Original Research

Association of Occupational Exposure to Chromium with Tumour Markers and Selected Biochemical Parameters

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Abstract

Twenty-four male industrial workers, exposed to various chromium compounds for 1-17 years (but deprived of overt symptoms of chromium toxicity) were the subjects of our study. The workers' urine chromium was measured serially by atomic absorption spectrometry. The simple kinetic model was proposed for tracking pre-shift and post-shift as well as day-to-day variation of urine chromium concentrations. This model proved to be useful in determining the biological effects of exposure to chromium compounds. Subsequently, the statistically significant associations of chromium status parameters (as delivered by the kinetic model) with biochemical indices, blood panel parameters, spirometric indices, and concentrations of tumour markers [carcinoembryonic antigen (CEA), squamous cell carcinoma antigen (SCC-Ag), cytokeratin 19 fragment (CYFRA 21-1), and neuron-specific enolase (NSE)] in those workers were evaluated by means of the hierarchical partial least squares method (PLS2 model). These relations should be further studied in larger groups of workers. In several individuals, outlined in the PLS2 model, cigarette smoking did not constitute any important source of chromium, although seniority at work in the chemical plant did influence biochemical status.

Introduction

Chromium(III) is an essential nutrient in humans, having significance in sugar metabolism, immune processes, and neuroendocrine responses [1-4]. However, numerous studies have shown that chronic occupational exposure to chromium compounds and their uptake can cause deleteri-ous long-term health effects to employees. The exposure to chromium due to chemical manufacturing of that element can occur predominantly by inhalation or through skin contact with chromium-contaminated dust and aerosols usually containing particularly harmful hexavalent chromium (Cr(VI)) compounds (most often – chromate or dichromate). People working in such conditions – mainly workers in chrome plating industry, stainless steel manufacturing, and pigment production, especially those having

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no proper outfit against ambient air (gloves, masks, protective clothing) – have been recognized as being at high risk of airway irritation and obstruction (bronchospasm, occupational asthma, nasal ulceration), allergic dermatitis and rhinitis as well as possibly lung cancer and other malignancies (e.g. prostate cancer, leukemia) [2, 5]. Bagchi et al. demonstrated that a cascade of cellular events, including DNA damage, is responsible for chromium(VI)-induced toxicity and carcinogenesis [6]. In contrast, trivalent chromium (Cr(III)), being the kinetically stable and not well soluble form, is poorly absorbed in the gastrointestinal tract and is generally assumed not to be toxic [7].

On the other hand, increased levels of tumour markers: carcinoembryonic antigen (CEA), squamous cell carcinoma antigen (SCC-Ag), cytokeratin 19 fragment (CYFRA 21-1), and neuron-specific enolase (NSE) may indicate the onset of the carcinogenic process. Moreover, elevated concentrations of these tumor markers are generally accepted to be unfavourable prognostic factors in lung cancer patients [8, 9].

Little information is available on possible relations between exposure to chromium compounds, tumour markers, and spirometric indices, as well as hematological and biochemical parameters in workers exposed to occupational doses of that metal. Therefore, the objectives of the current study were:
1) to formulate an adequate kinetic model of chromium intake and excretion;
2) to reveal the correlation structure of data by multivariate analysis (PLS2 model);
3) to check the importance of cigarette smoking and/or demographic parameters as potential sources of chromium in the individuals outlined in the PLS2 model.

Experimental

Subjects and Materials

The subjects of investigation were twenty-four male industrial workers of a small chemical plant, aged 23-53 years (mean 36.7), exposed continuously for 1-17 years (mean 7.2) to various chromium compounds at different concentrations, depending on the workplace. With only one exception, the subjects had no clinical or radiological symptoms of lung diseases. All of them were free of any acute disease. They underwent routine prophylactic examinations at the Małopolski Center for Occupational Medicine. Each subject gave his informed consent to participate in the study and was asked to provide information on height, weight, smoking habits, and medical history.

For the duration of the subjects' exposure, the chemical plant produced chrome oxides and chromate-pigments, including chromium(III) oxide, chromium(VI) oxide, chromium(VI) sulfate basic, sodium dichromate(VI), and potassium dichromate(VI). The concentration of chromate dust in the subjects' work environment ranged from 0.010 to 0.096 mg/m³ (geometric means for different days: 0.011

– 0.080 mg/m³), while the concentration of chromium(III) oxide was in the range of 0.008 to 0.100 mg/m³ (geometric means for different days: 0.009-0.080 mg/m³).

