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ORIGINAL ARTICLE

Biological monitoring of cobalt in hard metal factory workers

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Abstract
Purpose The main aim of this study was to investigate the cobalt (Co) concentrations in urine along 4 months and their relationship with Co concentrations in blood and haemoglobin (adducts) in 34 workers from a hard metal manufacturing plant where metallic Co and Co oxide were used. Furthermore, the excretion kinetics of Co was investigated and the half-lives of Co in blood, plasma and urine were calculated along 18 days of non-exposure in the same workers.
Methods Co was analysed, in all biological samples, by ICP/MS.
Results Wide fluctuations in the urinary Co concentration were observed throughout the work shift and during the work week. A highly significant linear correlation was found between Co concentration (geometrical mean) in urine samples provided each Thursday (end shift) during 16 subsequent weeks and levels of Co-haemoglobin adducts or blood Co concentrations at the end of the same period. The Co elimination kinetics in globin calculated along 18 days without Co exposure was slow, being related to the physiological metabolism of haemoglobin, while in blood, plasma and urine Co half-lives were 12.3, 9.1 and 5.3 days, respectively.
Conclusion Co concentrations in haemoglobin or blood are highly related to the geometrical mean concentration of urinary Co when samples are collected weekly for several subsequent weeks. The biological monitoring of occupational exposure to Co in hard metal facilities provides reliable results by using the Co concentrations in haemoglobin or in whole blood. The urinary findings, though, do not show the same reliability because of their wide daily and weekly fluctuations.

Keywords Biomonitoring · Urine · Blood · Haemoglobin adducts · Cobalt · Hard metal workers

Introduction

Cobalt is used in a variety of industrial and military applications, especially in the production of superalloys for gas turbines aircraft engines where elevated temperatures and high mechanical stress are encountered. Cobalt is also a binder for tungsten carbide cutting tools, and cobalt compounds are used as pigments in glass, ceramics, varnishes and paints, as catalysts in the petroleum and chemical industry, as paint driers and as trace element additives in agriculture and medicine. In factories where hard metal and cobalt–diamond tools are used or produced, workers are exposed to cobalt powders with size lower than 500 nm (IARC 2006; ATSDR 2004).

The main health effects of cobalt exposure are on the respiratory tract, including bronchial asthma, diffuse interstitial fibrosis or, more seldom, severe progressive alveolitis and dermal effects such as allergic eczema (Fischer and Rystedt 1983; ATSDR 2004; Sauni et al. 2010).

Cobalt and its compounds have been shown to induce different types of tumours in experimental animals. Inhalation exposure to soluble (sulphate heptahydrate) and insoluble (cobalt metal) forms of cobalt caused lung cancer in rats and mice, as well as systemic tumours in rats (Bucher et al. 1999; Behl et al. 2015; Suh et al. 2016). There are, however, no epidemiological data on the human

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carcinogenicity of cobalt, even in people living in areas where the metal is extracted, like Canada, Congo, Zambia and Australia (Lison 2000). IARC (2006) has classified cobalt metal with tungsten carbide as probably carcinogenic to humans (Group 2A) and cobalt sulphate and other soluble cobalt(II) salts as possibly carcinogenic to humans (Group 2B).

Studies on the biological monitoring of cobalt in workers often provided uneven results, due to different chemical forms of cobalt being used in industrial manufacturing, including metallic cobalt and cobalt oxide (with oxidation states +2 or +3) or salt, like Co-chloride, Co-sulphide and Co-sulphate. The solubility in water and that in biological matrices differ on the basis of the cobalt salt present: salts with S, P, Cl, N and cobalt(II) oxalate are soluble in water, while Co-carbonate and Co-hydroxide are not. Metallic Co and CoO are insoluble in water, but they are soluble in biological matrices (ATSDR 2004).

Information on the distribution of cobalt in the body after dermal, gastrointestinal or respiratory absorption is also inconsistent. According to some authors, the elimination kinetics is rather fast, for others it is slow. In their literature review, Lauwerys and Lison (1994) observed that "cobalt in urine and in blood expresses recent exposures". Data from Alexandersson (1988) and Leggett (2008) indicate that the blood Co decrease is quite slow suggesting a better relationship with less recent exposure. During long-term, systemic cobalt exposure in humans and laboratory animals, cobalt accumulates in tissues, particularly liver and kidney, and the cobalt concentration is increased in whole blood, serum and urine (Simonsen et al. 2012; Leggett 2008).

The different chemical forms of cobalt make a difference in biokinetics. Lison et al. (1994) described a large discrepancy in the biological behaviour of the metal between two groups of subjects, one exposed to metallic cobalt and the other to cobalt oxide, even if the air cobalt concentration was similar for the two groups: the average concentration of cobalt in urine was lower in the group exposed to cobalt oxides than in that exposed to cobalt metal.

After intravenous administration of the radionuclide ^{60}Co chloride in 11 healthy adult subjects, a fraction of about 90% of the radioactivity was removed from plasma within 30 min, while in the following 30 h the radioactivity of cobalt decays with an half-life of about one day, with urinary excretion prevailing over faecal excretion (Smith et al. 1972). For three subjects, the study continued for 1.000 days and the half-lives of 0.5, 6, 60 and 800 days were calculated according to a four exponential function.

Leggett (2008) described the biokinetics of inorganic cobalt, after reviewing the information available, proposed a model for the biological behaviour of inorganic cobalt in humans and laboratory animals. Biokinetics of cobalt depends on its chemical form and the mass administered, and varies among species, with humans having the highest long-term component of cobalt retention. The same author noted that the distribution of cobalt in the specific components of blood is not well known.

Finally, when the biological behaviour of accidentally inhaled Co^{60} was investigated for up to 11 years, a quick excretion of most of the absorbed cobalt was observed, while the remaining 10% had a half-life of 5–15 years suggesting a strong and significant binding to human tissues (ATSDR 2004).

In summary, due to the different and sometimes conflicting data and interpretations present in the literature there is no agreement yet about the best biological monitoring tools to be used in workers occupationally exposed to metallic cobalt and cobalt oxide. In order to further study the cobalt excretion kinetics in humans and, also, to try and address the critical point of which biomarker is best for biomonitoring long-term occupational Co exposure, we performed a three-stage study measuring Co concentrations in urine, plasma, whole blood and red blood cells (haemoglobin adducts) in workers from 3 different hard metal plants.

Materials and methods

Study rationale and design

This research based on an agreement between the factory management and the trade unions was designed and performed according to the national legal obligations of the occupational physicians concerned in monitoring workers' exposure (through environmental and/or biological monitoring) and health (Legislative Decree 81/2008). The workers involved in the study had undergone, along several years, regular clinical examinations by the factory's accredited occupational physicians without any specific changes related to cobalt exposure being found. All the workers involved in the study (mean age and range of 40 and 26–54 years, respectively) were in good health conditions and gave their individual written informed consent to participate.

