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Occupational Exposure to Cobalt: a Population Toxicokinetic Modeling Approach Validated by Field Results Challenges the Bei® for Urinary Cobalt

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Abstract

The objective of this study is to model the urinary toxicokinetic of cobalt exposure based on of 507 urine samples from 16 workers, followed up for one week, and 108 related atmospheric cobalt measurements to determine an optimal urinary cobalt sampling strategy at work and a corresponding urinary exposure threshold (UET). These data have been used to calibrate a population toxicokinetic model, taking into account both the measurement uncertainty and intra- and inter-individual variability. Using the calibrated model, urinary sampling sensitivity and specificity performance in detecting exposure above the 20 $\mu g/m^3$ threshold limit value (TLV-TWA) has been applied to identifying an optimal urine sampling time. The UET value is obtained by minimizing misclassification rates in workplace exposures below or above the TLV . Total atmospheric cobalt concentrations are in the 5 - 144 $\mu g/m^3$ range, and total urinary cobalt concentrations are in the 0.5 - 88 µg/g creatinine. A two-compartment toxicokinetic model best described urinary elimination. Terminal elimination half-time from the central compartment is 10.0 hours (95% confidence interval (8.3 - 12.3)). The optimal urinary sampling time has been identified as 3 hours before the end of shift at the end of workweek. If we assume that misclassification errors are of equal cost, the UET associated with the TLV of 20 $\mu g/m^3$ is 5 µg/L which is lower than the ACGIH recommended BEI® of 15 µg/L.

Introduction

Cobalt (Co) enters into the composition of hard metal alloys used in electrical, aeronautical or car industries as a binder for metallic carbides (tungsten, silicon, vanadium). Cobalt enhances the resistance of these alloys to high temperatures and corrosion. Cobalt is also used in steel production, glass and ceramic industries, and many other industrial applications. All personnel working in these industries are potentially exposed to cobalt or its compounds, both of which can induce chronic intoxication leading to respiratory, thyroid, cutaneous, cardiac and carcinogenic effects (1-5) under some circumstances.

The American Conference of Governmental Industrial Hygienists (ACGIH) has defined two exposure indices applicable to cobalt occupational exposures (6): an atmospheric threshold limit value time weighted average (TLV-TWA) of 20 $\mu g/m^3$ and a biological exposure index (BEI®) of 15µg/L total cobalt concentration in urine collected at the end of shift at the end of the last day of the workweek. The ACGIH has justified this BEI® through four studies (7-10) giving linear regression equations between cobalt concentration in ambient air and in urine obtained at the end of shift at the end of the workweek. In each of those studies, the regression equation was used to estimate cobalt levels in urine at 20 $\mu g/m^3$ exposure, assuming the TLV value to be reliable. No data on exposure or urinary cobalt were available before the last shift, except in one study (7). Thus the potential effect of cobalt exposure during the preceding days could not be assessed. Furthermore, in the absence of accurate information on cobalt excretion kinetics and of inter-individual variability, it is unclear whether urinary collection at the end of shift at the end of workweek is indeed the best sampling time. The BEI® value obtained from a linear regression equation is itself certainly not optimal because it ignores both the time-dependency of relationship between airborne exposure and urinary cobalt and the very large inter-subject variability.

This paper presents a toxicokinetic approach for determining an optimized urinary sampling strategy for cobalt and a consistent way of deriving an urinary exposure threshold (UET), corresponding to the ACGIH BEI®, for use in occupational hygiene. A population toxicokinetic model of cobalt intake and urinary excretion was developed from data on a series of cobalt-exposed subjects followed up during a period of one week. Atmospheric and urinary cobalt measurements were simultaneously collected for each subject. The model was then applied predictively to identify an optimal sampling strategy and back-calculate a UET corresponding to the current value of the TLV, which is assumed to provide valid protection for exposed workers.

Population and Methods

Population

A total of 16 male subjects exposed to cobalt dust were recruited in two plants producing tungsten carbide cutting tools. They were followed-up during one workweek respectively in 1988 and 1993.

Atmospheric and urinary sampling strategy

Airborne exposure to cobalt was measured by sampling the inhalable dust fraction in each individual breathing zone for all work shifts during the study week. The workweek comprised four, five or six days, depending on the plant and the subjects. For most subjects, exposure was measured for each half-shift (mean duration of half-shift = 3.5 hours), and for the others, exposure was measured for the total shift (mean of total shift = 7.5 hours).

Airborne sampling was performed in compliance with French AFNOR standard NF X 43457. Aerosol samples were collected on WHATMAN® QM-A quartz fibre filters fitted in closed configuration inside a MILLIPORE 37 mm cassette designed to collect the aerosol inhalable fraction by means of GILIAN® HFS113 (1 L/min flow) portable sampling pumps.

The personal pump flow rate was carefully checked at the beginning and end of each sampling sequence. The flow rate was also regularly checked at intermediate times.

All urine voiding of the study week were collected in parallel for each subject. Each subject was informed of the study aims and conditions, and agreed to collect urine based on a controlled sampling procedure: Subjects were requested to collect urine samples whilst avoiding contamination, without working clothes, with clean hands and, if possible, after a shower at the end of shift. The urine was processed, packaged and frozen on site. All equipment cups and tubes in contact with the urine for analysis were previously washed with a hot detergent, rinsed and immersed for 48 hours in a 10% nitric acid solution , rinsed with ultra-pure water and dried in a oven at 50°C. All these operations as well as storage were performed in airtight polyethylene containers, in which all devices were protected from dust and contact with operator fingers. Each urine sample was analyzed separately. For 12 subjects, all voiding of the following weekend were also obtained and analyzed.

Analytical methods

Air sample analysis

Total cobalt content of the filters was analyzed by two laboratories with a method developed by one of them (11). These laboratories belong to the French inter-laboratory ring trial network for occupational hygiene. Particles deposited on each filter, including dust on the filter holder inner walls, were dissolved in mixture of hydrofluoric (2 mL, 40%) and nitric (3 mL, 68%) acids. Cobalt was measured at a wavelength of 240.7 nm by atomic absorption spectrophotometry, using a flame technique based on a 10 µg/L detection limit.

Urine sample analysis

Total urinary cobalt concentration was evaluated by electrothermal atomic absorption spectrometry using a Perkin Elmer 3030/Zeeman instrument based on a method (12) involving a detection limit of 2 nmol/L ≈ 0.1µg/L in INRS internal quality system.

Urinary creatinine was determined by colorimetry (Roche 3667 kit) using a Cobas Mira S Plus (Roche Diagnostic System).

Statistical methods

A number of urine sampling strategies were compared on the basis of their efficiency at detecting an atmospheric cobalt concentration higher than a limit value. Optimal sampling was determined based on results of a multilevel (population) toxicokinetic data modeling (13), which estimates jointly the toxicokinetic parameters of each subject (subject level) and the inter-worker distribution of these parameters (population level).

Population toxicokinetic model

Figure 1 shows a conceptual graph of this model.

At the subject level, a toxicokinetic approach was used to model the urine-excreted quantity of cobalt in each voiding for each subject, as a function of the time-dependent atmospheric cobalt concentration in the worker's breathing zone, and of the urine sampling time.

All urine voiding were collected and analyzed separately. The absolute quantity of cobalt (in µg) excreted at each voiding was modeled as the dependent variable. This quantity was obtained by multiplying the cobalt concentration by the corresponding volume of excreted urine. Thus, this process overcame the need to use cobalt expressed per g creatinine. For each subject, the time course of cobalt quantity in urine (Q_u) was modeled as the output of a deterministic two-compartment model. This model included a central compartment and a

peripheral one, both without particular physiological interpretation. We checked that a simpler 1-compartment model would not correctly predict the data (results not shown). We also checked different parameterizations using various distribution shapes (see the Discussion section). The toxicokinetic model for each subject i had four transfer parameters $\theta_i = \{K_{in}, K_r, K_s, K_e\}_i$ characterizing transfers between compartments (the population distributions for K_r^i and K_s^i are not displayed on Figure 1 for clarity). The mathematical model and the precise meaning of these parameters are provided in online appendix 1. The toxicokinetic model can be viewed as a function relating cobalt urinary excretion to air exposure, time, and toxicokinetic parameters for a given subject:

$$Q_u = f(C_{in}, t, \theta_i) \quad (1)$$

At the population level, subjects were assumed to differ randomly from each other. The 4-component parameter vectors θ_i characterizing each subject were assumed to be log-normally distributed in the population:

$$\text{Log}(\boldsymbol{\theta}_i) \sim \text{Normal}(\boldsymbol{\mu}, \boldsymbol{\Sigma}) \quad (2)$$

in which $\boldsymbol{\mu}$ and $\boldsymbol{\Sigma}$ were respectively the population mean and variance (themselves vectors of four elements each, the variance measuring inter-individual variability) in a logarithmic scale.