The concentration of chromium in urine was used as a biomarker of occupational exposure to this metal [10]. Urine samples were collected serially, within a ca. two-week period, before and after a work shift, into plastic cups which had previously been washed with 5% HNO_3. To avoid contamination with dust during sample collection, specimens were collected outside the working area and subjects were asked to wash hands. The urine samples were divided into two aliquots: the first was used for creatinine analysis, the second was spiked with conc. nitric acid to obtain 1% v/v final acid concentration and kept in refrigerator until the analysis.

Blood samples were taken from the cubital vein: one part (on anticoagulant) was used to determine the hematologic parameters and another was used for the biochemical examination. Hemoglobin and blood cell count were assessed using the hematological analyzer Cell Dyn 3700. Serum was separated 30 min after collection, transferred with a pipette to four polypropylene Eppendorf tubes and then frozen.

Analytical Methods

Spectrometric measurements of electrothermal atomic absorption of urine chromium concentrations were performed on the Perkin Elmer® Model 5100 ZL with longitudinal Zeeman-effect background correction equipped with an AS-70 automatic sampler and a chromium hollow cathode lamp (Norwalk, CT, USA). Purge gas argon, active gas air, and pyrolytically coated graphite tubes with stabilized temperature platform furnace were used throughout. Absorption readings, measured as peak area, were determined at the wavelength of 357.9 nm and at the slit width of 0.7 nm. The detection limit was 1.1 µg/L, precision was 8.5%, and the calibration range was 0-250 µg/L. An internal quality control procedure was used to validate the analytical method. Seronorm reference urine enriched with toxic elements was purchased to assess accuracy of Cr measurements (Batch No. 403125, Nycomed Pharma AS, Oslo, Norway). The mean analyzed value was 21.1±1.8µg/L (certitified value 20.0µg/L).

The serum total proteins concentration was determined by the biuret method, and the levels of electrophoretical fractions (α1-globulin, α2-globulin, β-globulin, γ-globulin) were calculated on the basis of densitometry of electrophoregrams, after separating proteins in agarose gel. CEA and SCC-Ag were determined by microparticle enzyme immunoassays (MEIA method) using the Abbott Laboratories kit and AxSYM and IMx systems, respectively. CYFRA 21-1 and NSE were measured by an electrochemiluminescence immunoassay (ECLIA) method with materials from Roche Diagnostics and using the Roche Elecsys 2010 analyzer. Spirometric parameters (forced vital capacity, FVC; forced expiratory volume in

1s, FEV$_1$; middle expiratory flow 75% FVC, MEF$_{75}$) were obtained from flow-volume curves and the computer-aided system (Lungtest MES, Poland). Blood was taken for all analyses, and the respiratory examination was performed when the workers had their day off.

Statistical Approach

Having several clusters of predictor parameters which constituted the groups of potential diagnostic factors (chromium status parameters, spirometric indices, clinical parameters and some other biochemical parameters) and several response parameters (tumour markers), we employed the hierarchical partial least squares regression method (PLS2) to establish data structure as well as a possible causal relationship between dependent variables and potential explanatory variables which are contained in the blocks of data. The PLS2 method is a well-known multivariate pattern recognition method, whose algorithm is based on the disjoint principal component model. PLS2 finds the numerical solution by means of correct, reduced dimensionality of the original data. It is also worth noting that the assumption of the postulated distribution of data is not necessary in PLS2. The PLS2 algorithm in various applications has been described in many papers [11, 12]. For the PLS2 analysis all parameters were scaled to unit variance. The PLS2 model was validated by full cross-validation. The plots of the PLS2 score vectors were used to identify outlying subjects. Statistical computations were carried out using the commercially available packages: SIMCA-P v.9 (Umetrics, Umea, Sweden), STATISTICA PL v.6 (StatSoft, Tulsa, USA), and Statgraphics (Manugistics, Rockville, USA).

Results

The typical course of changes in chromium concentration in urine (uCr) is presented in Fig. 1. The data on chro-

mium concentration allowed us to formulate the simplified kinetic model of chromium absorption and excretion with the zero- and 1st-order rate constant, respectively. The details of the model are provided in Appendix 1. Main descriptive statistics of uCr, and of kinetic model parameters are shown in Table 1. Table 2 summarizes main descriptive characteristics of the blood panel, spirometric and smoking parameters in the subjects, while Table 3 shows main descriptive statistics of biochemical parameters and tumour markers in the study. Mean values of the blood panel and biochemical parameters were always within respective normal ranges (only in 3 subjects were Hct values slightly beyond the upper limit (49), being in the range 50-51). Similarly, spirometric parameters (FEV$_1$, FVC and MEF$_{75}$) were decreased in 3, 2 and 1 subject, respectively. With the exception of NSE, all other tumour markers exceeded the upper limits of normal ranges in several individuals.