In the first stage of the study, we involved 10 male subjects working at sintering processes in 2 different hard metal plants: they gave a urinary sample at the beginning and at the end of every work shift, every day for a work week (from Monday to Friday). Four workers provided an additional urine sample collected on the following Monday morning.

In the second stage of the study, we enrolled 34 workers (10 of which were female) employed in another hard metal factory who were asked to give a urine sample at the end of the shift (after a shower, to prevent external contaminations of the sample) every Thursday for 16 following weeks and a singular blood sample on Friday morning of the last

week of collection, before shift. At the end of the sampling period, we obtained 22 workers (6 females) giving at least ten out of the sixteen urine samples expected and also the blood sample. Among these workers, one used to weigh and blend the Co powders, 9 workers had the task to compacting powders, 2 workers were assigned to the sintering operations, 8 workers were involved in braze welding, and the last 2 workers were department supervisors. All working stations had local ventilation devices and workers used to wear protective clothing and gloves but not masks. Along the 16 weeks, several workers switched among different job sites, as it often happens in small factories, so little information is available on individual exposure. Co airborne concentrations were measured 4 years before our study (but no technical innovations were performed in that period), in thirteen personal air samples (inhalable fraction of dust) each taken, continuously for 4 h, from the breathing zone of the workers. The total dust collected on the filters was weighted and after filter dissolution in nitric acid Co was measured with an atomic absorption spectrophotometer. Quite different levels of exposure were found in the various departments of the factory. Mixing and granulation of Co powders were the tasks with the highest exposure to the metal (range 0.121–0.576 mg/m^3). These activities, however, were not carried out continuously during the work shift, nor daily. Middle exposure levels were found in the sintering and mould-filler units (range 0.011–0.074 mg/m^3), and low concentrations were present next the braze welding unit (range 0.003–0.008 mg/m^3).

Although measurement of Co concentration in workroom air may give useful information about occupational exposures, in hard metal plants the data reported in the literature are in fact very fluctuating. Apostoli et al. (1994) described exposures as wide as of 50–8000 μg/m^3 with leaks lasting 2–4 h per day, one or two times per week, whereas for the other days Co exposure was very low. Scansetti et al. (1983, 1985), Alexandersson (1988), Linnainmaa and Kiilunen (1997) and Ichikawa et al. (1985) also observed very wide ranges of Co exposure in hard metal tool production plants due to highly variable operating conditions. This suggests that biological monitoring should give more reliable results, when compared to environmental monitoring, about the actual Co intake.

In the third phase of the study, the Co excretion kinetics were studied in urine, plasma, whole blood and haemoglobin adducts. We gathered these biological samples from the same workers enrolled in the second phase: 22 urine and blood samples were collected at the end of shift before a long period of absence from work (Christmas holidays) and before resuming work 18 days later.

Sample collection: All urine samples provided by the workers on different times were frozen at −20 °C until analysis. In order to avoid contamination, nonmetal collection vessels were used and detailed instructions were given prior to sampling. Blood samples were drawn by butterfly needles using metal free Vacutainer® Trace Element K2EDTA tubes (BD, Franklin Lakes, NJ, USA). Blood samples were processed and prepared for analyses within a few hours after collection. In order to isolate haemoglobin to measure Co adducts, red blood cells (RBCs) were separated from plasma by centrifugation at 1500×*g* for 5 min. with Megafuge 1.0 (Heraeus Sepatech, Langenselbold, Germany). Plasma samples were stored at −20 °C until analysis. After removing the top layer (platelets and white blood cells), RBCs were washed three times by centrifugation at 1500×*g* for 10 min in 0.9% NaCl solution and finally frozen, after removal of the supernatant to obtain packed red blood cells. Just before analysis, after thawing and diluting 1:4 with low resistivity water, cellular debris was pelleted by centrifugation at 13,000×*g* for 20 min at 4 °C and the supernatant haemoglobin fraction was separated by decantation (Olivieri et al. 2001). As reported by Simonsen et al. (2011), cobalt in the cytosol is tightly and irreversibly bound to haemoglobin during the entire life span of the red blood cells and it should be therefore defined more properly as adduct to haemoglobin. Analysis of the cytosolic Hb was carried out using an ADVIA® 2120 System (Siemens).

ICP-MS analysis

For ICP-MS analysis, plasma and urine were diluted 1:10 with 0.5% nitric acid (Suprapur 65%, Merck, Darmstadt), while whole blood and RBC cytosol samples were diluted 1:10 with low resistivity water obtained from a Milli-Q Millipore system.

The measurements were performed using an ICP-MS (Thermo Fisher Scientific X Series II) instrument equipped with a Peltier cooled impact bead spray chamber, a single-piece quartz torch and the enhanced sensitivity PlasmaScreen Plus option, together with Xi interface cones.

The instrument operates with the collision cell technology (CCT) with kinetic energy discrimination (KED), by using helium as the collision gas and with in-sample switching between the standard and the collision cell modes. The ion lens settings, the nebulizer gas flow rate and the torch position of the instrument were optimized daily, using ^{115}In counts in order to obtain the maximum sensitivity. Sensitivity, stability and oxide levels were tested every day by performing a short-term stability test. The following isotopes were measured: KED mode, ^{59}Co, ^{103}Rh; standard mode, ^{59}Co, ^{103}Rh (the last isotope was used as the internal standard).

The following operating conditions were used: RF power, 1400W; nebulizer gas flow, 0,82 mL/min; auxiliary gas flow, 0,80 mL/min; cool gas flow, 13,0 L/min.

