation	>=50(=1)_vs_<50(=0)
BW	Bodyweight in kg
BW SD	Standard-deviation of Bodyweight
Range_BW	Minimum – Maximum in kg
BMI	Bodymassindex (kg/m2)
BMI SD	Stadard-deviation of BMI
BW Range	Minimum – Maximum in kg/m2
Analytical Techniques	See separate table below
Sensitivity	Sensitivity of the alalytical method or Limit of Detection
Sample_type	Urine, Blood, Serum, Bone
Analyzed_aliquot_urine	Volume analyzed in mL
Storage	Temperature in °C
Age_Adjustment	Yes or No
Lag_time	Time difference between max exposure and measurement in years
U-Cd_non_corrected	Cadium-content per liter urine
SD_U-Cd_non_corrected	GSD of U-Cd non corrected
Sample_Size_U-Cd_non_corrected	Number of samples of U-Cd non corr.
Range_U-Cd_non_corrected	Minimum – Maximum levels
U-Cd_unit non corr	µg / L urine
U-Cd(cr)	Cd-content per gramm creatinine
U-Cd(cr) SD	GSD of U-Cd(cr)
U-Cd(cr)_Sample_size	Number of samples of U-Cd(cr)
Range_U-Cd(cr)	Minimum – Maximum levels
Variance_U-Cd(cr)	=(LN(U-Cd(cr) SD))^2
Unit_U-Cd(cr)	µg/g cr
Cd-B_non_corrected	Cadmium-content per liter blood
SD_Cd-B_non_corrected	GSD of Cd-B non corrected
Sample_Size_CdB	Number of samples of Cd-B non corr

range_Cd-B_non_corrected	Minimum – Maximum levels
unit_Cd-B	µg/L blood
Cd-S	Cd-content per liter blood-serum
Cd-S SD	GSD of Cd-S
Cd-S Range	Minimum – Maximum levels
a1-MG(cr)	Concentration of a1-MG(=Protein HC) in urine per gramm creatinine
SD_a1-MG(cr)	GSD of a1-MG(cr)
Sample_Size_a1-MG(cr)	Number of samples of a1-MG(cr)
Range_a1-MG(cr)	Minimum – Maximum levels
Unit_a1-MG	mg/g creatinine
b2-MG	Concentration of ß2-MG per liter urine
SD_b2-MG	GSD of ß2-MG
Sample-Sizye_b2-MG	Number of samples of b2-MG
Range_b2-MG	Minimum – Maximum levels
Unit_b2-Mg	µg/L urine
b2-MG(cr)	Concentration of ß2-MG per gramm creatinine
SD_b2-MG(cr)	GSD of ß2-MG
Sample-Size_b2-MG(cr)	Number of Samples of ß2-MG(cr)
Range_b2-MG(cr)	Minimum – Maximum levels
Variance_b2-MG(cr)	=(LN(SD b2-MG(cr)))^2
Unit_b2-Mg(cr)	µg/g creatinie
NAG	Concentration of Biomarker NAG in urine
SD_NAG	GSD of NAG
Sample_Size_NAG	Number of samples of NAG
Range_NAG	Minimum – Maximum levels
Unit_NAG	U/l urine (=nameless unit per liter urine)
NAG(cr)	Concentration of Biomarker NAG per gramm creatinine
SD_NAG(cr)	GSD of NAG(cr)
Sample_Size_NAG(cr)	Number of Samples of NAG(cr)
Range_NAG(cr)	Minimum – Maximum levels
Unit_NAG(cr)	U/g cr (=nameless unit per gramm creatinine)
NAGa(cr)	Concentration of NAG-a per gramm creatinine
SD_ NAGa(cr)	GSD of NAG-a(cr)
NAGa(cr) range	Minimum – Maximum levels
Unit_NAGa	U/g cr (=nameless unit per gramm creatinine)
NAGb(cr)	Concentration of NAG-b per gramm creatinine
SD NAGb(cr)	GSD of NAG-b(cr)
Range NAGb(cr)	Minimum – Maximum levels