The statistically significant hierarchical PLS2 model with two significant components was derived. The eigenvalues were: 3.89 and 1.02. The first two components accounted for 98.2% and 86.1% of the variance in the predictor and response parameters, respectively. The predictor parameters included in this model were:
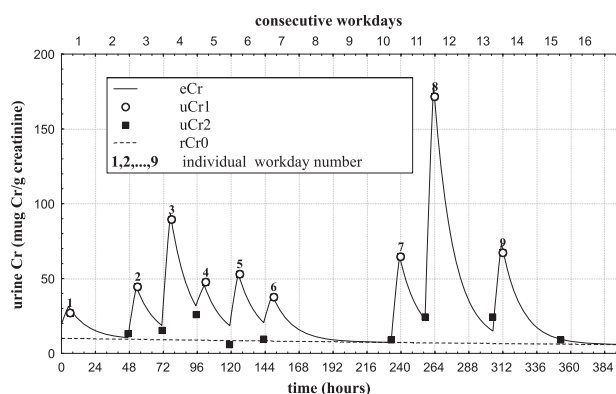

Fig. 1. Typical course of changes in chromium concentration in urine (for symbol meanings see Appendix).

Table 1. Main descriptive statistics of chromium concentration in urine (uCr), and of kinetic model parameters: mean half-life time of Cr elimination (t$_{1/2}$Cr), residual urine Cr concentration (rCr0), mean integral Cr elimination per workday (intCr), exposure-elimination zero-order rate constant (k^0_i) (see Appendix for explanation).

Parameter	Unit	Median	Quartile range	Range
uCr	μg Cr/g creatinine	17.9	12.9-27.5	1.3-61.2*
t$_{1/2}$Cr	hour	10.0	9.6-20.1	4.5-33.4
rCr0	μg Cr/g creatinine	6.0	4.9-11.3	1.0-36.4
intCr	μg Cr/g creatinine/workday	668	500-1291	253-4357
intCr normalized to 1 work-hour	μg Cr/g creatinine/hour	95.0	68.0-206.8	38.7-726.2
k^0_i	μg Cr/workday	2.52	2.1-6.1	0.06-19.0
k^0_i normalized to 1 work-hour	μg Cr/hour	0.41	0.28-0.91	0.01-3.17

* the reference value for urine Cr, determined in our laboratory in healthy adults, was 1.18±0.77 μg Cr/g creatinine.

Table 2. Main descriptive statistics of blood panel parameters (red blood cells – RBC, white blood cell – WBC, platelets – PLT, hematocrit – Hct, blood sedimentation rate – BSR), spirometric parameters (forced vital capacity – FVC, forced expiratory volume in 1s – FEV$_1$, middle expiratory flow 75% FVC – MEF$_{75}$), and smoking parameters (age of smoking commencement – ASC, Number of cigarettes smoked per day – NCSD).

Parameter	Unit	Mean	Min	Max	Normal range [13, 14]
RBC	cells /L	4.8	4.2×10^{12}	5.4×10^{12}	$4.3 – 5.7 \times 10^{12}$
WBC	cells/L	6.9	5.1×10^9	9.2×10^9	$4.5 – 11.0 \times 10^9$
PLT	cells/L	201×10^9	152×10^9	295×10^9	$150 – 450 \times 10^9$
Hct	%	45	40	51	39-49
BSR	mm/h	4.6	1.0	13.0	0-15
FVC	% N	97.6	64.7	113	≥ 80
FEV$_1$	% N	95.8	58.9	118	≥ 80
MEF$_{75}$	% N	99.3	39.5	172	≥ 60
ASC	yr	23	17	46	-
NCSD	cigarette/day	13	0	30	-

Table 3. Main descriptive statistics of biochemical parameters (TSP - total serum proteins, α1-globulin, α2-globulin, β-globulin, γ-globulin, albumin, LDH) and markers of carcinogenic effects (CEA -carcinoembryonic antigen, SCC-Ag - squamous cell carcinoma antigen, CYFRA 21-1 - cytokeratin 19 fragment, NSE - neuron-specific enolase).