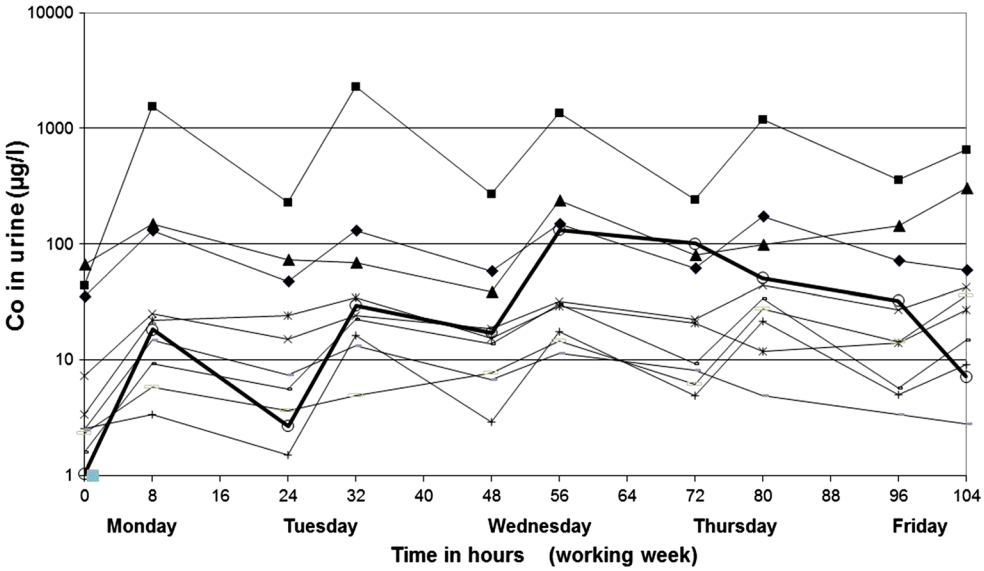

Fig. 1 Levels of Co in urine of ten workers who provided samples before and after the work shift for five consecutive days of a working week

In order to control matrix effects of serum and blood constituents, calibration curves were prepared by addition of known amounts of cobalt to each matrix (serum, urine, whole blood, RBC cytosol). Co detection limits were as follows: in urine, LOD = 0.02 µg/L, LOQ = 0.06 µg/L; in whole blood and plasma, LOD = 0.06 µg/L, LOQ = 0.20 µg/L; in globin, LOD = 0.06 ng/g, LOQ = 0.2 ng/g.

Certified internal quality controls were analysed during each analytical run: ClinCheck® Control (Recipe, München, Germany) was used for urine, and Seronorm® Trace Elements Serum and Seronorm® Trace Elements blood (Sero, Billingstad, Norway) for plasma and whole blood. The laboratory participates in the External Quality Assessment Scheme organized by the Network "*Organisers of EQAS for Occupational and Environmental Laboratory Medicine*" (Arnaud et al. 2009). Urinary creatinine was determined by the Jaffe method (Jaffe 1886).

Statistical analysis

Since the data were not normally distributed, as frequently occurs in biological and environmental measurements, nonparametric tests were used for the statistical analysis. Spearman correlation rates were calculated to identify possible associations between different variables, and the Mann–Whitney–Wilcoxon test (test *W*) was used to compare median levels with nonparametric distributions. The confidence level for hypothesis testing was 0.05. The abbreviation N.S. was used to describe a statistically not significant result. All statistical analyses and elimination rate constants (following the method described by Fichtl 1999) were carried out using a software Statgraphics® and Windows Excel (2007).

Results

First stage

In this stage, 10 male workers with different work tasks from two separate hard metal factories were involved. Co concentrations in urine samples collected from these workers before and at the end of the work shift during an entire work week were quite variable, nevertheless with a tendency to increase within the work day and throughout the work days. To better illustrate the variability of Co concentration, the data are shown in Fig. 1. Six workers, with Co levels on Monday morning lower than 7.3 µg/L, maintained fluctuating low Co levels at the end of the work shift, ever under 45 µg/L, indicating a quite low absorption of the metal. One worker with only 1 µg/L of Co in urine on Monday morning (marked with a thicker line in Fig. 1) reached urine Co concentrations of 100–130 µg/L in the middle of the working week. The last 3 workers (marked with fitted symbols) had urine Co concentrations between 35 and 66 µg/L on Monday morning: in two cases, Co concentration in urine raised up to 150–300 µg/L, with wide fluctuations, whereas in one case Co concentration in urine raised up to 2000 µg/L and even higher, at the end of some shifts. Interestingly, in 3 occasions the urine Co concentrations at the end of the work shift were lower as compared with those of the following morning. In any other case, and every day of the sampling week, in these workers urine Co concentrations at the beginning of the shift were lower than those measured at the end of shift and, generally, increased throughout the work week, showing that Co was gradually accumulating in the body.

Assuming that fluctuations of Co concentration in urine are due to rapid absorption during exposure and to fast but not complete excretion in urine afterwards, a decrease would be expected to occur during the 16 h between the end of shift and the beginning of a new work shift. Under the hypothesis of an exponential decrease in cobalt concentrations from the end of a shift and the start of the next shift on the following morning, a mean elimination rate constant (k) of 0.05433 h^{-1} was calculated for the 10 workers together, corresponding to an urinary half-life of cobalt in urine of 12.7 h.

Data obtained from the 4 workers which provided urine samples also on the following Monday morning after the sampling week described above allowed to calculate a mean elimination rate constant (k) in the time span of 64 h of 0.02402 with an half-life of Co in urine of 29 h. With such an half-life, the lapse of time without any exposure during the weekend is not long enough for a complete Co washout, thus resulting in slightly rising jags in the lines connecting urinary concentrations of cobalt in workers repeatedly exposed to the metal. Figure 1 shows that some subjects seem to have a higher body burden of cobalt than others and that their urinary levels of the metal are increasingly higher also at the start of the next shift, just a little more every day, with a quick increase during every shift. Other workers have urinary Co levels of about an order of magnitude lower, although with the same kind of increase throughout the shifts. So urinary excretion of cobalt appears to be quite fast but not complete throughout the work days, thus suggesting that the metal accumulates in the body.

Second stage

In the second stage of the study, 34 workers employed in a hard metal production plant and exposed to metallic cobalt and cobalt oxide were investigated by collecting their urine samples regularly (every Thursday at the end of the work shift along 16 weeks) and a single blood sample on the last Friday morning at the end of the 16 week biomonitoring period. Table 1 contains all the data obtained during this stage, i.e. the number of samples collected every week and the statistical parameters related to Co concentrations found in urine samples (median, arithmetic and geometric mean, range) expressed in μg/L and in μg/g creat. A large variability, spanning three orders of magnitude, was observed in Co concentrations, ranging 1.1–649.9 μg/L. Median values (8.5–17.6 μg/L) and geometric means (8.6 to 17.3 μg/L) were quite similar. In the second half of Table 1, the same data were recalculated according to the urinary concentration of creatinine and excluding all data from samples with a creatinine concentration in urine lower than 0.3 g/L or higher than 3 g/L. In this second analysis too, Co concentrations

Table 1 Concentrations of Co in urine samples provided by workers on Thursday at the end of their work shift along 16 weeks of exposure