At the measurement level, both cobalt concentration in air C_{in} and urine cobalt quantity Q_u were measured at finite accuracy. A measurement error model, assumed applicable to all subjects, was therefore set up to account for uncertainties affecting those data. We assumed that C_{in} was measured with a multiplicative log-normal error with GSD 1.5 (implying a 95% chance that the measured value was between 0.5 and twice the true value) (see the discussion for a justification of that model). The analytical measurement errors around Q_u were assumed to follow a normal distribution with mean zero and a standard

deviation, ξ, modeled as the sum of a constant error term SD_{min} and a term proportional to Q_u (eq. 3):

$$\xi = SD_{\min} + Q_u \cdot CoV \tag{3}$$

When Q_u is close to zero, ξ is at least equal to SD_{min} and, for high values of Q_u, that equation approximates a relative error model. The proportionality term (CoV) can be interpreted as a coefficient of variation.

All the parameters of the above models ($\boldsymbol{\theta}_i$, $\boldsymbol{\mu}$, $\boldsymbol{\Sigma}$, CoV, SD_{min}) were estimated in a Bayesian framework. Because for most parameters little prior information was available (mostly that they are non-negative and their order of magnitude), their prior distributions were chosen so that they had little influence on the final result (see online appendix 2). These prior distributions were then updated on the basis of the data to obtain posterior distributions, which were the Bayesian equivalent of estimated parameter and confidence intervals. Updating required calibration of the entire statistical toxicokinetic model with urinary and atmospheric cobalt concentrations data measured for the 16 subjects. Markov Chain Monte-Carlo (MCMC) methods (14-15) were applied using *MCSim* software (Free Software Foundation, Boston, MA, "http://www.gnu.org/software/mcsim/"). Two parallel Monte-Carlo Markov Chains with different starting points were run. After 10000 iterations, the two chains mixed well and converged, according to the R criterion of Gelman and Rubin (1992) (16). The following 50000 iterations of the two chains were used for identifying the posterior distribution. Model fit was checked and its predictive properties were tested by cross-validation (details on priors and model checks given in online appendix 2).

Determining an optimal sampling strategy

To determine the optimal strategy, we randomly sampled the toxicokinetic parameters for 200 virtual individuals from the posterior population distributions obtained as described

above. Each of these virtual individuals was exposed to a cobalt given dose for one workweek (four 8-hour shifts). This dose was assumed to be equal for all shifts. For each subject, a series of 28 atmospheric cobalt exposures was set, with exposure concentrations between 5 $\mu g/m^3$ and 30 $\mu g/m^3$ in steps of 1 $\mu g/m^3$, plus 35 and 40 $\mu g/m^3$. Thus 12 of the 28 exposure simulated exceeded the 20 $\mu g/m^3$ TLV.

For each of the 200 simulated subjects and 28 possible exposure levels, five urinary sampling strategies (USS) were compared: (A) Three hour urines collected each day at the shift end, and averaged over four workdays; (B) Three hour urines collected each day after 4 hours of exposure, and averaged over four workdays; (C) First urine of the last day of the workweek; (D) Urine from the last 3 hours of the last shift of the week; (E) Urine from the 3 hours following the end of the last shift of the week.

For any USS, the excretion rate of cobalt in urine was simulated every hour and the cobalt content of voiding, assumed to take place every three hours (close to the observed mean duration), were computed. A random error was added to that quantity based on the above urinary measurement error model (eq. 3). Predicted cobalt quantities were transformed into urinary cobalt concentrations by dividing them by 237 mL, the mean observed volume among the study subjects. That procedure takes into account the dependence of cobalt concentrations (inside the body and in urine) on the toxicokinetic parameters of each individual, and on the time-varying exposure (cobalt concentrations increase nonlinearly during exposure and decrease in the absence of exposure).

USS were compared according to their sensitivity and specificity in detecting an atmospheric value above a TLV of 20 $\mu g/m^3$. For each USS, a receiver operating characteristic (ROC) curve, giving the sensitivity for detecting above TVL excursions as a function of (1 minus specificity), was constructed by varying the urinary exposure threshold (candidate UET) by steps of 0.25 $\mu g/L$ between 0 and 9 $\mu g/L$ and by steps of 1 $\mu g/L$ between

10 and 20μg/L. For any given value of that threshold (say 8 μg/L), the sensitivity was computed as the number of simulated urinary concentration values exceeding 8 μg/L, divided by the number of simulations for atmospheric exposures equal or superior to the 20 μg/m^3 TLV (n=13x200=2600). Similarly, the specificity was computed as the number of simulated urinary concentration values below 8 μg/L, divided by the number of simulations for atmospheric exposures below the 20 μg/m^3 TLV (n=15x200=3000). The best USS was taken as the one with the largest area under the ROC curve (17).

After determining the optimal USS, the final step was to establish an optimal UET value for comparing future urinary results. For each candidate UET, each of the 5600 values of the USS-specific urinary measurements was either well-classified (both the atmospheric or urinary are either below or above their respective limits), or falsely positive (*i.e.*, with an urinary value above the UET with an atmospheric exposure below the TLV) or falsely negative (*i.e.*, with an urinary value below the UET with an atmospheric exposure above the TLV). We denote by C_{FP} the cost associated with a false positive classification, C_{FN} the cost associated with a false negative classification and FP and FN, respectively, the number of false positives and false negatives in our sample of 5600 values. Denoting by $C= C_{FP} /(C_{FN} + C_{FP})$, the relative cost of false positives over all false classifications, each value of the UET was associated with a total cost, given by:

$$Total\ Cost= CxFP+(1-C)xFN$$

A large UET resulted in wrongly classifying high urinary exposures as acceptable (large *FP* and low *FN*) while a small UET resulted in wrongly classifying low exposures as unacceptable (large *FN* and low *FP*). Increasing C, i-e increasing C_{FP} whilst keeping C_{FN} constant, would lead to put more emphasis on the costs associated with wrongly deciding that the atmospheric exposure exceeds the TLV based on the urine sample, while decreasing C would put more emphasis on the protection of the worker. The optimal UET among the

candidate UETs we considered (between 0 μg/L and 20 μg/L) , corresponded to a minimum total cost.

Results

Population characteristics

The atmospheric and urinary measurements for each of the 16 subjects studied are summarized in Table 1. The geometric mean of the atmospheric cobalt exposure measurements of plant A (subjects A to G) exceeded the TLV of 20 μg/m3 defined by the ACGIH in 3 out of 7 subjects. In plant B (subjects 1 to 9), the geometric means of the atmospheric cobalt measurements were even higher. Atmospheric cobalt exposures for each subject were usually followed by a peak in urinary cobalt within the next hours, which declined until the next exposure. Urinary cobalt excretion declined gradually over the week end.

Modeling results

The 2-compartment model gave a relatively close data fit, with model predictions following the described previously concentration time-course pattern. We chose the best model among the different ones we tested using different priors and distribution shapes. Those made little difference, but as soon as we chose a two-compartment model, the model fit improved markedly and did not depend much on the distributional shapes and priors we used. The overall correlation between observed data and model predictions is given in online appendix 2. Figure 2 illustrates in detail the fit for subject A. To provide a more familiar representation of the kinetics illustrated in Figure 2, all urinary measurements and predictions (urinary quantities in μg) were divided by time elapsed since the previous urine collection.

This led to an expression of the urinary cobalt excretion flow in µg/h. Table 2 presents a summary of the posterior distributions of population and individual model parameters.

The population geometric mean of K_{in} characterizing the quantity of inhaled cobalt entering the organism per unit time, was estimated at 60.4 L/h with a between-subject GSD of 2.0. Elimination parameter K_e corresponds to an elimination half-time $T_{e1/2}$ of approximately 10 hours, varying between 8 and 12 hours relatively constant for the studied subjects (GSD=1.16). Half-times $T_{r1/2}$ and $T_{s1/2}$ corresponding to exchanges with the peripheral compartment, were estimated at 9 and 20 hours respectively, with a rather high inter-individual variability. The coefficient of variation (*CoV*) for the urinary cobalt measurements was estimated at about 36% (CI 95%: 32% to 40%), with a baseline SD_{min} of 0.03 µg/voiding (CI 95%: 0.002 to 0.08)

Optimal sampling strategy

Detailed comparisons of the various strategies, on the basis of ROC curves, are presented in the online appendix 3. The best sampling strategies were to collect urine sampled during the last 3 hours of the last shift of the week (USS D) or during the 3 hours following the end of the last shift of the week (USS E). For ease of sampling, urine sampling from the last 3 hours of the last weekly shift can be considered as the optimal sampling strategy.

