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EXPOSURE TO SOME METALLIC ELEMENTS, POLYCHLOROBIPHENYLS AND 1-OH PYRENE: A RESEARCH IN FOUR METALLURGICAL SECTORS

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1. RESEARCH PRESENTATION

This research was promoted by Consortium for Environmental Research for Metallurgy (RAMET), originated from the need to provide companies with the necessary support to address issues related to exposure to chemicals, particularly carcinogens.

This also with reference to the project managed by the Lombardy Region on occupational cancer associated to the metallurgical activities in the Northern Italy area of Brescia.

As often happens, this issue has been addressed by individual companies on their own, referring to their expertise or their consultants, using guidelines more often generic and inclusive, not able to give a specific characterization of the risks for the sector of interest.

The result, therefore, has been to have different ways of approach and management of carcinogenic risk not only for the specific business, where it rightly requires a customization of the situations in detail, but for different sensitivity, knowledge and interpretations.

The research promoted by RAMET has the following objectives:

- To identify the carcinogens present in the metallurgical production cycle;
- To establish the criteria and methods of risk assessment in relation to the exposure characteristics and the results of health surveillance;
- To establish a system of risk management through training, organizational procedures and guidelines for environmental monitoring and health surveillance, as well as criteria to check their correct application;
- To provide for each metallurgical sector a photograph of the associated carcinogenic risk.

In essence, the project is aimed to identify a cognitive and pragmatic matrix on the topic that could represents a reference for the operating companies.

Such methodological uniformity can be presented and discussed with the Local Health Service (ASL) in order to share criteria and contents. In this way, a shared tool will be made available to the companies and, as such, it may represent a guarantee for companies that adopt it.

The set of available data from individual factories, together with a situation in which industrial production processes have been consolidated and almost standardized however aligned with the best technology standard (BAT) should, in fact, allow the achievement for

individual metallurgical sectors, of standardized exposure situations and risk conditions, and an initial epidemiological assessments.

In this way, it would therefore be possible to identify reference exposure and risk standards that would give individual companies a tool for comparison and planning of their management and improvement interventions.

RAMET, assisted by its Scientific Committee, has carried out supervision and coordination of the activities envisaged by the project.

A physician specializing in Occupational Medicine has collected the biological samples, as well as the occupational histories from workers; the analyses on biological matrices were entrusted to the Laboratory of Industrial Toxicology at the University Hospital ("Spedali Civili") of Brescia.

As regards to environmental monitoring, the RAMET Consortium gave the surveys to consultants already operating within the companies, after ascertainment that analytical methods and sensitivity met the specific project guidelines. This choice has allowed us to optimize the environmental sampling, as traders involved in the investigations were aware of the technological cycles of manufacturing companies as well as of any critical situation.

The companies involved in environmental sampling were: Chemiricerche Inc., INDAM chemistry laboratories Inc., SIECO Inc., Studio Sanitas Inc.

In this investigation, we focused on biological monitoring data and results of environmental monitoring are summarized for their better evaluation.

Biological monitoring is strictly related with risk assessment procedures, i.e. to quantify the probability that a specific agent or chemical will give rise to an adverse effect. The main factors that affect this practice include the intrinsic properties of the chemical, its use and the exposure levels, and the number and susceptibility of exposed individuals. In occupational situations, the number of individuals is usually relatively small and therefore the main determinant of the risk is the exposure level. In the overall procedure of risk assessment, which entails hazard identification, dose-response determination, exposure assessment and risk characterization, biological monitoring is mainly applied in the exposure assessment phase in order to identify exposed individuals and groups and determine their exposure levels. Biological monitoring may also be used in the risk characterization phase to assess health risks for exposed groups depending on exposure levels.

When dose-effect and dose-response relationships are known, a specific biomarker of dose may be sufficient to assess the risk of adverse effects.

The main objective of the research was to evaluate the exposure of different cohorts of metallurgy workers to toxic and carcinogenic compounds polluting the work environment.