Week	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	Total data
DATA in μg/L																	
Number of samples/workers	33	32	31	32	33	29	27	24	29	27	29	28	28	25	25	26	458
Median	11.1	13.9	13.4	13.1	12.5	17.6	9.5	9.1	16.8	8.8	10.1	8.5	14.9	10	11.8	9	11.3
Geometric mean	17.3	13.7	12.5	16.1	15.8	16.8	12.4	11.6	13.9	11.9	16.4	11.3	16.1	10.7	12.6	8.6	13.5
Geometric S.D.	1,51	1,29	1,21	1,20	1,43	1,27	1,00	1,21	1,18	1,00	1,25	1,39	1,21	1,35	1,37	1,27	1,3
Minimum	1.8	2.2	1.4	2.6	1.7	1.8	3.3	2.4	2.1	3.0	2.1	1.1	2.1	1.2	1.6	1.1	1.1
Maximum	649.9	331.0	133.0	398.0	164.5	189.0	96.1	101.1	84.7	74.4	405.6	134.3	201.5	102.5	138.2	124.2	649.9
DATA in μg/g creat																	
Number of samples/workers	33	31	29	32	32	29	24	23	29	26	28	27	28	21	25	26	443
Median	13.8	18.1	14.8	18.2	14.2	20.6	7.7	19.5	9.3	9.1	13.2	8.8	12.6	21.4	14.1	7.5	13.8
Geometric mean	16.4	15.6	14.5	17.6	14.4	20.2	13.0	13.1	11.9	13.3	16.3	12.3	12.7	15.2	12.4	8.5	14.1
Geometric S.D.	1.5	1.3	1.4	1.3	1.6	1.4	1.2	1.4	1.3	1.2	1.4	1.4	1.3	1.4	1.3	1.3	1.4
Minimum	1.5	1.6	1.1	1.7	1.1	2.0	1.6	1.6	0.9	1.7	1.2	1.3	1.1	1.9	1.2	0.7	0.7
Maximum	464.2	194.7	266.0	331.7	263.2	204.3	106.8	171.3	120.0	105.0	338.0	167.9	102.8	106.9	152.7	61.4	464.2

The number of urine samples collected in each week, the median, the geometric mean, the arithmetical mean, and the minimum and maximum values observed are reported for data expressed either in μg/L or in μg/g creat: the latter are a little less numerous on some days, due to the exclusion of 15 samples for which the creatinine concentration was out of the 0.3–3 mg/L range

Table 2 Concentrations of Co (μg/L) in each urine sample provided on Thursday at the end of the work shift along 16 consecutive weeks in 22 workers which gave each at least 10 urine samples throughout the 16 weeks and a blood sample at the 16th week

Worker/main job	1st week	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
1-Dept. head	2.9	22.4	2.1	2.6	1.8			2.6	2.5	3	2.1	2.3	2.1		1.6	1.2
2-Welding	1.8	2.2	1.9	6.5	3.9	3.5	3.8	2.5	2.1	3.4		3.1	7.2	3.8	6	3.6
3-Dept. head	3.6	5	5.7	7.8	3.8		3.9				7.3	2.9	2.2	1.8	1.9	3.6
4-Welding	5	63.7	3.5	2.6	3.7			4.4			7.3	1.1	4	1.8		1.1
5-Welding	4.4	7.3	5.3	3.8	5	5	4.3	4.5	3.2	5.1	4.5	3.6	5.2	3.6	3.3	2.9
6-Welding	3.6	45.6	4.9	4	6.4	8			4.1	3.7	6.7	2.4	3.8	1.2	4.2	1.4
7-Welding	5.1	8.5		6.3	12.5	5.4	4.8		4	7.5	6.7	5.1	6.6	4.7	4.3	2.8
8-Welding	3.8	15.4	8.1	2.9	4.1	8.2	6.6	2.4	9.9	6.7	22.7				9.7	8.7
9-Sintering	11.6	13.3	23.5	12.9	7.5	10.5	8.8		3.7	6.5	8	3.6	6.3	9	4.6	3.6
10-Welding			12.3	9.4	11	16.2	5.5	4.5	4.6		6.1	5.3		3.7		43.2
11-Welding	8.4	80.1	6.9	12.3	4.2	2.9	10.9	10.9	9.9	10.5	30.4	9.4	12.6	9.5	7.1	6.8
12-Pressing	18	103	20.2	23.4	18.7	19.7	6.2	7	5.4	8.8	9	8.3	29.5	7.2	11.8	9
13-Sintering	39.9	8.9	20.5	21.2	19.3	7.7	9.5	10.3	7.2	8	14.4	8.8	25.2	11.9	14.1	21.5
14-Pressing	222	3.2	15.7	7.5	40.8	189	24.6	49.8	21.1		9.2	3.8	9.4		3.7	3.1
15-Pressing	37.3	31.7	15.2	28.9	23.1	20.6	13.8		23.1	20.4	18.7	16.6	17.6		12.3	10.1
16-Pressing	10.6	38.4	7.4		9.5	25.8	7.1	16.8	53.2	33	31.2	133.5	24.1	19.7		13.9
17-Pressing	25.5	2.3	18.3	48.7	49	25			42.2	21.7	23.4	51.7	44.5	20.5	80	17.4
18-Pressing	119.3		13.4	37.7	164.5	14.8	36.4	27.2	18.5	73.5	63.7	13.3	51.3	10	49.6	23
19-Pressing	119.7	72.3	35.3	43	136.6	98.2	15.7	51.4	48		32.7	47	51.4	10.3	45.8	11.4
20-Pressing	42.4	27	123.4	36.2	153.1	25.3	18		48.8	54.3	79.9	45.8	34.3	102.5	43.4	30.7
21-Pressing	104.2	2.7	127.6	135	138.9	111.5	71.6	101.1	41	74.4	170.6	123.5	201.5	88.3	138.2	124.2
22-Mixing powders	649.9	41.1	133	398	157.9	183.9	65.7	30.8	79.8	37.3	405.6	134.3	141.4	96.2	135.6	59.2

Main job of workers is reported in the first column

were ranging widely, 0.7–464.2 μg/g creat., and values of medians and geometrical means of total data were similar to those obtained previously, before creatinine correction.

Each of twenty-two subjects gave 11–16 urinary samples, plus a blood sample in the last sampling week, on Friday morning before starting the work shift (Table 2). Five other subjects provided only 2–8 biological samples each, plus the blood sample, and the remaining 7 subjects provided 10–16 urinary samples without the blood sample (the data of these latter 12 workers were not included in the statistical analysis).

Figure 2 shows the Co levels in the urine of the 6 workers who provided all the 16 urine samples required. Values are substantially not uniform and there is a high inter- and intra-individual variability. One subject had levels constantly lower than 10 μg/L, others had levels always fluctuating under 100 μg/L, while 2 subjects had Co levels largely exceeding 100 μg/L, up to 300–650 μg/L.