Parameter	Unit	Median	Min	Max	Normal range [13, 9]
TSP	g/L	76.3	71.3	83.8	64.0 – 83.0
albumin	g/L	46.2	38.9	50.7	35 - 50
α1-globulin	g/L	3.2	2.8	4.2	1 - 3
α2-globulin	g/L	6.2	5.2	8.8	6 - 10
β-globulin	g/L	9.2	7.4	12.0	7 -11
γ-globulin	g/L	11.9	6.8	17.0	8 -16
CEA	µg/L	1.3	0.1	8.0	0.0 – 7.1
SCC-Ag	µg/L	1.2	0.6	2.4	0.3 – 2.1
NSE	µg/L	8.8	7.2	14.5	4.8 – 25.4
CYFRA 21-1	µg/L	1.6	0.6	3.3	0.2 – 3.0

1) chromium status parameters: mean chromium concentration in urine (after rejecting outlying values), median chromium concentration in urine after one workday, mean half-life time of chromium elimination via urine, mean integral Cr elimination per workday, residual urine Cr concentration (see Fig. 1 and Appendix); the latent variable calculated for this group of parameters is referred as 'Cr status.'
2) biochemical indices: concentrations of total serum proteins, albumin, α1-globulin, α2-globulin, β-globulin, γ-globulin; the latent variable calculated for this group of parameters is referred to as 'proteins.'
3) blood panel: BSR, Hct, RBC, WBC, PLT; the two latent variables calculated for this group of parameters are referred to as 'bp' and 'bp(BSR),' respectively. The variable 'bp' is mainly loaded by Hct, RBC, WBC, PLT, while the variable 'bp(BSR)' is almost solely loaded by BSR.
4) spirometric indices and smoking status: FVC, FEV$_1$, MEF$_{75}$, number of cigarettes smoked per day, age when subject commenced smoking; the latent variable calculated for this group of parameters is referred to as 'Spirometry.'

The response parameters included in the model were: CEA, SCC-Ag, CYFRA 21-1, NSE (tumour markers). The parameters of age, BMI, and k^0_i (exposure zero-or-

der rate constant) were omitted from this model as uninformative. The final plot, which comprised the weights (denoted as w*) that combined the predictor parameters with latent components, and the weights (denoted as c) that combined response parameters with latent components, showed that bp(BSR) and CEA were interrelated, while three other tumour markers were influenced by all the remaining parameters (Fig. 2). The plots showing the values predicted by the PLS2 model versus the observed ones for all response parameters enabled us to find the outlying subjects, i.e. those which were most distant from the regression curve. For example, Fig. 3 illustrates such a plot for CYFRA 21-1 where two points were regarded as outliers. Generally, of the 24 subjects, 7 were identified as outliers. The characteristics of these outlying subjects are listed in Table 4. The outlying subjects differ from the rest of the study group in respect to seniority at work, but neither in the age they started smoking nor in the number of cigarettes they smoke per day (Table 5, Fig. 4).
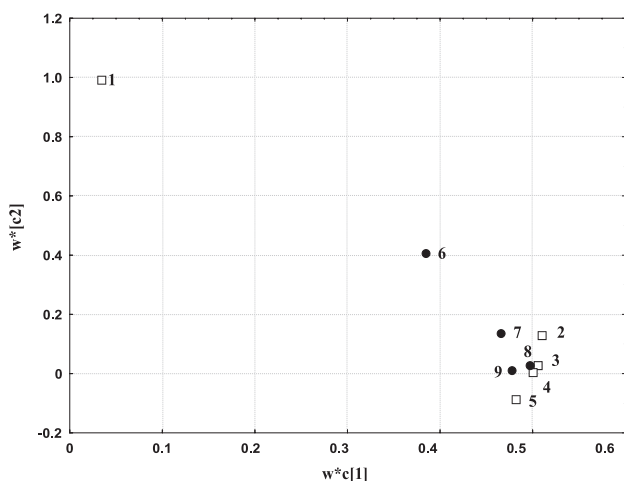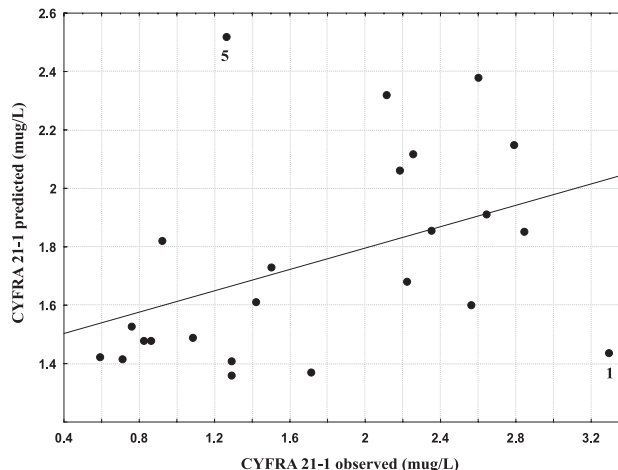