Figure 3 illustrates cost functions for two misclassification costs, *C*. The heavy line represents the cost associated with a false positive (*FP*, wrongly deciding that the exposure exceeds the TLV) and a false negative (*FN*, wrongly deciding that the atmospheric exposure is below the TLV). These were set to the same value, meaning that the user gave as much importance to an *FN* as to an *FP* ($C = 0.5$ and $C_{FN}=C_{FP}$). The optimal UET value is 5 µg/L in that case. For the exposure values considered and given the chosen optimal USS, this UET yields an FP percentage of 40% and of an FN percentage of 23%. For the light line, the cost of *FN* was considered to be twice as high as that of *FP* meaning that the user gave twice as

much importance to an *FN* as to an *FP* ($C = 0.33$ and $C_{FN} = 2C_{FP}$). The optimal UET was then approximately 3.5 µg/L, yielding 58% *FP* and 12% *FN*.

Discussion

This paper presents an optimized strategy for urinary sampling in the workplace based on a population toxicokinetic modeling of the urinary cobalt excretion kinetics in 16 workers over a period of one week. We find that the optimal strategy was to sample urine during the last three hours of the workweek. In implementing this strategy, we have calculated a UET value corresponding to the TLV of 20 µg/m^3 depending on costs assigned with wrong decisions. A UET value of 5 µg/L is obtained when the cost of wrongly deciding that exposure is acceptable is considered equal to the cost of deciding that the exposure is unacceptable. Our results agree with international recommendations (6) with respect to the urinary sampling time (end of shift at the end of workweek). They also suggest that sampling after the end of shift at the end of workweek would not provide any additional benefit.

However, the optimal biological value (UET) we obtained (5µg/L or 3.5 µg/L, depending on the error relative cost) is lower than the value (15 µg/L) recommended by the ACGIH. When applying the 15 µg/L value, we estimated 80% of false negatives (that is, workers with urinary cobalt concentrations below the UET during exposure atmospheric concentrations exceeding the TLV), and 3.5% of false positives (data not shown). The high percentage of false negatives shows that the ACGIH BEI® may not sufficiently protect the workers. The difference depends probably to a large extent on how correspondence between TLV and BEI® was established. The ACGIH have used only cross-sectional end-of-shift urinary data linearly regressed on the atmospheric exposure. Most notably, no individual kinetics were observed. Conversely, our results stem from modeling of actual individual data for workers followed-up over a full week. It should be noted (data not shown) that, if we

apply the ACGIH strategy to our data with a simple linear regression, the corresponding value of the UET is 15 µg/L when we regress urinary concentration on the log atmospheric concentration in the last shift of the workweek, and is 12 µg/L when using atmospheric concentration on the natural scale. If we apply the ACGIH strategy to our data, we obtain an UET close to the BEI®. However, our data and method also allow to actually estimate misclassification rates and, allowing for them, yield a much lower UET. This is largely due to the fact that our approach takes inter-worker variability into account; the ACGIH approach does not do this explicitly. Thus our results represent a challenge not only to the BEI® for cobalt, but, more generally, to the ACGIH approach to deriving these BEI®s.

Our results may be discussed in relation to a number of issues. We did not consider varying volume of voiding when determining an optimal strategy and the corresponding UET. However, we do not believe that this is a serious limitation. What was measured, and used as data to calibrate the model, is the cobalt quantity in µg in each voiding. That quantity depends on the time between voiding (the voiding times were recorded and those actual times were used in input to the model). The urine volume also depends on time between voiding, but the quantity excreted between two voiding is conditionally independent of the urine volume given the voiding times. However, we used the mean volume observed among our subjects, when converting this quantity into a concentration in µg/L, for display purposes.. If we wanted to ensure protection for all workers, we would need to use a minimum urine volume and this would lead to even higher concentrations.

All urines of the week were collected and atmospheric exposure was measured for all the shifts worked. The range of cobalt urinary and atmospheric concentrations was very wide (geometric mean from 4.89 to 144.22 µg/m^3 for atmospheric data, and from 1.62 to 12.33 µg/g creatinine for urinary data). The range of applicability of our results should therefore be

relatively wide too. Moreover, the total number of workers (16) studied is not very large, but for each worker all exposure and urinary follow-up is complete.

The model estimates at 36% the coefficient of variation of the urine analytical cobalt concentration measurement . This is higher than the laboratory value, supposedly to be 4% (12). We therefore confirm that the true accuracy of field studies is probably lower than pure laboratory uncertainty. However, the estimated 36% CV also includes other sources of errors (modeling error or intra-individual variability) and represents an upper limit in terms of analytical accuracy.

Atmospheric exposures measurements were collected over half shifts or complete shifts because instantaneous readings were not feasible. Therefore, in contrast to usual pharmacokinetic modeling, only mean exposures values were available in this case. This created a degree of uncertainty in the estimated parameters. Secondly, atmospheric measurements cannot be assumed to correspond exactly to the inhaled quantity. This was taken into account by assuming a log-normal measurement error with GSD 1.5 around the true inhaled concentration value. Other GSDs between 1.2 and 2 were tried, but the model's overall fit did not change much and that had no influence on the UET. Only one model is presented in this paper. However, alternative toxicokinetic models were considered and fitted, including one-compartment models and other parameterization of the measurement sub-models. For instance, several different prior statistical distributions were tested. The model shown here was chosen because of its better fit to the data.

The parameters of our model are subject to only limited physiological interpretation. The elimination half-time $T_{e1/2}$ (equivalent to rate constant K_e) can be interpreted as the body elimination half-time of cobalt (central compartment). The parameters determining flow rates between the central compartment and other non-specified organs (lumped into a single peripheral compartment) cannot be easily interpreted. The estimated geometric mean for K_{in},

the parameter determining the cobalt quantity inhaled into the organism per unit time, is 60.4 L/h. That is quite lower that the physiological respiratory flow rate, which is about 500 L/h for a man at rest. A possible explanation for this is that 88% (1-(60.4/500)=0.88) of the inhaled cobalt is exhaled or does not enter the body (*i.e.* is eliminated by the lungs into the gastro-intestinal tract and feces without absorption). It has been estimated that approximately 30% of cobalt inhaled as cobalt oxide can be absorbed (18).

It is interesting to note that, when a single compartment model was applied (data not shown), the estimated parameter K_{in} was virtually identical to the one estimated in the two-compartment model. Elimination half-time from the one-compartment model was approximately 20 hours (data not shown), which is close to the values given by Lauwerys and Hoet (19) (p88) quoting Christensen *et al* (20) and Apostoli *et al* (21). In the two-compartment model, the apparent elimination time depends on the rate-limiting exit from the peripheral compartment rather than on the elimination from the central compartment. But here also , the elimination half-life is estimated at approximately 20 hours ($T_{s1/2}$=20.38 hours) whilst the 2-compartment model fit to the data was far better than that of a one-compartment model.

The results given in this paper were based on a large number of data (507 urinary cobalt determinations and 108 atmospheric cobalt measurements), requiring very close co-operation of the study subjects and availability of research personnel for every working shifts during the week. Such a protocol is expensive and difficult to organize. Therefore, it should only be used if its results are expected to be interpretable and useful. An important constraint in this respect is the elimination half-time. If it is longer than a few days, this procedure involving atmospheric and urinary data for a whole week will not allow it to be accurately identified it with any precision, yet it would still condition the overall body burden. Thus for metals with a longer elimination half-time, this protocol would be unsuitable. A similar

protocol with sparser sampling over a longer period of time would be more appropriate. Optimal design methods could be applied to this issue (22). Lauwerys and Hoet (19) suggest there is another component (possibly due to kidney or liver storage) of cobalt kinetic, which may persist for 2 years. The protocol applied in this study would naturally be incapable of identifying phase associated with such a compartment. However, for all practical purposes in occupational health, two compartments described the data and simulations closely enough (data not shown), showing that a dynamic steady-state is reached.