Table 3 shows the results of those 22 workers who provided both the urinary end-shift samples and the blood sample. From this blood sample, Co levels were measured in whole blood, in plasma and in haemoglobin adducts. Co concentration shows a considerable variability, both in blood and in urine. The first 9 subjects listed in Table 3 had average concentrations of cobalt in urine lower than 10 μg/L, 9 other subjects had Co levels between 10 and 50 μg/L, and the last 4 subjects had average levels between 54 and 171 μg/L. Also the concentrations of Co measured in whole blood, in plasma and in haemoglobin were ranging within two orders of magnitude in each matrix. Co concentrations in whole blood and in plasma were very similar to each other, indicating that there were no significant differences in the distribution of the metal between the different blood components.

Figure 3 shows the correlations between the concentrations of Co in urine expressed in μg/L (geometric mean of the data from each worker, with samples collected over 16 weeks), and those in whole blood samples or in haemoglobin, collected at the end of the 16 weeks from the same 22 workers. Data are reported on a logarithmic scale and show that the average Co concentrations in urine are closely correlated with the Co concentrations measured, both in the whole blood and in haemoglobin, in the blood samples collected at the end of the 16 weeks. Similar results were obtained with urine Co concentrations expressed in μg/g creat (results not shown).

In Table 4, the correlation coefficients between cobalt concentrations in urine and those in blood samples (whole

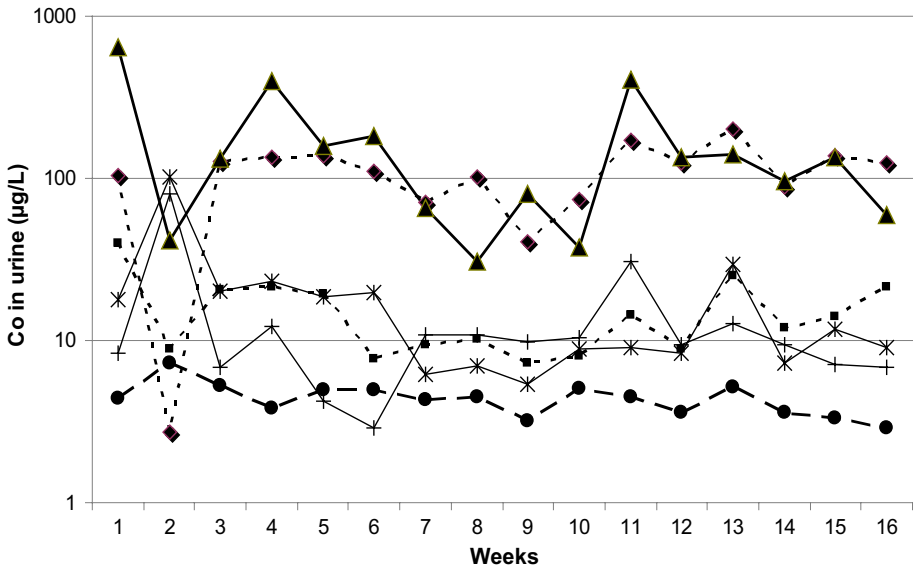

Fig. 2 Logarithmic concentrations (in µg/L) of Co in the end-of-shift urine of 6 workers who provided an urine sample regularly, every Thursday, at the end of the working day, along 16 consecutive weeks

Table 3 Concentrations of Co in urine samples (µg/L and µg/g creat) provided by each worker at the end of the work shift on Thursday along 16 consecutive weeks and reported as geometric mean, median and range

Worker/main job	Cobalt in urine (µg/L)			Cobalt in urine (µg/g creat.)			Cobalt in blood		
	Geometric mean	Median	Range	Geometrical mean	Median	Range	Globin ng/g	Whole blood (µg/L)	Plasma µg/L
1-Dept. head	2.6	2.3	1.2–22.4	1.5	1.2	0.7	0.6	0.35	0.5
2-Welding	3.4	3.5	1.8–7.2	4.4	4.2	1.7–8.6	2.7	0.88	1.1
3-Dept. head	3.7	3.7	1.8–7.8	4.3	3.8	1.9–19	3.6	0.83	0.9
4-Welding	3.9	3.7	1.1–63.7	6.0	3.9	3.3–49	1.8	0.65	0.8
5-Welding	4.3	4.5	2.9–7.3	2.6	2.7	1.5–4.1	1.6	0.53	0.7
6-Welding	4.4	4.1	1.2–45.6	4.3	4.0	2.0–45.6	1.5	0.64	0.8
7-Welding	5.7	5.3	2.8–12.5	4.1	4.0	2.8–7.4	3.7	0.72	0.8
8-Welding	7.0	8.1	2.4–22.7	6.4	5.8	2.0–38.5	2.3	1.05	1.4
9-Sintering	7.7	8.0	3.6–23.5	16.7	16.3	6.0–58.8	8.3	1.72	2.2
10-Welding	8.3	6.1	3.7–43.2	14.7	17.8	4.6–28.8	8.3	2.37	3.0
11-Welding	10.2	9.7	2.9–80.1	6.9	5.7	2.8–89.0	6.2	1.31	1.4
12-Pressing	13.4	10.4	5.4–103	29.4	29.4	13.5–79.2	9.6	2.67	3.6
13-Sintering	13.7	13.0	7.2–39.9	10.9	9.9	4.7–29.3	6.5	1.61	2.1
14-Pressing	15.8	12.6	3.1–222	11.4	8.7	1.8–148	16.9	1.84	1.4
15-Pressing	19.4	19.6	10.1–37.3	11.4	10.4	5.9–35.2	8.0	1.34	1.3
16-Pressing	21.4	21.9	7.1–133.5	57.2	64.6	21.2–123.3	45.8	7.44	7.2
17-Pressing	26.6	25.3	2.3–80	28.8	30.3	7.7–68.3	16.4	5.24	6.6
18-Pressing	34.1	36.4	10–164.5	60.3	65.5	23.1–170.4	20.1	3.64	4.1
19-Pressing	42.7	47.0	10.3–136.6	109.5	131.7	38.0–195.1	37.8	6.52	6.4
20-Pressing	47.9	43.4	18–153.1	70.6	67.5	30.5–159.8	65.2	8.39	7.9
21-Pressing	86.9	117.5	2.7–201.5	59.9	62.5	13.5–135.0	45.9	10.67	13.7
22-Mixing powders	118.3	133.7	30.8–649.9	116.4	105.6	20.6–464.2	137.0	17.74	14.7

Co concentrations measured in blood samples provided on Friday morning before work shift at the end of the 16 working weeks period are also reported; data in blood and plasma are expressed in µg/L, while those in haemoglobin are expressed in ng/g globin