Unit_NAGb	U/g cr (=nameless unit per gramm creatinine)
RBP(cr)	Concentration of Biomarker RBP per gramm creatinine
SD_RBP(cr)	GSD of RBP(cr)
sample size RBP	Number of samples of RBP (cr)
Range_RBP(cr)	Minimum-Maximum levels
Unit_RBP(cr)	µg/g creatinine
Urinary_creatinine	Concentration of creatinine in urine
SD_U-cr	Standard deviation of U-cr
range_U-cr	Minimum – Maximum levels
Unit_cr	g (gramm)
GFR	Globulal filtration rate
GFR SD	GSD of GFR
GFR_Sample-Size_n	Number of samples of GFR

GFR range	Minimum – Maximum levels
GFR_unit	ml / min / m2
Proteinuria	Concentration of Proteinuria
Proteinuria SD	GSD of Proteinuria
Proteinuria_Sample_size_n	Number of samples for Proteinuria
Proteinuria range	Minimum – Maximum levels
unit_proteinuria	mg/g creatinine
BMD	Bone mineral density
SD_BMD	GSD of BMD
Sample_Size_BMD_n	Number of samples for BMD
Range_BMD	Minimum – Maximum levels
unit_BMD	g/cm2
U-Ca	Urinary calcium_concentration
S.D._U-Ca	GSD of U-Ca
Sample-Size_U-Ca	Number of samples for U-Ca
Unit_U-Ca	mg/L
Serum Calcium	Calcium concentration in Serum
SD_S-Ca	GSD of S-Ca
Range_S-Ca	Minimum – Maximum levels
unit_S-Ca	mg/L
bALP	Bone alkaline phosphatase(=BAP)
SD_bALP	GSD of bALP
Sample_Size_bALP	Number of samples for bALP
Range_bALP	Minimum – Maximum levels
unit_bALP	µg/L or ng/mL
T ALP	total serum alkaline phosphatase
SD_T ALP	GSD of T ALP
Sample_Size_ T ALP	Number of samples for T ALP
Range_total serum ALP=T ALP	Minimum – Maximum levels
unit_bALP	Nameless Unit IU
PTH	Parathyroid hormone
PTH SD	GSD PTH
Sample_Size_PTH	Number of samples for PTH
PTH Range	Minimum – Maximum levels
unit_PTH	ng/L
U-DPD	Deoxypyridinoline concentrations in urine reflecting bone turnover
SD_U-DPD	GSD of U-DPD
Sample_Size_U-DPD	Number of samples of U-DPD
range_U-DPD	Minimum – Maximum levels
unit_U-DPD	g/cm2
other_biomarkers_analysed	Listing of other biomarkers analysed
Urinary levels co-exposed	e.g.: Levels of Hg,Pb,As,Zn in urine
Urinary levels co-exposed SD	Standard deviation for values above
Blood levels co-exposed	e.g.: Levels of Hg,Pb,As,Zn in blood
Blood levels co-exposed SD	Standard deviation for values above
Serum levels co-exposed	e.g.: Levels of Hg,Pb,As,Zn in serum
Serum levels co-exposed SD	Standard deviation for values above
Bone levels co-exposed	e.g.: Levels of Hg,Pb,As,Zn in bone
Bone levels co-exposed SD	Standard deviation for values above

ANNEX 3: LIST OF THE PUBLICATIONS USED FOR THE BENCHMARK DOSE MODELLING OF B2-MICROGLOBULIN

REFERENCE	Code
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ANNEX 4: CONSOLIDATED DATABASE USE FOR B2-MICROGLOBULIN

				U-Cd			b2-MG					
Study code	gender	ethnicity	age	GM	GSD	N	GM	GSD	N	Study type	workers	coexp