The study design relied on exposure assessment through combined external and internal dose assessment. We planned to determine airborne concentrations of metallic elements (As, Be, Cd, Cr, Ni, Pb, Al, Cu, Zn, Mn) and organic compounds (polycyclic aromatic hydrocarbons) by environmental sampling. The internal dose was characterized by biological monitoring of validated exposure biomarkers in blood/urine of exposed workers. In all settings, we determined therefore a set of carcinogenic and toxic elements (As, Be, Cd, Cr, Ni, Pb), some metallic elements relevant for the different investigated sectors (Al, Cu, Zn, Mn), urinary 1-hydroxypyrene (1-OHP) as tracer of exposure to polycyclic aromatic hydrocarbons (PAH) mixtures, and 34 congeners of polychlorinated biphenyls (PCB) (PCBs 28, 31, 52, 74, 77, 81, 99, 101, 105, 114, 118, 123, 126, 128, 138, 146, 153, 156, 157, 167, 169, 170, 172, 177, 180, 183, 187, 189, 194, 196+203, 201, 206, 209).

2. METALLURGICAL SECTORS PRESENTATION

The sample of selected companies was represented by companies participating in the RAMET consortium and significant in relation to the main topics of the Project.

Aluminum remelting foundry: three companies, representing more than 50% of aluminum production in the Lombardia region, were included in the study.
The production of secondary aluminum has some peculiarities as compared to other productive cycles of non ferrous materials, given the variety of raw materials and furnaces used in the remelting process. Schematically, two main melting technologies may be used:
– Production of aluminum billets starting from new aluminum scrap: the raw material is poorly contaminated by other metals and it is remelted in reverberatory furnaces.
– Production of aluminum starting from scrap of different source: the scrap is remelted in rotating furnaces with the addition of sodium chloride in the melting pot.
The investigated companies may be considered representative of the sector for the Brescia province. Their productive cycles are different since one uses reverberatory furnaces, another salt rotating furnaces and the third both the technologies.

Copper alloy remelting foundry: three companies, representing more than 50% of the copper alloy production in the Lombardia region, were included in the study.
The most important product of copper and copper alloy secondary metallurgy is represented by brass from scrap. Schematically, two main melting technologies may be used, according to the characteristics of the final product:
– semi-finished brass for further mechanical working (bar, coil, laminated, skein, band, roll): the raw material is high quality copper/copper alloy, mostly deriving from mechanical turning;
– ingot bar brass for further remelting: the starting raw material is constituted by lower quality scrap, including metallic grain recovered by grinding of the blast-furnace slag.
The investigated companies may be considered representative of the sector at a National level, one of them producing also secondary copper.

Electric steel plants*:* five companies, representing more than 30% of steel production in the Lombardia region, were included in the study and they may be considered representative of the electric arc furnace sector.

In the Italian ironworks, two main technologies are employed:

– integrated cycle: steel is obtained by reducing iron minerals;

– electric arc furnace cycle: steel is obtained by re-melting of iron scraps, with continuous cast and further steel transforming processes.

Cast iron remelting foundry*:* four companies representing more than 30% of the production of cast iron in in the Lombardia region, were included in the study.

On the basis of the raw material and the final product, these may be considered representative of the sector. The plants included either electric arc or cupola furnaces, the latter more characteristic of the sector.

3. METHODS FOR ENVIRONMENTAL AND BIOLOGICAL MONITORING

3.1 Environmental monitoring

Environmental monitoring was carried out by personal air samplings in the same days of biological monitoring. For each sector, the number of samplings was determined taking into account specific questionnaires compiled by selected industries and biological samplings.

The following substances were determined:

✓ Polycyclic aromatic hydrocarbons (PAH): benz[a]pyrene; benz[a]anthracene; benzo[b]fluoranthene; benzo[j]fluoranthene; benzo[k]fluoranthene; dibenz[a,h]anthracene; benzo[e]pyrene; naphtalene; indeno[1,2,3-cd]pyrene; chrysene; acenaphtylene; acenaphtene; fluorene; fenanthrene; anthracene; fluoranthene; pyrene; 5-methylchrysene; benzo[ghi]perylene; dibenz[a,e]pyrene; debenz[a, h]pyrene; dibenz[a, i]pyrene; dibenz[a, j]achridine; dibenz[a, h]achridine; 7H-dibenzo[c, g]carbazole;

✓ Metallic elements: chromium (Cr); Nickel (Ni); Cadmium (Cd); Arsenic (As); Lead (Pb); Copper (Cu) and Zinc (Zn) (copper alloy foundries); Aluminum (Al) (aluminum foundries).

In any case, the duration of personal airborne sampling corresponded to the workshift preceding the biological sampling.

For PAH, the NIOSH 5515/94 method was applied and compounds were determined by gas-cromatography coupled to mass spectrometry.