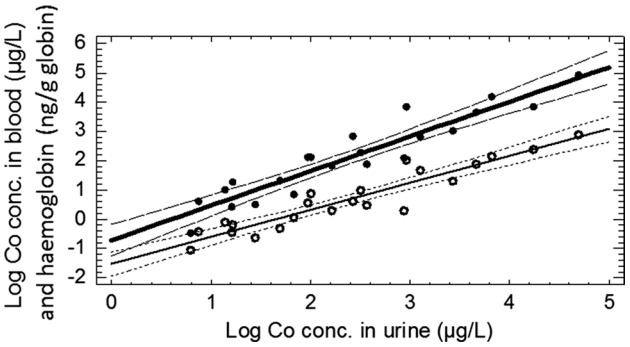

Fig. 3 Correlation between average (geometric) log concentration of Co in urine (in µg/L) collected throughout 16 continuous weeks, and log concentration of Co measured at the end of the 16 weeks either in haemoglobin (solid circles, thicker straight line, and dashed lines for 95% confidence interval): Log Co Conc. in haemoglobin (in ng/g globin) = –1.5302 + 0.9208 × (Log Co conc. in urine); $n = 22$, $r = 0.9389$, $p < 0.001$ or in blood (*open circles*, *thinner straight line* and *pointed lines* for 95% confidence interval): Log Co conc. in blood (in µg/L) = –0.7123 + 1.18 × (Log Co conc. in urine); $n = 22$, $r = 0.9379$, $p < 0.001$)

Table 4 Regression lines between Co concentrations in urine samples provided throughout 16 weeks (geometric mean) and those in blood (whole blood, plasma and globin) collected at the end of this period of study

Co (µg/L in blood) = 0.332 + 0.141 Co (µg/L in urine)	$r = 0.9579$
Co (µg/L in plasma) = 0.7324 + 0.133 Co (µg/L in urine)	$r = 0.9461$
Co (ng/g globin) = −2.019 + 0.9851 Co (µg/Lin urine)	$r = 0.9148$
Co (µg/L in blood) = 0.3714 + 0.1095 Co (µg/g creat. in urine)	$r = 0.8829$
Co (µg/L in plasma) = 0.8591 + 0.0999 Co (µg/g creat. in urine)	$r = 0.8459$
Co (ng/g globin) = −2.157 + 0.7794 Co (µg/g creat. in urine)	$r = 0.8589$
Co (µg/L in blood) = 0.8503 + 0.1319 Co (ng/g globin)	$r = 0.9650$
Co (µg/L in plasma) = 1.4181 + 0.1143 Co (ng/g globin)	$r = 0.8785$
Co (µg/L in blood) = 0.4753 + 0.9245 Co (µg/L in plasma)	$r = 0.9713$

Number of samples = 22 for all regression lines; $p < 0.001$ for all regression lines

The correlation among the three different components of blood is reported too

blood, plasma or haemoglobin) are reported. Correlation coefficients for the data expressed in µg/L are slightly higher than those for the data expressed in µg/g creat. There are good correlations also between Co concentrations in plasma and those in haemoglobin, between plasma and whole blood, and between haemoglobin and whole blood.

Third stage

The third phase of the study investigated the kinetics of Co after a fairly long period of non-exposure in 22 workers, 18 of whom had participated in the second phase of the study too, who provided urine and blood samples at the end of the work shift on the last day at work before Christmas holidays, and after 18 days, at the start of shift before resuming work. Table 5 reports the geometric means of Co concentrations in blood, plasma, urine and haemoglobin of these subjects, male and female together, as no gender-dependent statistically significant difference was found in Co kinetics in any of the different media.

During the period of non-exposure to Co, the per cent decrease in Co concentration was 64% in blood, 75% in plasma, 90.4% in urine and only 15% in haemoglobin. This is not surprising since Co is tightly bound to haemoglobin (Simonsen et al. 2011), and therefore, the decline is just due to haemoglobin replacement, that is of about 1.2% each day, exactly corresponding to 15% in 18 days. In the general population, the Co concentrations in urine and in blood corresponding to the 95th percentile are lower than 1.5 µg/L and 1 µg/L, respectively (SIVR 2011).

Assuming that the decrease in Co in whole blood and in plasma was both exponential, the data shown in Table 5, obtained after 18 days of non-exposure, allowed to calculate the Co elimination rate constant (k) in each biological matrix. Figure 4 illustrates the decrease in Co concentration in blood, plasma and haemoglobin. From the corresponding elimination rate constants (k), we calculated the half-life of Co in whole blood and in plasma that were 12.3 and 9.1 days, respectively. A similar calculation was also performed on the urinary concentrations from the 13 workers who had relatively high levels of cobalt in urine at the end of shift, above 10 µg/L. This calculation resulted in an elimination rate constant (k) of 0.1304, corresponding to a biological half-life of 5.3 days.

Table 5 Levels of Co (geometric mean) in blood, plasma, urine and haemoglobin from samples provided by 22 workers at the end of the last day of a working week before and at the beginning of a new work shift after a period of 18 days without exposure

Type of sample	Co concentrations at shift end before holidays	Co concentrations after 18 day without exposure	% of the starting level
Blood	3.77 µg/L	1.37 µg/L	36
Plasma	3.85 µg/L	0.98 µg/L	25
Urine	53.20 µg/L	5.09 µg/L	9.6
Haemoglobin	3.97 ng/g globin	3.39 ng/g globin	85

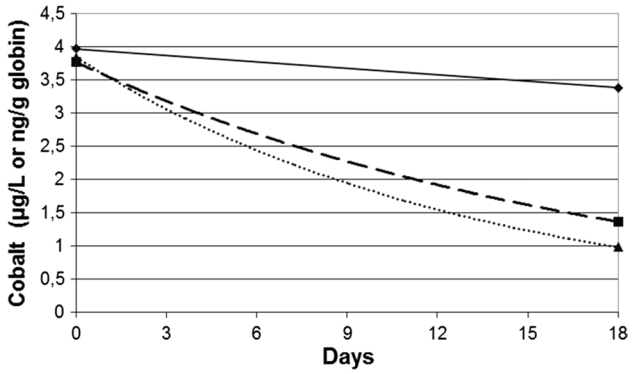

Fig. 4 Kinetics of Co in haemoglobin (ng/g globin, continuous line: $Y = 3.9738 - 0.0326\ X$), in whole blood (µg/L, *dashed line*: $Y = 3.7729\ e^{-0.0564X}$) and in plasma (µg/L, *dotted line*: $Y = 3.8463\ e^{-0.0758X}$), assuming an exponential decrease in blood and plasma. Data at time 0 and after 18 days are the geometric means of values obtained from 22 workers who provided the biological samples before (at the end of the work shift) and after (before the work shift) a rest period of 18 days