7	F	Asians	70	17.2	1.6	53	11500	3	53	1	no	no
9	M	Caucasians	37.4	0.23	1.97	21	43	4.4	21	1	yes	yes
9	M	Caucasians	33.6	1.09	1.47	23	72	1.7	23	1	yes	yes
9	M	Caucasians	38.1	3.84	1.32	8	42	3.2	8	1	yes	yes
9	M	Caucasians	39.9	6.4	1.16	8	57	3.5	8	1	yes	yes
9	M	Caucasians	34.7	26.3	1.75	9	126	2.8	9	1	yes	yes
11	M/F	Caucasians	55	0.45	1.02	417	52	1.1	342	1	no	no
11	M/F	Caucasians	55	0.79	1.05	129	41	1.1	114	1	no	no
11	M/F	Caucasians	55	1.03	1.1	59	34	1.2	53	1	no	no
11	M/F	Caucasians	55	1.04	1.07	117	83	1.1	106	1	no	yes
11	M/F	Caucasians	55	1.63	1.25	22	65	1.2	25	1	no	yes
11	M/F	Caucasians	55	1.7	1.81	9	57	1.6	10	1	no	yes
11	M/F	Caucasians	55	1.33	1.11	85	66	1.1	86	1	no	yes
11	M/F	Caucasians	55	0.81	1.29	15	92	1.2	17	1	no	yes
11	M/F	Caucasians	55	1.01	1.66	7	87	1.4	8	1	no	yes
14	F	Asians	47.5	1.22	1.89	927	103	1.7	927	1	no	no
14	F	Asians	47.5	1.4	2	1042	121	1.8	1042	1	no	no
14	F	Asians	47.5	3.16	1.87	1028	129	1.9	1028	1	no	no
14	F	Asians	47.5	1.4	1.9	994	116	1.8	994	1	no	no
14	F	Asians	47.5	0.98	1.88	1323	114	1.7	1323	1	no	no
14	F	Asians	47.5	1.48	1.96	1213	120	1.9	1213	1	no	no
14	F	Asians	47.5	1.11	1.98	1131	121	1.8	1131	1	no	no
14	F	Asians	47.5	0.96	1.93	1104	102	2	1104	1	no	no
14	F	Asians	47.5	1.16	1.8	998	106	1.7	998	1	no	no
14	F	Asians	47.5	0.76	1.95	993	114	1.7	993	1	no	no
17	F	Asians	55.5	2.87	1.72	908	46	3.7	908	1	no	no
18	M/F	Asians	.	0.86	6.16	123	115	1.9	123	1	no	yes
18	M/F	Asians	.	2.16	3.88	122	214	4.1	122	1	no	yes
20	F	Asians	45.8	2.14	1.59	39	94	2	39	1	no	no
20	F	Asians	53.3	2.53	1.86	58	147	2.1	58	1	no	no
20	F	Asians	65.4	3.1	1.65	71	19	2.6	71	1	no	no
20	F	Asians	45.8	2.72	1.78	34	119	2	34	1	no	no
20	F	Asians	54.3	3.2	1.65	75	121	2.3	75	1	no	no
20	F	Asians	65.5	4.15	1.61	59	169	2.6	59	1	no	no
20	F	Asians	45.4	2.16	1.7	57	103	1.8	57	1	no	no
20	F	Asians	53.3	3.54	1.53	52	114	2	52	1	no	no
20	F	Asians	64.4	3.95	1.59	70	160	2	70	1	no	no
20	F	Asians	45.1	2.69	1.82	80	111	1.9	80	1	no	no
20	F	Asians	54.1	3.53	1.54	78	144	2.1	78	1	no	no
20	F	Asians	63.2	4.27	1.65	29	133	2	29	1	no	no
20	F	Asians	45.3	3.59	1.75	88	111	1.9	88	1	no	no
20	F	Asians	54.6	4.01	1.68	175	164	2.2	175	1	no	no

20	F	Asians	64.5	4.5	1.72	221	178	2.3	221	1	no	no
22	M	Caucasians	50.4	0.13	5.23	208	20	4.9	208	1	no	yes
22	F	Caucasians	51.2	0.3	4.09	385	15	5.4	385	1	no	yes
23	F	Asians	53.8	2.31	2.3	378	252	1.9	378	1	no	no
27	M/F	Asians	35	5.44	1.97	19	70	1.9	18	0	no	no
27	M/F	Asians	45	11.37	1.68	29	949	11.3	28	0	no	no
27	M/F	Asians	35	4.41	2.05	19	57	2.2	18	0	no	no
27	M/F	Asians	45	10.98	1.79	29	1354	10.9	28	0	no	no
27	M/F	Asians	35	3.47	2.25	19	126	1.6	18	0	no	no
27	M/F	Asians	45	8.67	1.68	29	2000	8.8	28	0	no	no
32	M	Asians	48.8	10.3	2.3	33	466	2.5	33	1	yes	no