For metallic elements, the UNICHIM 271/1977 sampling method and the NIOSH 7300/94 (EPA 200.8-1994) analytical method in inductively coupled plasma mass spectrometry were applied.

3.2 Biological monitoring

3.2.1 Subjects

The job tasks (workers) undergoing biological monitoring were identified by the RAMET Consortium through the following procedural steps:

✓ Preliminary inspections, useful to characterize the productive cycles of the factories selected for the Project;

✓ Identification of specific work areas and related job tasks.

In every investigated sector, we identified the following main areas, also after compilation of a questionnaire by every factory:

- raw material area;
- furnace(s);
- waiting/refining;
- continuous casting;
- others (maintenance; cleaning; forklift drivers)

For biological monitoring, workers were differentially selected depending upon the kind of analyte(s) to be determined:

- urinary concentrations of metals and 1-OHP: all workers in shift during environmental monitoring;

- blood concentrations of Pb (Pb-B) and serum levels of PCB (ΣPCB-S): 50% of workers employed in the scrap and furnace areas.

The table below synthesizes the above mentioned criteria:

<table>
<tr><td rowspan="2">Compounds</td><td></td><td>Blood</td><td>Urine</td><td colspan="6">Areas</td></tr>
<tr><td></td><td></td><td></td><td>Scrap</td><td>Furnace</td><td>Refining</td><td>Casting</td><td>Cast iron</td><td>Others</td></tr>
<tr><td rowspan="5">Carcinogenic elements</td><td>As</td><td></td><td>X</td><td colspan="5" rowspan="5">100%</td><td rowspan="10">variable</td></tr>
<tr><td>Ni</td><td></td><td>X</td></tr>
<tr><td>Cd</td><td></td><td>X</td></tr>
<tr><td>Cr</td><td></td><td>X</td></tr>
<tr><td>Be</td><td></td><td>X</td></tr>
<tr><td>Toxic elements</td><td>Pb</td><td>X</td><td></td><td>50%</td><td>50%</td><td></td><td></td><td></td></tr>
<tr><td rowspan="3">Sector tracers</td><td>Al</td><td></td><td>X</td><td colspan="5">100 % (aluminum foundries)</td></tr>
<tr><td>Cu-Zn</td><td></td><td>X</td><td colspan="5">100 % (copper alloys)</td></tr>
<tr><td>Mn</td><td></td><td>X</td><td colspan="5">100 % (electric steel foundries)</td></tr>
<tr><td>PCB</td><td></td><td>X</td><td></td><td>50%</td><td>50%</td><td></td><td></td><td></td></tr>
<tr><td>PAH</td><td>1-OHP</td><td></td><td>X</td><td colspan="5">100%</td></tr>
</table>

Selection criteria for biological monitoring of Pb-B and ΣPCB-S included:

- continuity of engagement in the job task;
- employment duration in the factory and in the job task;
- age: older workers were preferred.

Maintenance workers were included if they worked in selected areas during the workweek of environmental and biological sampling.

In any case, biological sampling was performed by the RAMET Consortium on a voluntary basis.

Before sample collection, enrolled worker were interviewed by a physician about socio-demographic information and lifestyle habits.
Biological monitoring was performed through the determination of urinary concentrations of carcinogenic metallic elements (As-U, Be-U, Cd-U Cr-U, Ni-U), metals characteristic of different sectors (Al-U, Cu-U, Zn-U, Mn-U) and 1-hydroxypyrene (1-OHP).
Urine spot samples were collected at the end of the workshift.
In addition, blood samples were collected for determination of PbB and of the sum of 34 PCB congeners in serum (ΣPCB-S).