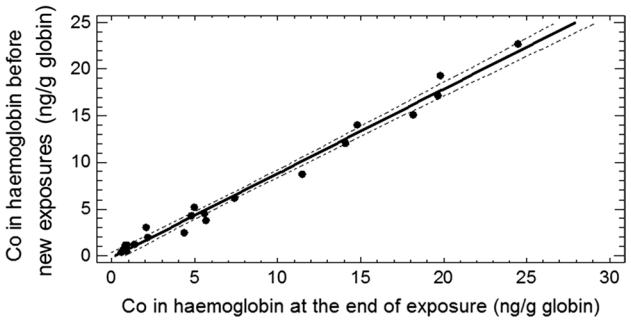

Fig. 5 Correlation between Co concentrations in haemoglobin (ng/g globin, dashed lines for 95% confidence interval) in blood samples collected from 22 workers before and after a period of 18 days without exposure (Y): Co in haemoglobin = –0.2097 + 0.9931 Co in haemoglobin; $n = 22$; $r = 0.9927$; $p < 0.001$

After calculating specific elimination rate constants, the statistical correlation was studied between the two collection times, before and after 18 days of non-exposure, for the levels of Co in each biological matrix. Figure 5 shows the highly significant linear correlation for Co levels in haemoglobin between the two collection times. The levels of Co in whole blood, plasma or urine also show a highly significant correlation as shown here:

Whole blood: $Y = 0.3405 + 0.28X$; $n = 22$; $r = 0.8552$; $p < 0.001$.

Plasma: $Y = 0.39 + 0.15X$; $n = 22$; $r = 0.9050$; $p < 0.001$.

Urine: $Y = 1.68 + 0.0669X$; $n = 18$; $r = 0.8475$; $p < 0.001$.

with $Y =$ Co concentration in samples collected at the start of shift after a non-exposure period of 18 days and $X =$ Co concentration in samples collected at the end of the last shift after several weeks of occupational exposure to Co.

Discussion

The levels of Co in blood and urine in this study are very often higher than the corresponding current biological exposure indexes suggested by ACGIH (2015) of 1 and 15 µg/L, respectively. The technical exposure limit proposed by DFG until 2004 was 60 µg/L in urine, corresponding to a concentration of 0.1 mg/m^3 in the air (DFG 2004), whereas after 2004 no limits were proposed, given the carcinogenicity of Co (DFG 2015). Studies on the biomonitoring of Co in urine following occupational exposure only provide partly consistent results. Differences in kinetics are probably due to the different chemical forms of cobalt used in industry (metallic cobalt, cobalt oxide, cobalt chloride, etc.), which can result in different kinetic behaviours. For this reason, any proposal for which biological marker of exposure should be used in workers occupationally exposed to Co should be diversified according to the specific type of cobalt salt used. Our study concerns workers exposed to particles of metallic cobalt.

The correlation between occupational exposure to Co and its concentration in urine was investigated by Pellet et al. (1984). In that study, five workers with different tasks in 3 factories provided urinary spot samples at the beginning and at the end of every work shift from Monday to Friday, in addition to a 24-h collection sample on the following Saturday and Sunday. Co concentrations in end-shift urine samples were increasing day after day, while on the following Monday, after a weekend without any exposure to Co, they decreased to early week pre-shift values. The trend of Co concentrations in urine on Monday through Friday reported by Pellet et al. (1984) was not much different from the one found in the present study (Fig. 1). These results overall sustain the hypothesis that Co partly accumulates in the body throughout the work week, particularly in those workers with an initial lower level of Co in urine. The Co fluctuations in urine samples provided at the beginning and at the end of a working exposure observed by Alexandersson (1988) and by Ferri et al. (1994) provide further confirmation that occupational exposure to Co produces a fast increase in Co level in urine, while urinary excretion is not so fast and the metal progressively accumulates in the body.

Occupational exposure to cobalt was also studied by Scansetti et al. (1985) in 26 workers of a hard metal manufacturing plant by analysing urinary samples collected at the end of the work shift on the last day before a period of rest, and after resuming work 4 weeks later, on Monday, Wednesday and Friday, for a period of four following

weeks. After holidays, the levels of Co in the first set of samples (collected on Monday morning) were similar to those of non-exposed subjects and lower than 1 µg/L. At the end of the first work week, cobalt levels in urine increased fourfold, and the following Monday, at the beginning of the work shift, they got back to starting levels. However, after three weeks of occupational exposure the rest period of 60 h during the weekend was no more adequate to reduce urine Co concentrations at low levels, suggesting that accumulation had occurred. The same group of researchers, after further studies on the absorption and the urinary excretion of Co, concluded that the biological monitoring of occupational exposure to cobalt by urine analysis is not very reliable, since Co excretion kinetic data are variable and because of the potential confounding effect of skin absorption (Scansetti et al. 1983, 1994, 1998).

Mosconi et al. (1994) also provided interesting kinetics data on the urinary excretion of Co in 12 subjects employed in a factory producing diamond grinding tools. In that study, workers provided urinary samples at the end of the work shift for five following days, after having been exposed to airborne concentrations of cobalt of 0.02–1.1 mg/m^3. Co concentrations in the range 30–1.200 µg/g creat., with elimination rate constants (k) ranging -0.0115 to -0.0230, corresponding to half-lives of 60 and 30 h, respectively. Five workers provided urinary samples for 24 days after the end of exposure, and from these data the authors calculated two Co elimination rate constants: the first one with an average half-life of about 2 days (43.9 h) and the second one with an average half-life of 10.3 days. These findings are not in contrast with those of Apostoli et al. (1994) who calculated a half-life of 19–22 h in subjects exposed to cobalt powders and providing urine samples until 70 h after exposure.

Using the results taken from the first and the third phase of our study and bearing in mind the results by Mosconi et al. (1994) and Apostoli et al. (1994), we calculated the elimination rate constants resulting from the three different non-exposure time spans of our study. The first one, calculated considering 16 h of non-exposure, was equal to 0.055853 (corresponding to a half-life of 12.4 h). The second k resulting from a 64-h non-exposure period, was equal to 0.02425 (half-life = 28.6 h). The third k was 0.0053 (half-life = 130.7 h), as obtained from a period of 432 h of non-exposure during the 18-day vacation period of the workers.