On the other hand, we should note a number of limitations on the possibility of extrapolating our study results. First, despite the wide range of exposure levels, all data were obtained in hard metal factories at which cobalt was always combined with tungsten. It cannot be assumed that the results would be exactly the same in other forms of cobalt exposure (coating, recycling, ceramics, polymers). However, the data do originate from two different plants, which contributes to our confidence in results representative of such this form of occupational exposure. It is known that the close-faced cassette sampler is slightly biased in relation to the ISO curve. However, the exposure is never overestimated so the low percentage of cobalt retained in the body cannot be explained by this bias. Furthermore, this study assumes that all the cobalt intake is via inhalation. It is probable that some intake is via ingestion, on which we have no information (19). However, the low percentage of cobalt retained in the body does not support the hypothesis of a major impact from a source of exposure other than the respiratory. A final limitation of our study is that no physiological data were available on the subjects (*e.g.*, respiratory flows, smoking habits and so on).Therefore, individual characteristics could not be taken into account using, for example, a physiologically-based pharmacokinetic model. However, this may not be central issues, given the aim of our study (obtaining valid USS and corresponding UET).

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TABLE I. Summary statistics of the atmospheric and urinary cobalt concentration data in µg/g crea and in µg/L.

	Atmospheric cobalt measurements						Urinary cobalt measurements									
Subject	**Number of days**	**Number of samples**	**GM[(A)] µg/m^3**	**GSD [(B)]**	**Min µg/m^3**	**Max µg/m^3**	**Number of days**	**Number of samples**	**GM[(A)] µg/g crea**	**GSD [(B)]**	**Min µg/g crea**	**Max µg/g crea**	**GM[(A)] µg/L**	**GSD [(B)]**	**Min µg/L**	**Max µg/L**
A	4	8	4.9	1.4	4	9	7	35	1.6	2.1	0.5	21.9	2.3	2.3	0.5	22.7
B	4	8	9.4	1.6	4	18	7	34	3.6	1.7	0.6	8.4	5.7	2.2	1.2	20.7
C	4	8	10.7	1.4	7	16	7	34	2.9	1.9	1.0	7.8	3.9	2.2	0.8	16.4
D	4	8	6.2	1.8	4	17	7	33	2.5	1.8	0.8	6.2	2.7	2.2	0.2	10.1
E	4	8	61.6	2.8	22	276	7	34	12.3	2.3	1.9	87.9	14.3	2.7	2.0	197.8
F	4	8	26.3	1.2	20	32	7	36	6.0	1.6	2.5	13.1	8.1	2.2	1.4	30.2
G	4	8	25.6	2.0	10	74	7	35	4.2	2.1	1.2	16.4	4.9	2.2	1.2	38.4
1	5	10	144.2	2.0	66	449	7	34	16.4	1.9	5.4	65.1	14.5	1.9	1.8	47.7
2	4	4	26.9	3.4	6	72	4	25	11.5	2.2	1.9	55.1	15.5	2.3	2.0	71.0
3	6	6	41.8	2.9	17	228	7	34	8.6	2.0	3.0	69.7	5.7	2.4	0.3	34.8
4	6	6	74.5	2.8	21	205	7	34	11.4	1.6	5.2	28.6	15.0	1.5	7.1	43.5
5	5	5	14.8	2.9	6	72	4	25	4.5	1.5	2.1	12.7	6.6	1.7	2.0	15.8
6	5	5	19.8	1.3	14	25	7	29	8.7	1.6	2.9	20.2	9.6	1.5	3.7	22.1
7	5	5	26.8	1.8	18	77	4	26	7.5	1.7	2.2	13.8	7.2	1.5	3.2	16.6
8	5	5	23.3	1.6	12	35	4	25	7.2	1.7	2.6	15.9	10.0	1.3	5.6	19.1
9	6	6	5.9	2.0	3	19	7	34	3.0	1.7	1.1	8.0	2.6	1.9	0.3	6.9

[(A)] GM = Geometric Mean
[(B)] GSD= Geometric Standard Deviation

TABLE II. Summary statistics of the posterior distributions for population and individual parameters.

Parameter (unit)	Population geometric mean μ (95% CI)	Between subject geometric SD Σ (95% CI)	Minimum of mean subject-specific value (subject)	Maximum of mean subject-specific value (subject)
K_{in} (L/h)[(A)]	60.4 (27.3-97.3)	2.04 (1.47-4.57)	29.9 (1)	123 (D)
K_e (1/h)[(B)]	0.068 (0.056-0.083)	1.16 (1.02-1.54)	0.059 (F)	0.076 (9)
$T_{e1/2}$ (h)[(C)]	10.19 (8.35-12.37)	1.16 (1.02-1.54)	11.75	9.12
K_r (1/h)[(D)]	0.078 (0.040-0.25)	1.93 (1.15-4.89)	0.054 (G)	0.171 (8)
$T_{r1/2}$ (h)[(C)]	8.88 (2.77-17.32)	1.93 (1.15-4.89)	12.8	4.0
K_s (1/h)[(D)]	0.034 (0.013-0.10)	2.22 (1.17-4.98)	0.015 (3)	0.117 (1)
$T_{s1/2}$ (h)[(C)]	20.38 (6.93-57.76)	2.22 (1.17-4.98)	46.2	5.92

[(A)] K_{in}: intake coefficient
[(B)] K_e : elimination coefficient
[(C)] $T_{x1/2}$ = half time = $\ln(2)/K_x$
[(D)] K_r and K_s : transfer coefficients between central and peripheral compartment, (see online appendix 2 and text).

1. Toxicokinetic model

For each subject, the time course of cobalt excretion (in µg) in urine noted Q_u, was modelled in a deterministic two-compartment model with central and peripheral compartments. Figure 1.1 presents that model graphically: arrows represent the flows between compartments or the external environment. K_{in} (in L/h) conditions the quantity of cobalt inhaled (C_{in} in µg/L) entering the organism. K_e, K_r and K_s are transfer coefficients (in 1/h) and describe the flow between the compartments (K_r, K_s) or excretion (K_e). The corresponding half-times can be computed as ln(2)/K_i were i is either r, s or e.

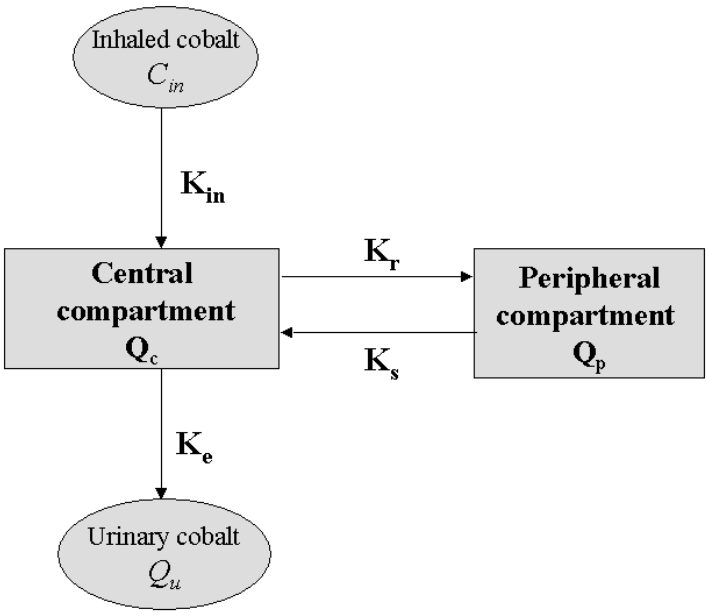

Figure 1.1 : The two-compartment model used.

The following differential equation system describes the temporal evolution of the quantities of cobalt quantities Q_c, Q_p and Q_u in the central compartment, peripheral compartment and urine, respectively:

$$\frac{dQ_c(t)}{dt} = K_{in} \cdot C_{in}(t) + K_s \cdot Q_p(t) - K_e \cdot Q_c(t) - K_r \cdot Q_c(t) \tag{A1}$$

$$\frac{dQ_p(t)}{dt} = K_r \cdot Q_c(t) - K_s \cdot Q_p(t) \tag{A2}$$

$$\frac{dQ_u(t)}{dt} = K_e \cdot Q_c(t) \tag{A3}$$

Equation (A1) represents the instantaneous variation of the quantity of cobalt in the central compartment (that is the quantity entering in the compartment, minus the quantity leaving the it). Equation (A2) represents in the same way the instantaneous variation of the quantity of cobalt in the peripheral compartment. Equation (A3) gives the instantaneous variation of the quantity of cobalt excreted in urine.