3.2.2 Analysis

All laboratory analyses were performed at the laboratory of Occupational Hygiene, Toxicology and Prevention Unit of the University of Brescia. The lab participates from more than ten years in external quality assessment schemes organized by the German Society of Occupational and Environmental Medicine (Erlangen, Germany) and has shown a high degree of proficiency in metal analysis. Metallic elements were determined in urine and blood samples collected at the end of the shift by inductively coupled mass spectrometry (ICP-MS) methods on an ELAN DRC II (PerkinElmer SCIEX, Shelton, CT, USA) instrument (Al-U, Be-U, Cd-U, Cr-U, Cu-U, Mn-U) or by atomic absorption spectrometry on a Varian AA240Z Zeeman (Varian Inc., palo Alto, CA, USA). Accuracy and precision of the methods were assessed using certified materials from the German Society of Occupational and Environmental Medicine (Erlangen, Germany).
1-OHP was determined in end of shift urine samples applying a previously described method (Jongeneelen et al, 1988), with minimal changes. Briefly, samples underwent enzymatic hydrolysis with beta-glucuronidase-arylsulfatase at 37℃ overnight and concentration on SPE C18 3 ml columns. About 20 ml of eluate were analyzed by high pressure liquid chromatography with fluorimetric detection (λ_{ecc} 242 nm; λ_{em} 388 nm)using Supelcosil LC-18 DB columns. Urinary creatinine was determined by the method of Jaffe (Kroll et al., 1986).
PCBs were determined by gas chromatography coupled to mass spectrometry (GC-MS) using an Agilent Technologies GC 6890N equipped with a quadrupole mass spectrometer 5973 (electron impact ionization). A capillary PONA column (Agilent Technologies, 50 m x 0.20 mm ID; 0.50 μm film thickness) was used, carrier gas was helium at 0.8 ml/min

(constant flow), and splitless sample injection of 2 µl at 250 ℃ was employed. Temperatures were programmed as follows: MS interface temperature 320 ℃; oven temperature 100℃ for 1 min, 30℃/min to 180℃, and then 10℃/min to a final temperature of 300℃, held for 25 min. The total runtime was 40 .67 min. The GC-MS system was tuned before each analytical batch. Quantifier and qualifier monitored ions pairs were respectively: for triCBs 256.0-258.0, for tetraCBs 289.9-291.9, for pentaCBs 325.9-327.9, for hexaCBs 359.8-361.8, for heptaCBs 395.8-393.8, for octaCBs 427.8-429.8, for nonaCBs 465.8-463.8, for decaCB 497.8-499.7.

In order to allow comparisons among biomonitoring data and available biological limit values/reference values, markers were expressed either as weight/volume concentrations (µg/l: Al-U, Cr-U, Ni-U; µg/dl: Pb-B; ng/ml: ΣPCB-S) or as a function of urinary creatinine (µg/ g creatinine: Cd-U and 1-OHP).

The detection limits (L.O.D.) of investigated biomarkers are below reported.

Biomarkers	**LOD**	**Biomarkers**	**LOD**
Cd-U (µg/g creatinine)	0.01	Cu-U (µg/l)	0.20
Cr-U (µg/l)	0.01	Mn-U (µg/l)	0.07
Ni-U (µg/l)	0.20	Zn-U (µg/l)	100
As-U (µg/l)	1.00	1-OHP (µg/g creatinine)	0.05
Be-U (µg/l)	0.01	Pb-B (µg/dl)	0.40
Al-U (µg/l)	0.20	ΣPCB-S (ng/ml)	0.1 for each congener

For statistical analyses, a value corresponding to ½ of the LOD was attributed to values falling below the LOD (Helsel 1990).

3.2.3 Statistics

Raw data were supplied by the RAMET Consortium as Excel® datasheets and these underwent several corrections of input errors and modifications, in order to gain the best possible accuracy of information and to format the data for processing by the statistical software package S.P.S.S®. The project members have had several meetings, to discuss about the project aims, the data verification and the interpretation of the results.

Data distributions have been evaluated by non parametrical "Z Kolmogorov-Smirnov" test (to verify if two sample groups are drawn from the same population; the test, based on the maximal difference between two cumulated distributions, is sensible to any difference between distributions) on raw data, and both log and ln transformed data (the latter for exponential distributions). Thereafter, we have performed a descriptive analysis of the data, to obtain both arithmetical and geometric means (for variables following a log-normal distribution) with standard deviations, medians, minimum and maximum values. The asymmetry indices were calculated for further confirmation of the results obtained by the "Z Kolmogorov-Smirnov" test. Asymmetry values higher than 1 identify non normally distributed variables; 0 indicates normal and symmetric distributions; positively asymmetric distributions have a tail on the right, the negative ones a tail on the left.

The descriptive analysis has been performed pooling together the data from the numbers of plants. Separated analyses have been performed on workers stratified by job title (both the general and that of the sampling week), age groups, work age, smoking habits, diet, ethnic groups, residence. Finally, a "z-score" transformation of the variables (metallic elements without aluminum, hydroxypyrene, PCB) has been performed, to allow a non-weighed analysis to formulate an exposure classification list.