32	M	Asians	48.6	9.12	1.85	66	321	2.5	66	1	no	no
32	M	Asians	50.3	1.91	2.17	66	176	2.9	66	1	no	no
32	F	Asians	48.8	18.97	2.08	11	729	3.6	11	1	yes	no
32	F	Asians	48.6	10.84	1.99	22	431	6	22	1	no	no
32	F	Asians	50.3	1.55	4.15	22	154	3.3	22	1	no	no
33	M	Asians	34.1	2.01	1.81	30	86	2.9	30	1	no	yes
33	M	Asians	38.8	5.77	1.68	35	266	3.2	35	1	yes	yes
35	M/F	Asians	75.8	9.04	1.61	18	8910	5.6	18	1	no	no
35	M/F	Asians	78.2	10.4	1.62	22	18700	4.1	22	1	no	no
35	M/F	Asians	76.5	2.86	1.64	18	264	2.6	18	1	no	no
35	M/F	Asians	76.8	5.52	1.71	22	396	2.6	22	1	no	no
36	M/F	Asians	62.5	1.53	2.88	102	234	1.7	102	1	no	yes
36	M/F	Asians	61.7	1.2	2.72	149	197	1.9	149	1	no	yes
43	M	Asians	55	4.1	1.8	598	79	4.4	598	1	no	no
43	M	Asians	65	4.6	1.8	485	192	5.1	491	1	no	no
43	M	Asians	75	5.1	1.9	262	413	6.3	265	1	no	no
43	M	Asians	85	6.4	1.8	64	1178	8.1	65	1	no	no
43	M	Asians	55	2	1.7	60	67	2.7	62	1	no	no
43	M	Asians	65	2.1	1.6	37	129	2.3	38	1	no	no
43	M	Asians	75	2.2	1.8	26	275	7.3	26	1	no	no
43	M	Asians	85	2.8	1.4	7	276	5.6	7	1	no	no
43	F	Asians	55	6.8	1.8	696	116	3.8	709	1	no	no
43	F	Asians	65	7.2	1.8	581	259	5	586	1	no	no
43	F	Asians	75	7.6	1.8	334	708	6.5	340	1	no	no
43	F	Asians	85	8.1	2.1	105	2698	8.8	110	1	no	no
43	F	Asians	55	3.1	1.8	64	99	2.4	64	1	no	no
43	F	Asians	65	3	1.5	49	174	3.2	49	1	no	no
43	F	Asians	75	3.5	1.7	33	156	2.8	34	1	no	no
43	F	Asians	85	2.9	2	13	386	4.4	14	1	no	no
46	M	Asians	57.3	2.69	2.62	44	102	2.2	44	1	no	no
46	F	Asians	62.8	4.68	2.53	54	183	2.6	54	1	no	no
46	M	Asians	62.4	1.08	2.15	21	116	2.2	21	1	no	no
46	F	Asians	64.5	1.69	2.17	29	171	2.7	29	1	no	no
47	F	Asians	65	17.77	1.66	3	16391	8.6	3	1	no	no
47	F	Asians	75	15.41	1.61	4	103098	1.2	4	1	no	no
47	F	Asians	85	11.92	1.23	3	76836	1.3	3	1	no	no
48	F	Asians	70.2	16.92	3.07	50	173628	1.6	50	1	no	no

49	M/F	Asians	67	13.23	1.63	30	64079	1.7	30	1	no	no
50	M	Asians	.	0.79	.	68	125	.	68	1	no	yes
50	F	Asians	.	0.94	.	55	107	.	55	1	no	yes
50	M	Asians	.	2.22	.	72	221	.	72	1	no	yes
50	F	Asians	.	2.06	.	50	197	.	50	1	no	yes
53	M	Caucasians	48	1.74	1.98	11	53	1.7	12	1	yes	no
53	M	Caucasians	50	5.59	2.23	8	73	1.5	8	1	yes	no
53	M	Caucasians	55	8.8	.	3	106	2.1	4	1	yes	no
54	M	Caucasians	38.6	0.71	2.34	47	57	3.2	47	1	no	no
54	M	Caucasians	42.5	4.97	1.64	36	64	1.6	36	1	yes	yes
54	M	Caucasians	53.5	1.1	1.67	35	67	2.8	35	1	no	no
54	M	Caucasians	54	4.69	1.47	31	63	2	31	1	yes	yes
54	M	Caucasians	55.5	11.1	1.47	12	298	15.7	12	1	yes	yes
54	M	Caucasians	62.8	1.72	1.58	10	45	2.5	10	1	no	no
54	M	Caucasians	62.6	10.87	1.43	8	7447	5.9	8	1	yes	yes
57	M	Asians	36.7	0.38	1.95	53	20	4	53	1	no	yes
57	F	Asians	38.1	0.44	1.91	65	18	2.7	65	1	no	yes
58	M	Asians	35.8	0.5	1.86	27	52	3	27	1	no	yes
58	M	Asians	38.5	0.45	2.32	16	34	4.3	16	1	no	yes