The input parameter K_{in}, the transfer rate constants K_r and K_s, and the excretion rate constant K_e have a specific value for each subject.

2. Priors, model checking, cross validation

Priors and posteriors

The Bayesian framework in which our model was calibrated requires to specify priors for its parameters.

Figures 2.1 to 2.10 show the prior and posterior distribution of the population parameters. These Figures show clearly that the data have strongly modified the parameter distributions and that the priors are likely to have little influence on the final results. Of course, if prior knowledge on these parameters had been available in addition to the rough possible range, we would have included it in informative priors.

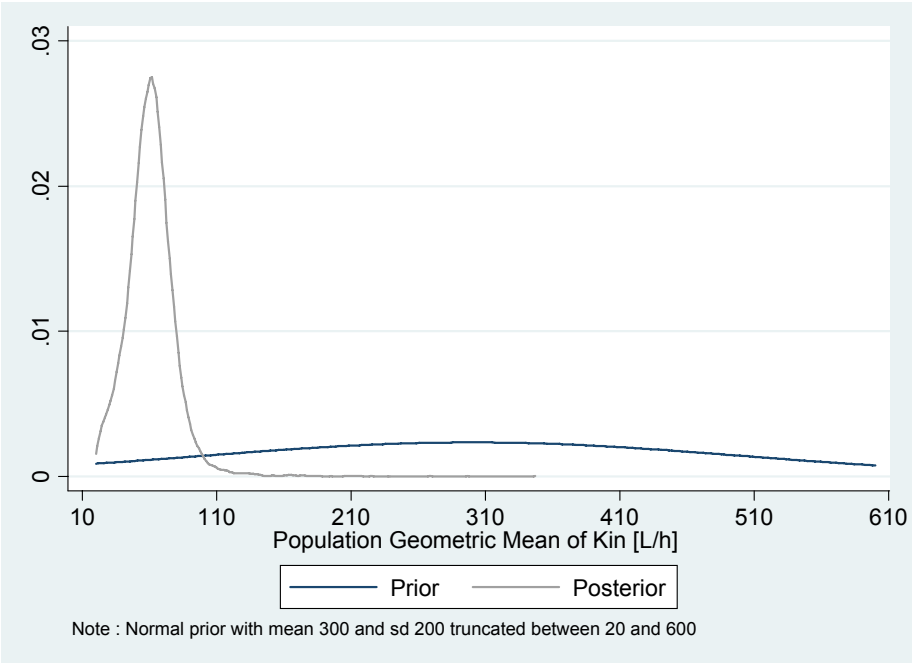

Figure 2.1 : Prior and posterior distribution of the population geometric mean of K_{in} [L/h]

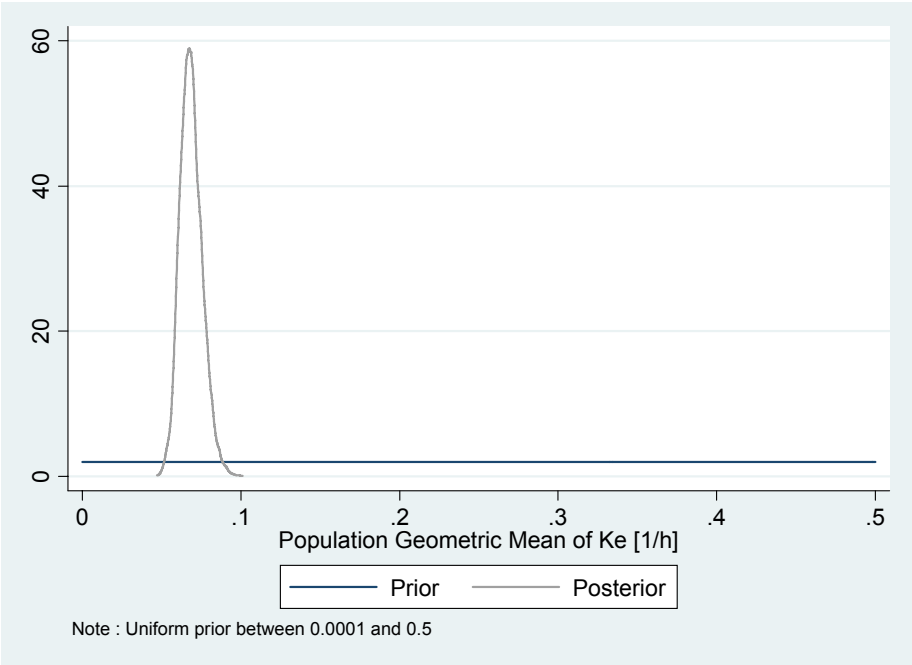

Figure 2.2 : Prior and posterior distribution of the population geometric mean of K_e [1/h]

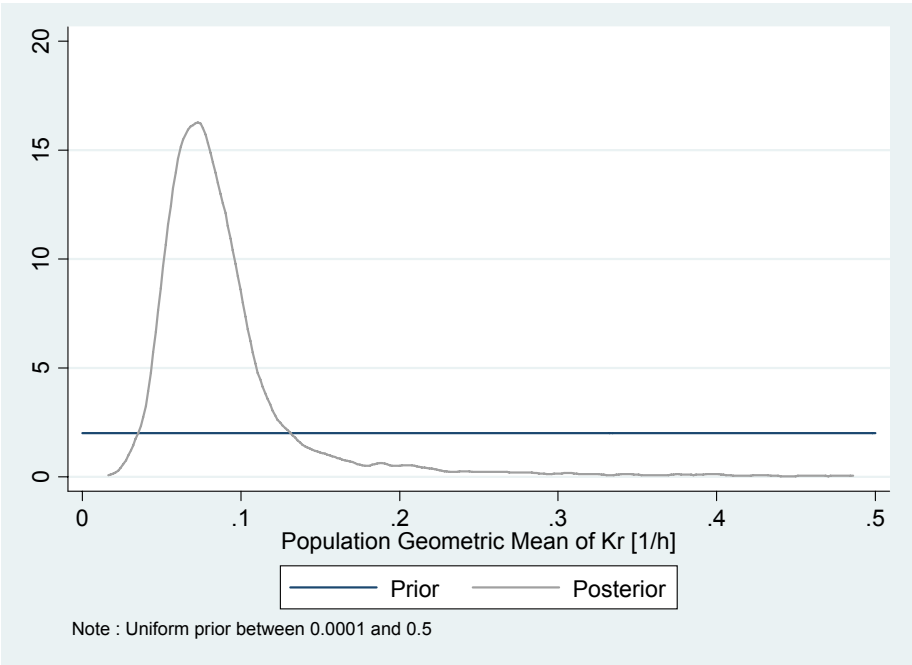

Figure 2.3 : Prior and posterior distribution of the population geometric mean of K_r [1/h]

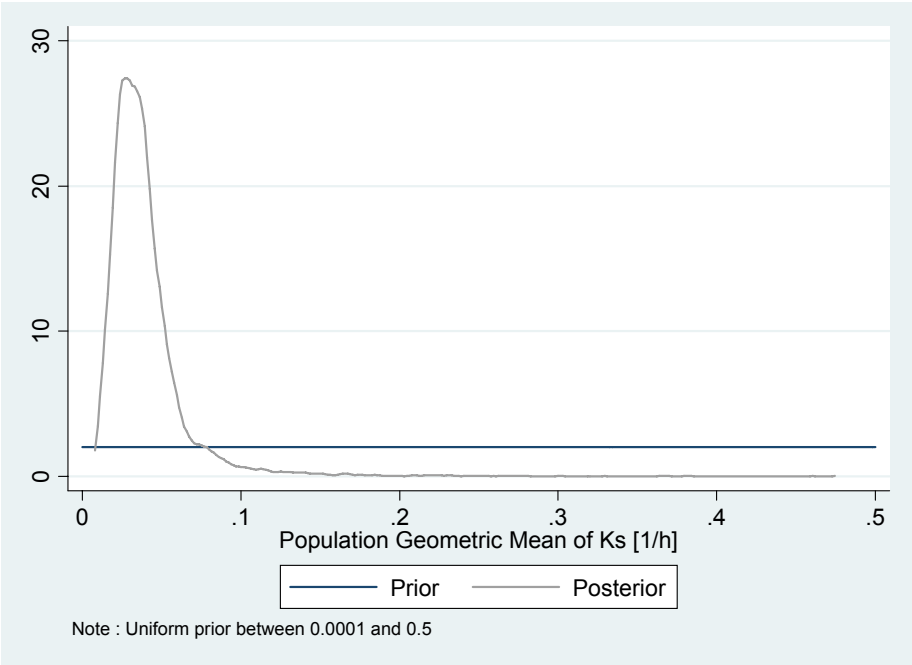

Figure 2.4 : Prior and posterior distribution of the population geometric mean of K_s [1/h]