Following the classical steps of methodological statistical analysis, e.g. descriptive, stochastic, inferential, we have performed the following statistical tests:

– "Mann-Whitney U test" for two independent samples: non-parametric test equivalent to the "t" test that verifies if two independent samples are drawn from the same population. It is more powerful than the median test as it is based on the cases' ranks. It has been applied to evaluate significant differences among marker values between pairs of factories;

– "Kruskal-Wallis H test" when comparing more than two independent samples; the test has been applied to evaluate differences among the five job tasks (both general and weekly) both on the whole sample and the sample classified by factory;

– Multiple stepwise linear regressions to investigate relationships among biomarkers (as dependent variables, "Y") and lifestyle factors, as alcohol and tobacco consumptions (as multiple independent continue covariates, "X")

– Analysis of variance (AN.O.VA) followed by the post-hoc tests to evaluate differences among biomarkers in the sample stratified by residence or diet;

– Parametric Pearson's and non-parametric Spearman's correlations among biomarkers;

All the performed tests have been based on the "p value", based on asymptotic distribution, e.g. the probability to obtain so extreme results as those observed and in both the directions, when the null hypothesis is true. If probability value (p) is 0.05 or lower, the null hypothesis "H 0" can be rejected. This significance level is applied to hypotheses with unspecified direction of the effect (two tailed tests at the α level 0.025 right and 0.025 left).

4. RESULTS

4.1 Secondary aluminum foundries

4.1.1 Environmental monitoring

The general descriptive results (as μg/m^3) of 30 personal airborne sampling are below reported:

	As	Be	Cd	Cr-tot	Ni	Pb	BaP**
Minimum	<0.6	<0.6	<0.3	<0.6	<0.6	<0.6	<0.0006
Maximum	<1.3	<1.3	<0.8	3.2	1.6	16.1	0.1193
Mean	-	-	-	0.7	0.4	3.0	0.0094
Geometric mean	-	-	-	0.5	0.4	1.7	0.0028
***TLV-TWA (μg/m^3)**	10	2	10	500	100	75***	n.a.

*Treshold limit values according to ACGIH; **Benz[a]pyrene; n.a.: not available; ***according to the Italian Law (DLgs 81/2008)

All the determined compounds fell below the corresponding TLV-TWA value.

4.1.2 Biological monitoring

The participating rate was of 88% (i.e. 172 subjects) and 93% (i.e. 37 subjects) for urinary and blood sample collection, respectively. Enrollment failure was due to: concomitant ill, or vacation or change of shift or refusal to participate.

Before sample collection, enrolled worker were interviewed by a physician about socio-demographic information and lifestyle habits.

The enrolled people belonged to the following main working areas:

- scrap: 20 %;
- furnace/casting 45 %;
- maintenance: 12 %;
- jolly/heads: 9%;
- others: 6%.

The workers' age was mostly (73%) comprised between 30 and 50 years and 92% of subjects were Caucasians, other ethnic groups including Africans (n=5), Arabians (n=3) and a mixed group (n=5, from India, Pakistan and Maghreb). Most of workers lives in rural

zones or municipalities of the Brescia province, whereas only 26 subjects live in the Brescia hinterland.

Sixty-eight workers were current smokers and 50% of non smokers were former smokers.

Table 1 resumes, the descriptive analysis of evaluated biomarkers on a whole group basis. On average, the values were well below the biological limit values (BLV) indicated by either DFG or ACGIH, or, in the case of 1-OHP, by authoritative published results in this field (Jongeneelen FJ 2001).

A fair fraction of workers showed levels of Al-U, As-U, PbB and ΣPCB higher than the corresponding upper limits of the reference values (18%, 8%, 19% and 11%, respectively). For Cd-U, Cr-U and Ni-U the corresponding figures were negligible, whereas no worker exceeded the upper limits of the RV for Be-U and 1-OHP. Be-U could be determined in 12% of workers, only.

The analysis of data stratified by plant is summarized in Table 2. Apart from Ni-U and PbB, the other biomarkers showed significant differences among factories. Workers from factory 2 showed the lowest values of both Al-U and ΣPCB and the highest levels of As-U.

Table 3 resumes the data distributions by different job tasks (referred to the week of sampling) evaluated on the whole sample, in order to have sufficient subgroup sizes. Cd-U, Cr-U and Ni-U showed significant differences among the five categories. On average, Cd-U prevailed in the scrapping, furnace /casting and maintenance tasks; the lower Cr-u levels were observed among jolly workers and heads; the highest Ni-U values were determined among maintenance workers.