Fig. 3. The values predicted by the PLS2 model versus the observed ones for the CYFRA 21-1 parameter. The outlying subjects were denoted as 1 and 5, and their characteristics are provided in Table 4.

Fig. 2. The weights of the first two components of the PLS2 model (w*c1, w*c2). The meaning of labels: 1 – bp(BSR), 2 – bp, 3 – proteins, 4 – spirometry, 5 – Cr status, 6 – CEA, 7 – CYFRA 21-1, 8 – NSE, 9 – SCC-Ag.
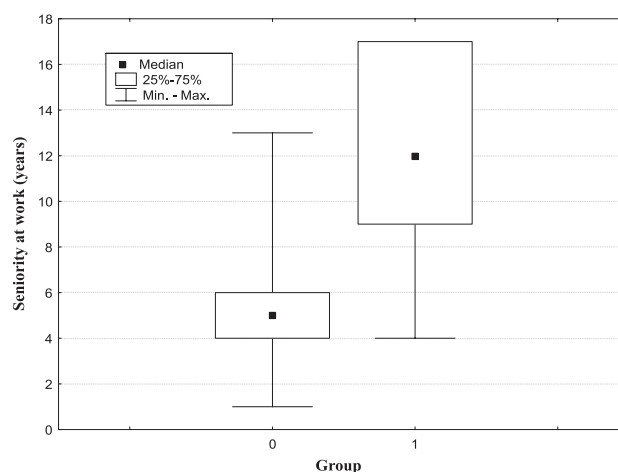

Fig. 4. Seniority at work in a chemical plant in seven outliers and in the rest of the study group (0 – rest of the study group, 1 – group of seven outliers).

Table 4. The clinical and demographic parameters data in 7 outlying subjects.

Subject number	Parameter for which subject is outlying	Age of smoking commencement /current age (yr)	Number of cigarettes smoked per day	Seniority at work in the chemical plant (yr)
1	CEA, CYFRA 21-1	20/40	10	17
2	CEA	20/36	20	9
3	SCC-Ag	21/40	10	9
4	SCC-Ag	19/44	20	13
5	CYFRA 21-1	19/30	15	12
6	NSE	17/23	15	4
7	NSE	22/53	15	17

Discussion of Results

Caution is needed when interpreting the results of this relatively small study. Nevertheless, the results showing the increase in chromium excretion during the workday are in reasonable agreement with results of other studies [15, 16]. The data provided by the current study show that large amounts of absorbed chromium were excreted into urine on exposure day, within several hours after exposure (Fig. 1). This finding, and the high degree of variation in urinary chromium among subjects, are also in concordance with the results reported by other authors, who – however – did not formulate any model of chromium absorption and excretion [5].

The experimental points and the calculated curve shown in Fig. 1 support the view that the proposed simple kinetic model provides an adequate fit to data on chromium absorption and excretion via urine in the study group, and it is useful in determining the biological effect of exposure to chromium compounds. It is worth stressing that the type of fitted curve was identical for all individuals, but particular parameters of the kinetic model were different for each of them, as shown in the Appendix.

The values of tumour markers higher than the upper limits of normal ranges may reflect the potential risk of some disturbances due to chronic exposure to harmful chromium components – without any acute symptoms of toxicity at the time of examination. However, the above-mentioned parameters were within normal ranges for almost all subjects.