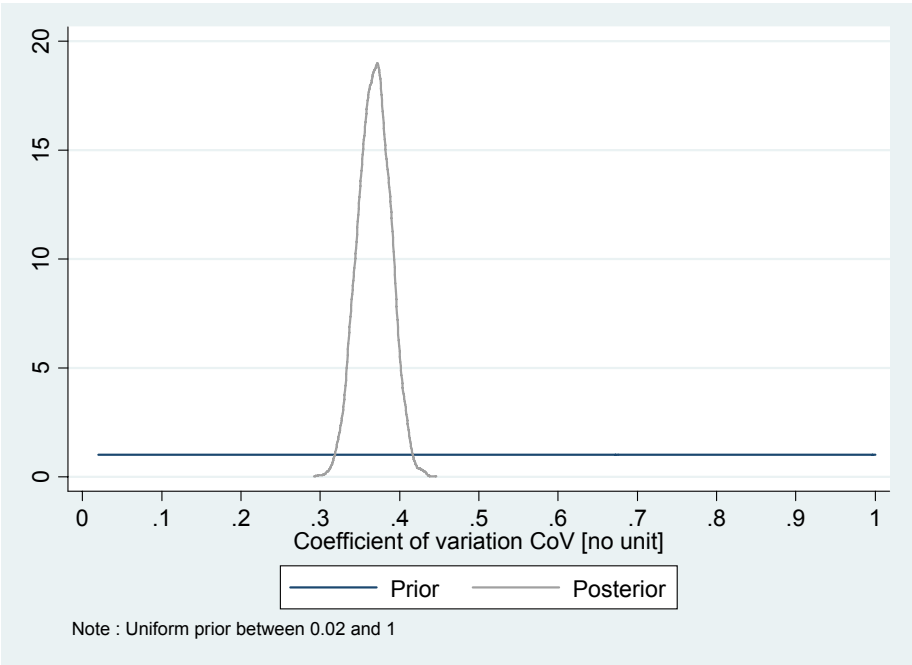

Figure 2.5 : Prior and posterior distribution of CoV

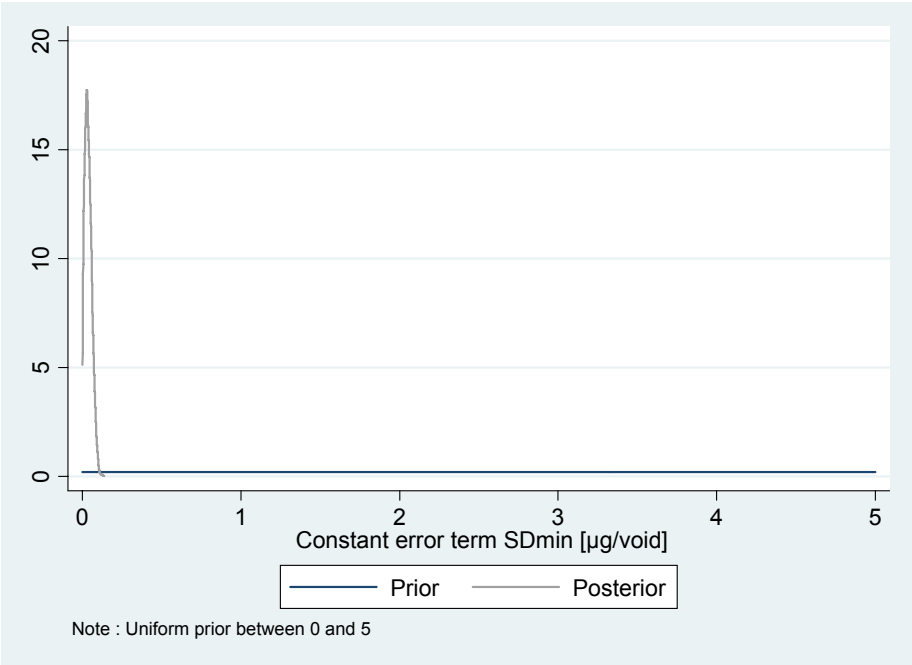

Figure 2.6 : Prior and posterior distribution of SD_{min} [µg]

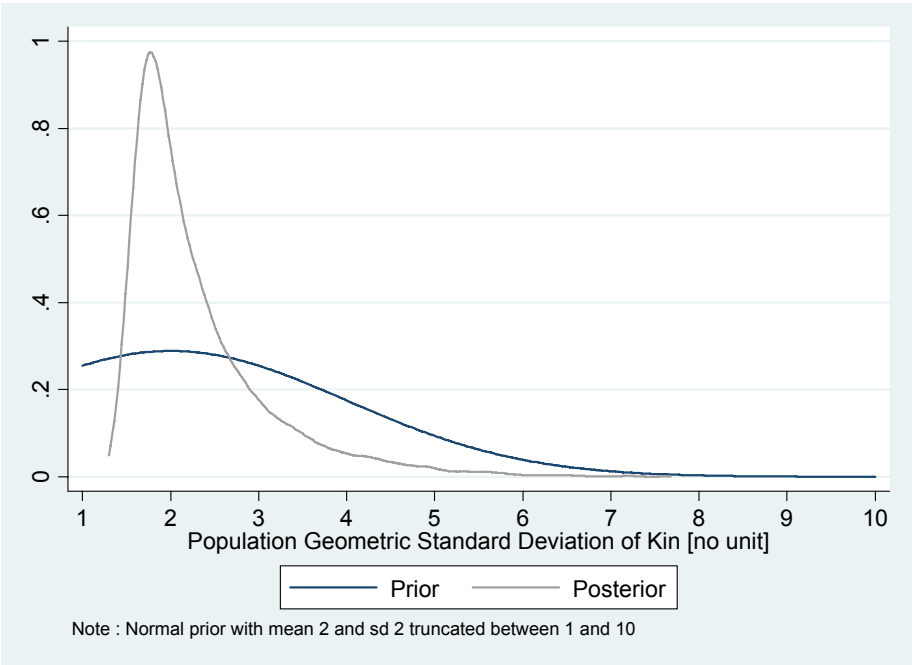

Figure 2.7 : Prior and posterior distribution of the population geometric standard deviation of K_{in}

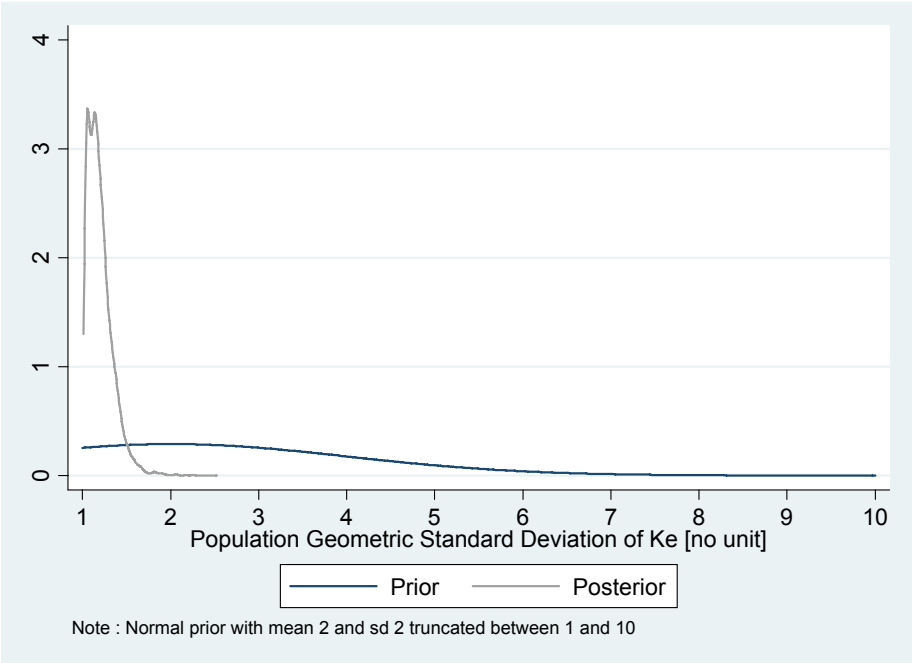

Figure 2.8 : Prior and posterior distribution of the population geometric standard deviation of K_e