Age was available only as ordinal variable (age brackets). The analysis of data by age groups (Table 4) evidenced significant differences for Al-U, Cd-U, 1-OHP and ΣPCB values. The highest Al-U values were determined in workers elder than 50 years; Cd-U showed a trend toward higher values with increasing age, that was even clearer for ΣPCB. 1-OHP values prevailed in the intermediate age groups.

The distributions of data by groups of work seniority did not give rise to statistically significant differences (data not shown). Only the ΣPCB values showed a trend toward higher values with increasing of exposure duration, but this was not significant, probably for the low number of subjects in the highest duration group.

Table 5 summarizes distribution of biomarkers in workers classified by ethnic group. Both Al-U and As-U values showed statistically significant differences among groups. The

lowest Al-U values were observed among Caucasians, whereas As-U prevailed among Africans and the "others" groups.

Apart from Cd-U, other investigated biomarkers failed to show significant differences in workers classified by residence area (Table 6). The highest Cd-U levels were measured in workers living in villages neighbouring to Brescia.

The analysis of data by lifestyle factors (smoking habits; consumption of fish/jellyfish and grilled foods in the days preceding the biomonitoring) demonstrated only an interference by tobacco smoke on Cd-U, 1-OHP and As-u levels (Table 7). Current smokers showed significantly higher Cd-U and 1-OHP levels than smokers, whereas the opposite was true for As-U levels.

Stepwise multiple regression analyses were performed for all investigated biomarkers in models including them as dependent variables and lifestyle factors (smoking and alcohol intake) as independent variables.

Al-U was significantly influenced by the extent of daily alcohol intake (as gr of alcohol). The relationship was described by the equation: *Al-U=0.07(gr/alcohol)+9.271 (p=0.002)*.

Cd-U and 1-OHP levels were significantly associated to the extent of smoking habits (as packyears). The respective equations were: *Cd-U=0.008(packyears)+ 0.33* and *1-OHP=0.009(packyears)+0,186 (p<0.000 for both)*.

In the following tables, the concentration of analytes are expressed as µg/l, with the exception of Cd, 1-OHP (both as µg/gcreat), PbB (µg/dl) and the sum of PCB (as ng/ml). The main parameters emerging from descriptive analysis are reported (GM= geometric mean), together with the reference values (RV), the biological limit values (BLV) according to both ACGIH (BEI®) and DFG (BAT, EKA), and the number of subjects showing values lower than the limit of detection (N°< LOD) or exceeding the upper limit (UL, 95th ntile) of RV (N°> ULRV).

Table 1 Distribution of biomarkers in workers of secondary aluminum foundries.

Parameters	**Al-U**	**Cd-U**	**Cr-U**	**Ni-U**	**As-U**	**Be-U**	**1-OHP**	**PbB**	**PCB**
N°	164	163	164	164	164	164	171	37	37
GM	7.72	0.32	0.14	0.46	4.73	0.005	0.19	6.04	2.73
Mean	10.50	0.38	0.28	0.79	8.33	0.005	0.24	6.81	4.60
SD	9.57	0.25	1.17	0.98	8.94	0.001	0.19	3.16	5.26
Asymmetry	2.38	2.67	12.41	3.62	2.31	3.52	1.63	0.27	2.07
Minimum	0.70	0.05	0.02	0.10	0.10	0.005	0.02	1.90	0.20
Maximum	60.00	2.00	15.00	8.00	52.20	0.01	1.13	13.00	19.40
Percentiles									
5th ntile	2.22	0.12	0.03	0.10	0.15	0.005	0.05	2.17	0.38
95th ntile	32.75	0.84	0.60	2.37	32.30	0.01	0.63	12.01	18.77
Reference Values									
5th ntile	1.00	0.10	0.05	0.10	0.10	0.01	0.03	0.50	0.80
95th ntile	15.00	1.50	2.20	4.00	20.00	0.06	0.70	10.00	14.10
BLV									
ACGIH	N.A.	5	25	N.A.	35	N.A.	2.7†	30.00	N.A.
DFG	200	N.A.	12-40	15-45* 25-70°	50-300	N.A.		40.00	N.A.
N°< LOD	0	0	9	0	0	145	0	0	0
N°> 95th ntile of RV	29	1	1	3	13	0	0	7	4

*Ni metal, oxide, carbonate, sulfide, sulfidic ores; $^{\circ}$easily soluble Ni compounds, e.g. Ni acetate and similar soluble salts, Ni chloride, Ni hydroxide, Ni sulfate.
†NOAEL according to Jongeneelen et al.
N.A.: not assigned.