Using these three elimination rate constants together, after some simulations we obtained that the decrease in cobalt in urine can be well described by the function below, as graphically represented in Fig. 6.

$$\text{Co}\ (\mu\text{g/L}) = 1.25^{(-0.055853\times t)} + 1.75^{(-0.02425\times t)} + 50.2^{(-0.0053\times t)}$$

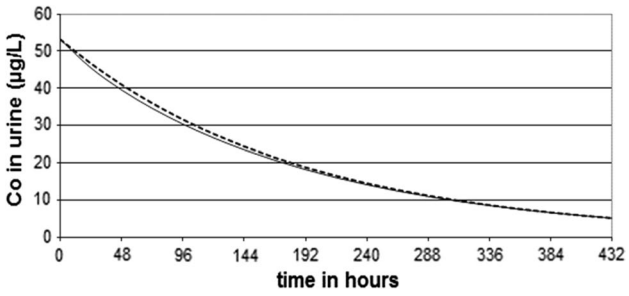

Fig. 6 Comparison between tri-exponential (*continuous line*) and mono-exponential (*dotted line*) decrease in cobalt in urine: the starting and final values (53.2 and 5.09 µg/L, respectively, see Table 5) correspond to the geometric means of Co concentrations in urine samples provided by 22 workers at the end of the last day of a working week before holidays, and before a new working shift, after a period of 18 days without exposure

with t = time in hours.

The corresponding mono-exponential function Co (µg/L) = $53.2^{(-0.13\times t)}$ is also reported in Fig. 6 which highlights that the cobalt excretion kinetics in urine is mainly determined by the slow elimination rate constant, while the rapid excretion constants (first 24 or 64 h) little alter the overall excretion rate of cobalt.

Taking all the evidence together, it would appear that the use of urinary samples for the biological monitoring of long-term occupational exposure to Co may bear some uncertainty because of the overlapping of different kinetic phases, the last of which is rather slow. Our data indicate that during the day-after-day working exposure Co gradually tends to accumulate in the body, despite the fast urinary excretion occurring for some hours after exposure.

Studies reporting cobalt concentration and kinetics in blood of exposed workers are not copious. Lison et al. (1994) reported that blood concentrations of cobalt in different chemical forms (-salt, -metallic, -oxide) are mainly due to recent occupational exposure. Despite the fact that the airborne Co concentrations in the oxide and the metal groups of workers were similar (median values of 383 and 467 µg/m^3, respectively, with a very wide range: 17–10,767 µg/m^3), Co concentration in urine and blood was lower in the oxide (median 70 µg/g creat and 1.9 µg/L, respectively) than in the metal group (median 161.6 µg/g creat and 2.8 µg/L, respectively). Strong correlations with the air concentration of Co were observed in samples collected at the end of shift on Monday, and even on Friday, for these workers except for those exposed to cobalt oxides.

Alexandersson (1988) investigated workers occupationally exposed to Co by collecting their urine samples for a week, at the beginning and at the end of each work shift. Air concentration of cobalt was 0.09 and 0.01 mg/m^3 on average in workers with high (n. 5) and low (n.5) exposure, respectively. Punctual urinary levels detected in that study

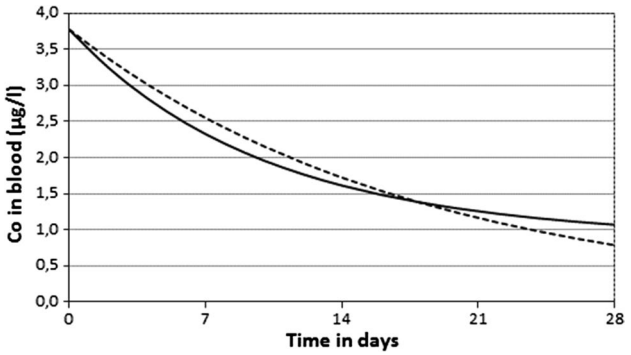

Fig. 7 Decrease in Co in blood during 28 days of non-exposure. Starting concentration (3.77 µg/L) and concentration of cobalt at the 18th day (1.37 µg/L) correspond to those shown in Table 5. The *continuous line* shows the Co kinetics using the biexponential decrease constants acquired from Alexandersson (1988), while the *dashed line* describes the mono-exponential decrease obtained with our results

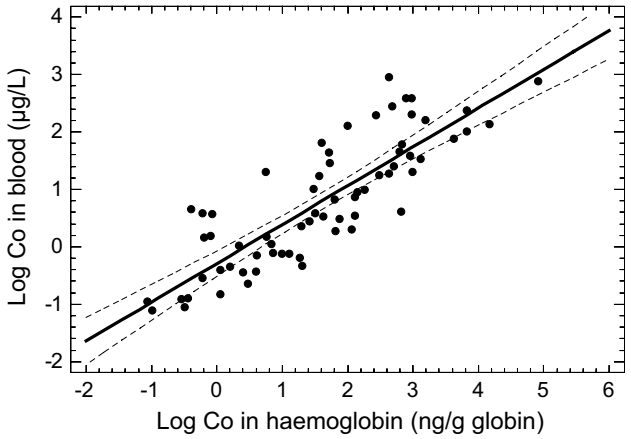

Fig. 8 Correlation between log concentrations of Co in blood (µg/L) and haemoglobin (ng/g globin): log Co conc. in blood = −0.2894 + 0.6758 (log Co conc. in globin); $n = 66$; $r = 0.8440$, $p < 0.001$)

were similar to ours, as shown in Fig. 1, which confirms a rather fast excretion kinetics for daily exposures. The same authors also collected blood samples at the end of the exposure and after 2 (48 h) and 28 (672 h) days. The calculated Co elimination rate constants (k) in blood from these two lapses of time of non-exposure were: $k_{48} = -0.104$ and $k_{672} = -0.034$, corresponding to an half-life of 6 and 20.3 days, respectively. When we applied these two rate constants to our data, the results were similar to those obtained using our own rate constant (Fig. 7): the model obtained with Alexandersson's elimination rate constants (k) in blood shows the predominant contribution of the slow component in the removal of Co from blood, with only small differences from the model built with the mono-exponential decrease.

Figure 8 shows the statistically significant linear correlation between blood and haemoglobin Co concentration of using all the blood samples collected during the second and third stages of our study together, including those collected before starting shift and those collected at the end of the work shift (as data are not normally distributed, logarithmic scales were used to describe the statistical correlation).

Our results confirm and reinforce previous observations indicating that Co excretion kinetics in blood and in haemoglobin are much slower than in urine, suggesting that Co in urine is a reliable parameter only to monitor recent exposure. They also indicate that the biological monitoring of occupational exposure to cobalt by using blood concentrations of the metal, or its adducts to haemoglobin, may also provide consistent and useful information on the absorption of Co during long periods of time, up to a few months. Our findings are in agreement with those of Simonsen et al. (2011), who concluded that: "for biomonitoring of long-term Co exposure it could be appropriate to measure the Co content in red cells to give an average value for the exposure over the 120 days life span of red cells". In conclusion, the results of our study overall support the idea that Co blood levels, which were shown to be in good correlation with Co adducts to haemoglobin, should be preferred to urinary measurements for the biomonitoring of workers with a long-term occupational exposure to Co in hard metal facilities.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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