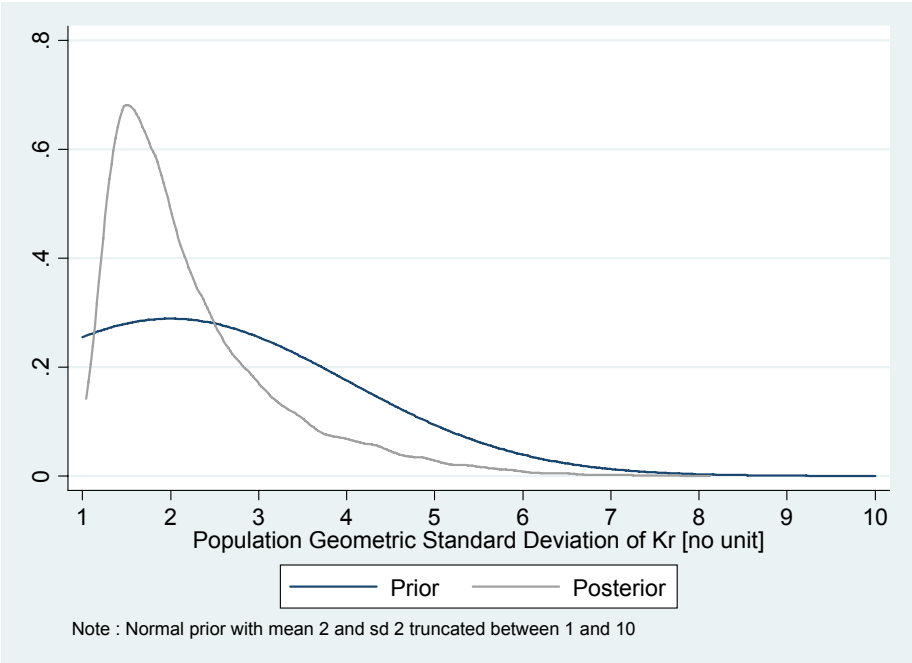

Figure 2.9 : Prior and posterior distribution of the population geometric standard deviation of K_r

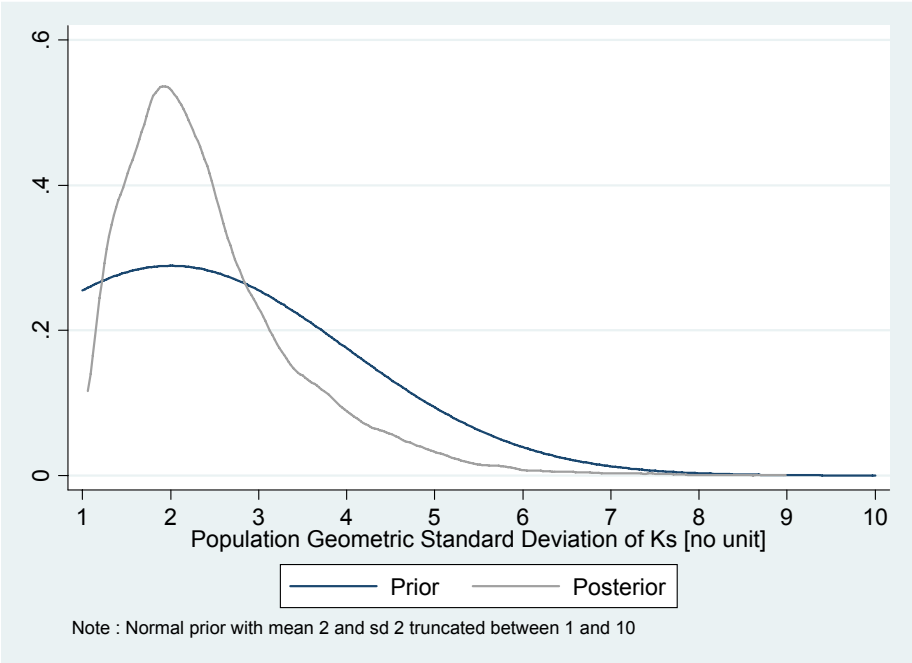

Figure 2.10 : Prior and posterior distribution of the population geometric standard deviation of K_s

Model checking

The fit of the model to the data was examined by plotting the observed vs predicted urinary and atmospheric measurements.

The resulting Figures 2.11 and 2.12 are shown below .

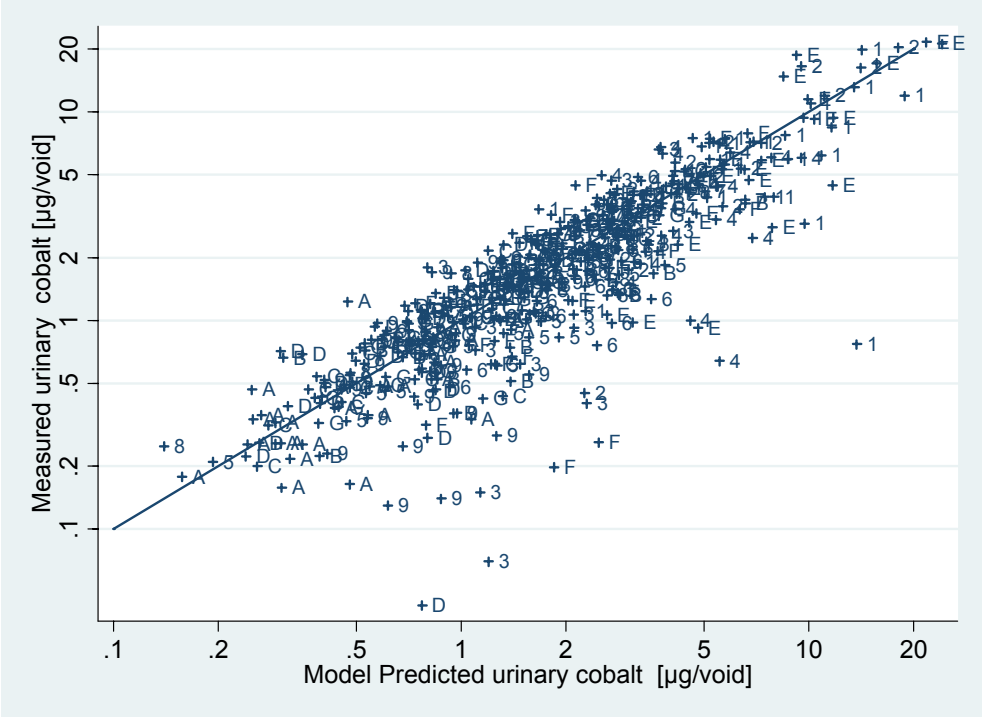

Figure 2.11 : Scatter plot of observed and predicted urinary data .

The corresponding coefficient of determination is 0.77 on the natural scale and 0.72 on the logarithmic scale. Although some of the measured values are below the corresponding predicted values, the overall fit seems adequate. Note that (with the possible exception of subject 9 which has one of the lowest atmospheric exposures) the various "outliers" correspond to different subjects.

For atmospheric measurements, one clearly sees the effect of rounding in the lower measurements. The overall fit is however adequate. The corresponding coefficient of determination is 0.82 on the natural scale and 0.88 on the logarithmic scale.