The PLS2 model confirmed the link between biochemical indices, blood panel parameters, spirometric parameters, and the concentrations of tumour markers – in absence of overt signs of chromium toxicity. No systemic investigation of such dependency has been published so far. This link could have been even stronger if the ambient air had contained only Cr(VI) salts, which are readily absorbed through inhalation and are classified as potent carcinogens in humans. However, the air in the chemical plant included the mixture of different forms of chromium with diverse solubilities. It is also worth bearing in mind that inhaled hexavalent chromium is converted to trivalent chromium by the acidic environment in the stomach and in some parts of the respiratory tract, such as epithelial lining fluid, pulmonary alveolar macrophage or peripheral lung parenchyma cells. These incompletely defined defence mechanisms reduce the health risk [5, 7]
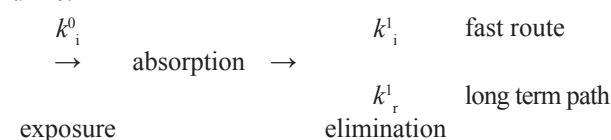
For people not occupationally exposed to chromium, cigarette smoking might constitute an important source of chromium intake and heighten the risk of lung cancer. In the individuals working in an atmosphere containing compounds of this metal, cigarette smoking (as declared by most of the subjects in our study) does not seem to constitute a source of chromium comparable to occupational exposure doses. In our study the subjects outlying in the PLS2 model differ from the rest only in respect to seniority at work. It is even debatable if cigarette smoke can react synergistically in strengthening chromium's carcinogenic effects and promoting tumour growth [17].

In conclusion, our study points to the necessity of using the proper kinetic model in evaluating chromium status of workers occupationally exposed to chromium compounds. The revealed association between chromium status parameters (as delivered by the kinetic model), biochemical indices, blood panel parameters, spirometric indices, and the concentrations of tumour markers should be further studied in larger groups of workers.

It is worth adding that the same chemical plant from which the subjects of our study have originated has produced hazardous waste dumped in the vicinity. It contains a large amounts of chromium and causes detrimental changes in the environment as was proved recently by means of specific biological indicators of soil contamination. Thus, further monitoring of health status of chemical plant workers can be helpful in assessing a potential risk for the neighbouring human population.

Appendix

Kinetic model construction of Cr elimination via urine:

k^0_i k^1_i fast route
$\rightarrow$ absorption $\rightarrow$

                                 k^1_r long term path

exposure   elimination

k^0_i –   exposure: zero-order rate constant
k^1_i –   elimination: fast route 1-st order rate constant, initially estimated from Cr concentrations as: $-\ln(uCr_1 / uCr_2) / t_w$
where: i – individual workday number
         uCr_1 and uCr_2 – urine Cr concentration (µg/g creatinine) after one workday and before the next one, respectively (see Fig. 1)

Table 5. Comparison of demographic parameters for seven outlying subjects and for the rest of the study group.

Parameter	Outliers			Rest of the study group			
	Mean	Min	Max	Mean	Min	Max	p
Age of smoking commencement	19.7	17.0	22.0	24.4	17	46	0.2434
Number of cigarettes smoked per day	15.0	10.0	20.0	11.9	0	30	0.2291
Seniority at work in the chemical plant	11.6	4.0	17.0	5.4	1.0	13.0	0.0066

$t_w = 6$ or 8 hours (workday period)

k^1_r – elimination: long-term 1-st order rate constant

Constituents of kinetic model:

1) exposure-elimination component (0-order rate of exposure, 1-st order rate of elimination):

$$EX(t_i)=(k^0_i / k^1_i) * (1-\exp(-k^1_i * t_i))$$

2) elimination-after-exposure component:

$$EL(t_i)= EX(t_w) * \exp(-k^1_i * t_i)$$

3) residual long-term component of elimination:

$$rCr = rCr0 * \exp(-k^1_r * t)$$

where: t_i – work time count from 1-st hour of the monitored workday;

$t_i \in <0; t_w>$

rCr0 – residual (background) urine Cr concentration (see Figure 1)

t – internal time count (in hours from 1-st day of monitoring); $t \in <0; 400$ hours>

Total kinetics equation for Cr elimination:

$$eCr = \sum_i^N [EX(t_i) + EL(t_i)] + rCr$$

where: N – number of workdays monitored

The parameters: k^0_i, k^1_i, k^1_r, and rCr0 were fitted by means of the least-square method of minimization using the equation: $Y = \sum_i^N [eCr - uCr]^2$ (sum of squared deviations of the model's calculated values (eCr) from observed data (uCr)).

For each subject the following parameters were derived:

1) $t_{1/2}Cr$ – half-life time of Cr elimination;

$t_{1/2}Cr = \ln(2) / k^1_i$

2) rCr0 – residual urine Cr concentration

3) mean integral Cr elimination per workday:

$$\sum_{j=0-400}[eCr(t_i) * (t^j_{ij}-t^{j-1}_i)]/N$$

where: j – subsequent time count (in hours) from the beginning of monitoring.

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