66	M	Asians	55	1.6	2.7	256	126	2.9	256	1	no	no
66	M	Asians	65	1.9	2.4	476	151	2.9	476	1	no	no
66	M	Asians	75	1.9	2.3	319	186	3.4	319	1	no	no
66	M	Asians	89.5	1.9	2.6	54	214	4.4	54	1	no	no
66	M	Asians	55	1.1	3.5	98	125	2.8	98	1	no	no
66	M	Asians	65	1.5	2.7	167	173	2.9	167	1	no	no
66	M	Asians	75	1.7	2.4	138	172	3.4	138	1	no	no
66	M	Asians	89.5	1.4	2.6	26	218	3.8	26	1	no	no
66	F	Asians	55	2.4	2.8	446	136	2.4	446	1	no	no
66	F	Asians	65	2.4	2.6	705	147	2.6	705	1	no	no
66	F	Asians	75	2.4	2.6	456	152	2.8	456	1	no	no
66	F	Asians	89.5	2.4	4.9	47	274	5.1	47	1	no	no
66	F	Asians	55	2.4	2.8	431	136	2.4	431	1	no	no
66	F	Asians	65	2.4	2.6	692	146	2.6	692	1	no	no
66	F	Asians	75	2.4	2.6	448	151	2.9	448	1	no	no
66	F	Asians	89.5	2.4	5	46	278	5.1	46	1	no	no
71	M/F	Asians	56	9.22	2.07	128	970	5.4	128	1	no	no
71	M/F	Asians	59	6.43	1.51	12	9058	3.1	12	1	no	no
71	M/F	Asians	59	11.07	1.76	12	6813	3.7	12	1	no	no
71	M/F	Asians	58	7.16	1.6	12	89	3.6	12	1	no	no
71	M/F	Asians	60	7.49	1.83	60	1486	4.1	60	1	no	no
72	M	Asians	62.2	6.6	1.93	109	160	11.2	97	1	no	no
72	F	Asians	63.6	11.2	1.71	153	658	10	153	1	no	no
73	M/F	Caucasians	18	0.5	1.5	128	41	3.2	79	1	no	yes
73	M/F	Caucasians	18	1.5	1.5	128	37	2.5	26	1	no	yes
73	M/F	Caucasians	18	2.5	1.5	128	71	2.5	20	1	no	yes
73	M/F	Caucasians	46	0.5	1.5	170	26	3.2	24	1	no	yes
73	M/F	Caucasians	46	1.5	1.5	170	29	3.1	43	1	no	yes
73	M/F	Caucasians	46	2.5	1.5	170	46	3.3	98	1	no	yes

75	F	Asians	44.3	1.27	1.87	34	133	1.6	34	1	no	no
75	F	Asians	44.3	1.09	2.14	34	112	1.7	34	1	no	no
76	F	Asians	44.6	1.08	1.98	1190	113	1.8	1190	1	no	no
77	M	Asians	73.8	9.1	1.7	28	7245	6.8	28	1	no	no
77	F	Asians	73.2	11.8	1.6	44	10657	5.7	44	1	no	no
77	M	Asians	72.8	2.5	1.6	23	173	3.5	23	1	no	no
77	F	Asians	68.8	4.1	1.5	47	158	3	47	1	no	no
78	M	Asians	65	7.5	1.8	23	3692	4.5	23	1	no	no
78	F	Asians	65	8	1.8	20	18215	4	20	1	no	no
78	M	Asians	65	2.5	1.3	18	92	5.7	21	1	no	no
78	F	Asians	65	4.4	1.4	37	89	4.1	44	1	no	no
80	M	Asians	50	2	1.47	145	155	1.5	145	1	no	no
80	M	Asians	49	0.8	1.69	121	64	1.6	121	1	no	no
80	M	Asians	49.5	0.4	1.67	144	108	1.5	144	1	no	no
80	F	Asians	49	4.2	1.38	147	200	1.4	147	1	no	no
80	F	Asians	49	1.7	1.48	128	104	1.4	128	1	no	no
80	F	Asians	49	0.8	1.44	143	155	1.4	143	1	no	no
87	F	Asians	48.6	2.71	1.33	5	110	1.9	5	1	no	no
87	F	Asians	40	1.45	1.98	17	116	1.4	17	1	no	no
88	M	Asians	55	1.2	3.1	137	147	2.6	137	1	no	no
88	M	Asians	65	1.4	2.6	250	184	2.7	250	1	no	no
88	M	Asians	75	1.4	2.5	155	221	2.9	155	1	no	no
88	M	Asians	85	0.8	2.8	16	335	2.4	16	1	no	no
88	F	Asians	55	1.4	2.6	207	153	2.5	207	1	no	no
88	F	Asians	65	1.4	2.7	327	143	2.3	327	1	no	no
88	F	Asians	75	1.2	2.7	198	171	2.7	198	1	no	no
88	F	Asians	85	0.5	3	11	308	4.3	11	1	no	no