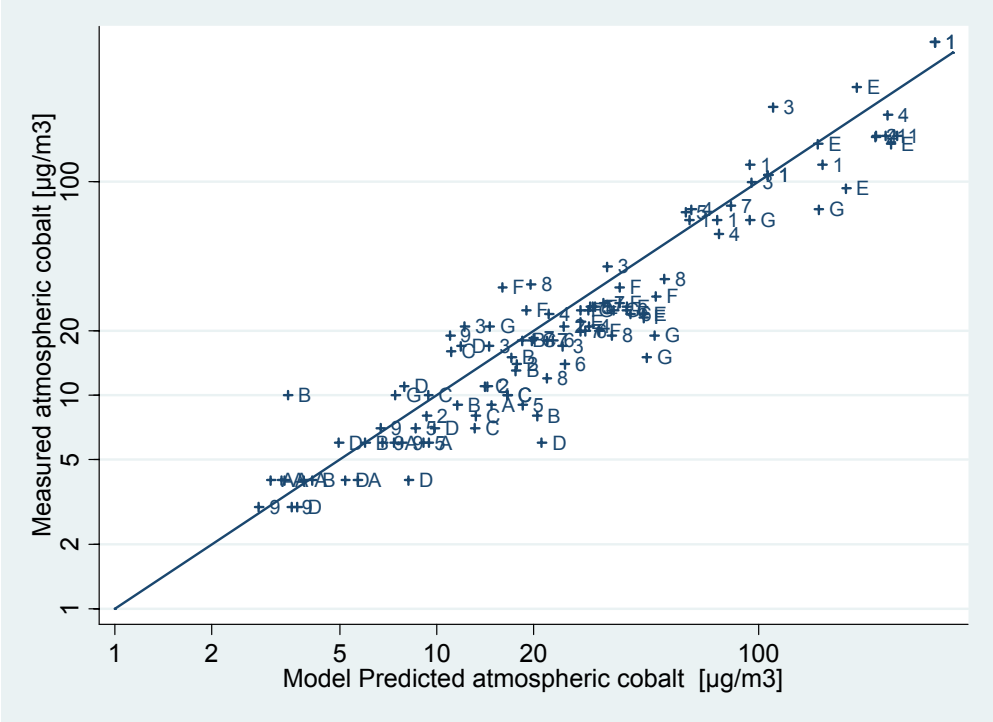

Figure 2.12 : Scatter plot of observed and predicted atmospheric data

Cross-validation

To cross-validate the model it was first calibrated using 11 randomly selected subjects out of the 16 study subjects. Predictions of the data for the 5 subjects left out was examined by simulating 50 urinary samples based on their actual atmospheric exposure measurements and plotting the observed values together with the simulation results. Figure 2.13 shows an example for subject E. These results did not show any inconsistencies between model predictions and data

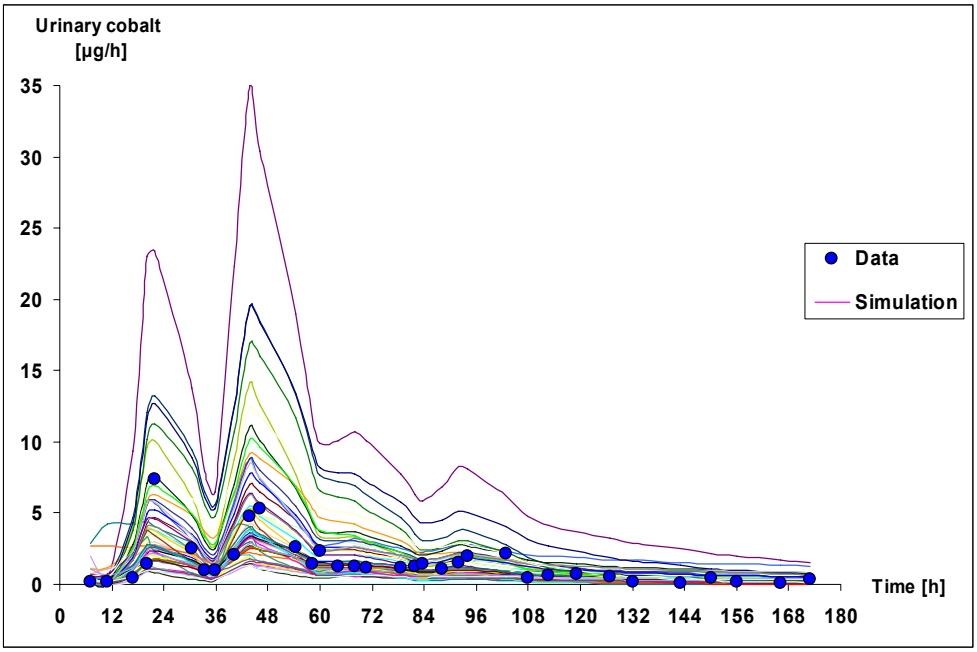

Figure 2.13 : Fifty urinary simulations based on the subject E atmospheric measurements, with observed data.

3. Comparison of ROC curves for various Urinary Sampling Strategy (USS)

As explained in the main text, the USS were compared on the basis of their sensitivity and specificity in detecting an atmospheric value above a TLV of 20 μg/m^3. For each USS, a receiver operating characteristic (ROC) curve, giving the sensitivity for detecting above TVL excursions as a function of (1 minus specificity), was constructed by varying the urinary decision threshold by steps of 0.25 μg/L between 0 and 9 μg/L and by steps of 1 μg/L between 10 and 20μg/L. For any given value of this threshold (say 8 μg/L) the sensitivity was computed as the number of simulated USS-specific urinary exceeding 8 μg/L, divided by 12x200=2400, the simulations corresponding to an atmospheric exposure equal to or exceeding the 20 μg/m^3 TLV. Similarly the specificity was computed as the number of simulated USS-specific urinary below 8 μg/L, divided by 15x200=3000, the simulations corresponding to an atmospheric exposure below the 20 μg/m^3 TLV.

Figure 3.1 shows the graphical comparison of these ROC curves. Five urinary sampling strategies (USS) were compared: (A) urine collected each day at the shift end, and averaged over four workdays; (B) urine collected each day after 4 hours of exposure, and averaged over four workdays; (C) first urine of the last day of the workweek; (D) urine from the last 3 hours of the last shift of the week; (E) urine from the 3 hours following the end of the last shift of the week. It is to be noted that the curves corresponding to the last two strategies USS (D) and (E) are virtually identical and are the ones with the maximal area under the curve.

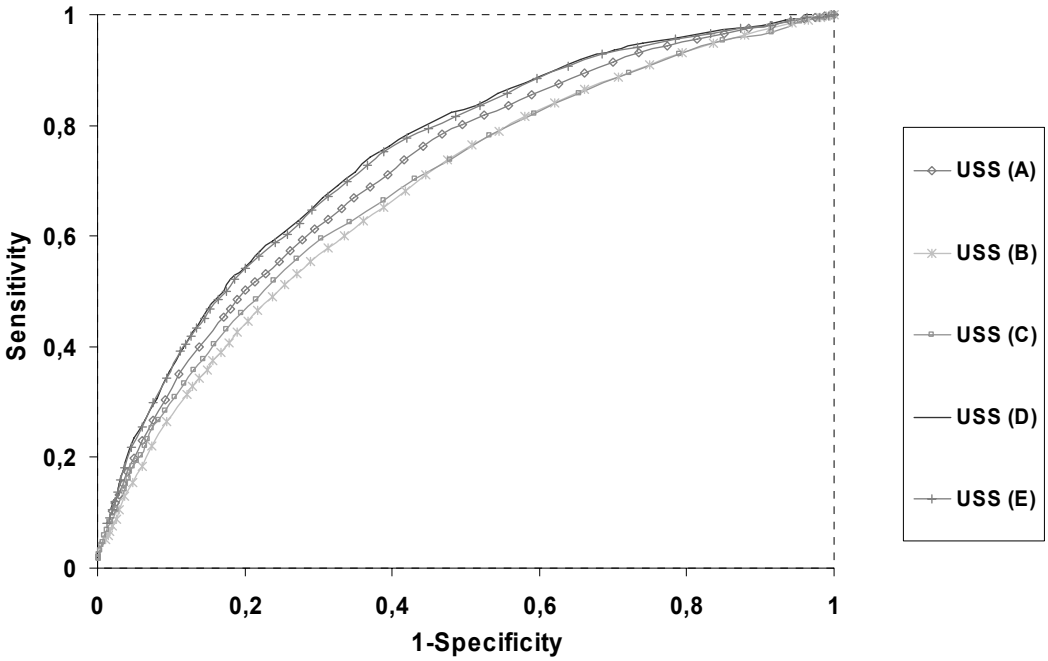

Figure 3.1 : Receiving Operator Curves of five urinary sampling strategies (USS). USS(A) corresponds to urine collected each day at the shift end, and averaged over four workdays; USS(B) corresponds to urine collected each day after 4 hours of exposure, and averaged over four workdays; USS(C) corresponds to first urine of the last day of the workweek; USS(D) corresponds to urine from the last 3 hours of the last shift of the week and USS(E) corresponds to urine from the 3 hours following the end of the last shift of the week.

Table 3.1 shows the areas under the curve for each of the strategies. As the these areas are between 0.70 and 0.76, there is no great difference between strategies. Strategy D however is virtually identical to the strategy recommended by the ACGIH, so that the fact that we found it to be the best is reassuring.

Table 3.1 : Areas under the ROC curves corresponding to USS

Urinary Sampling Strategies (USS)	Areas under the ROC curves
(D)	0.76
(E)	0.75
(A)	0.73
(C)	0.71
(B)	0.70