Table 2 Distribution of biomarkers in aluminum workers stratified by plants.

Biomarkers	Plant 1			Plant 2			Plant 3		
	N°	GM	Min.- Max.	N°	GM	Min.- Max.	N°	GM	Min.- Max.
Al-U	60	8.99[a]	1.60-51.00	42	4.49[a,c]	0.70-13.60	62	9.62[c]	2.80-60.00
Cd-U	60	0.26[a,b]	0.05-0.94	41	0.41[a]	0.18-1.28	62	0.34[b]	0.10-2.00
Cr-U	60	0.11[b]	0.02-0.74	42	0.13[c]	0.02-15.00	62	0.20[b,c]	0.08-100
Ni-U	60	0.44	0.10-8.00	42	0.45	0.10-4.60	62	0.48	0.10-4.50
As-U	60	5.48[a,b]	0.30-52.20	42	8.61[a,c]	1.90-38.40	62	2.73[b,c]	0.10-35.90
Be-U	60	0.005	0.005-0.01	42	0.005	0.005-0.01	62	0.005	0.005-0.010
1-OHP	63	0.19	0.03-0.71	45	0.15[c]	0.02-0.66	63	0.22[c]	0.06-1.13
PbB	14	6.01	2.20-11.60	11	5.38	1.90-13.00	12	6.75	3.30-11.90
Σ PCB	14	3.58	0.40-19.40	11	1.75[c]	0.60-3.40	12	3.00[c]	0.20-18.30

[a,b,c]p<0,05 Mann-Whitney U test; a = plant 1 *vs* 2; b = plant 1 *vs* 3; c = plant 2 *vs* 3.

Table 3 Distributions of biomarkers in aluminum workers stratified by job tasks.

Biomarkers	Scrap			Oven/casting			Jolly, heads			Maintenance			Others		
	N	GM	Min-Max	N	GM	Min-Max	N	GM	Min-Max	N	GM	Min-Max	N	GM	Min-Max
Al-U	36	7.91	1.70-33.00	78	8.14	1.60-60.00	14	5.74	0.70-21.40	19	8.65	2.10-37.50	17	6.47	1.80-19.30
Cd-U*	36	0.38	0.14-1.28	78	0.34	0.09-1.18	13	0.26	0.12-0.68	19	0.30	0.13-2.00	17	0.21	0.05-0.85
Cr-U*	36	0.13	0.02-15.00	78	0.15	0.02-0.64	14	0.08	0.02-0.21	19	0.23	0.02-1.00	17	0.12	0.02-0.39
Ni-U*	36	0.35	0.10-4.60	78	0.49	0.10-3.60	14	0.41	0.10-1.50	19	0.91	0.10-8.00	17	0.30	0.10-2.90
As-U	36	5.61	0.30-39.90	78	5.16	0.10-52.20	14	4.86	1.40-38.40	19	3.36	0.10-18.80	17	3.16	0.10-31.70
Be-U	36	0.005	0.005-0.010	78	0.005	0.005-0.01	14	0.005	0.005-0.005	19	0.005	0.005-0.01	17	0.005	0.005-0.005
1-OHP	37	0.19	0.05-0.71	82	0.20	0.02-1.13	15	0.12	0.03-0.33	20	0.19	0.04-0.69	17	0.21	0.06-0.60
PbB	20	6.12	1.90-13.00	15	5.73	2.20-11.30	0	-	-	2	7.87	5.20-11.90	0	-	-
Σ PCB	20	2.04	0.20-18.70	15	4.21	0.80-19.40	0	-	-	2	2.005	0.60-6.70	0	-	-

*$p<0.05$ Kruskall Wallis's H test

Table 4 Distributions of biomarkers in aluminum workers stratified by age brackets.

Biomarkers	**< 30 years**			**30-40 years**			**40-50 years**			**> 50 years**		
	N	GM	Min-Max	N	GM	Min-Max	N	GM	Min-Max	N	GM	Min-Max
Al-U*	13	5.96	2.70-37.00	51	8.06	2.20-41.00	67	6.76	0.70-37.50	33	10.464	1.60-60.00
Cd-U*	13	0.19	0.05-0.39	51	0.27	0.06-0.64	66	0.37	0.08-2.00	33	0.37	0.17-1.28
Cr-U	13	0.14	0.02-0.59	51	0.13	0.02-0.64	67	0.15	0.02-15.00	33	0.16	0.02-0.69
Ni-U	13	0.33	0.10-1.30	51	0.51	0.10-4.60	67	0.45	0.10-4.50	33	0.46	0.10-8.00
As-U	13	2.65	0.10-16.00	51	4.22	0.10-39.90	67	5.16	0.10-38.40	33	5.95	1.70-52.20
Be-U	13	0.005	0.005-0.005	51	0.005	0.005-0.01	67	0.005	0.005-0.01	33	0.005	0.005-0.01
1-OHP*	13	0.17	0.04-0.34	56	0.22	0.02-0.86	68	0.20	0.04-0.69	34	0.14	0.03-1.135
PbB	1	5.20	5.20-5.20	11	4.34	1.90-9.20	14	6.87	4.00-11.90	11	7.24	2.50-13.00
Σ PCB*	1	0.60	0.60-0.60	11	1.28	0.20-5.40	14	3.17	0.40-19.40	11	5.52	2.10-18.700

*$p<0.05$ Kruskall Wallis's H test

Table 5 Distributions of biomarkers in aluminum workers stratified by ethnic groups.

Biomarkers	Caucasians			Africans			Arabians			Others		
	N	GM	Min-Max	N	GM	Min-Max	N	GM	Min-Max	N	GM	Min-Max
Al-U*	153	7.4	0.70-60.00	5	12.11	7.90-24.50	2	15.22	12.20-19.00	4	15.75	10.40-33.00
Cd-U	152	0.32	0.05-2.00	5	0.30	0.18-0.44	2	0.33	0.21-0.52	4	0.32	0.19-0.55
Cr-U	153	0.14	0.02-15.00	5	0.14	0.10-0.30	2	0.13	0.09-0.19	4	0.15	0.02-0.58
Ni-U	153	0.46	0.10-8.00	5	0.50	0.10-3.60	2	0.22	0.10-0.50	4	0.46	0.10-2.30
As-U*	153	4.32	0.10-38.40	5	23.69	9.50-52.20	2	6.08	5.60-6.60	4	17.52	5.90-39.90
Be-U	153	0.005	0.005-0.01	5	0.005	0.005-0.005	2	0.005	0.005-0.005	4	0.005	0.005-0.005
1-OHP	159	0.19	0.02-1.13	5	0.11	0.03-0.26	3	0.23	0.15-0.30	4	0.25	0.18-0.43
PbB	35	5.96	1.90-13.00	0	-	-	1	11.60	-	1	5.10	-
Σ PCB	35	2.86	0.20-19.40	0	-	-	1	3.60	-	1	0.40	-

*$p<0.05$ Kruskall Wallis's H test

Table 6 Distributions of biomarkers in aluminum workers stratified by residence area.

Biomarkers	**Country / Mountain**			**Brescia**			**Neighbouring villages**			**Brescia hinterland**		
	N	GM	Min-Max	N	GM	Min-Max	N	GM	Min-Max	N	GM	Min-Max
Al-U	56	7.31	0.70-51.00	11	7.36	2.20-33.00	85	7.86	1.60-60.00	12	9.16	2.30-21.0
Cd-U*	55	0.30	0.10-0.97	11	0.20	0.05-0.53	85	0.37	0.12-2.00	12	0.23	0.06-0.52
Cr-U	56	0.16	0.02-0.75	11	0.11	0.02-0.58	85	0.14	0.02-15.00	12	0.12	0.02-0.74
Ni-U	56	0.46	0.10-4.60	11	0.39	0.10-1.90	85	0.47	0.10-8.00	12	0.42	0.10-1.80
As-U	56	3.49	0.10-38.40	11	8.71	0.90-39.90	85	5.32	0.10-52.20	12	4.88	1.10-31.70
Be-U	56	0.005	0.005-0.01	11	0.005	0.005-0.005	85	0.005	0.005-0.01	12	0.005	0.005-0.005
1-OHP	57	0.17	0.02-0.86	13	0.15	0.03-0.47	89	0.21	0.03-1.13	12	0.17	0.11-0.32
PbB	14	4.80	1.90-10.10	3	4.69	2.20-9.20	18	7.21	2.80-13.00	2	8.88	6.80-11.60
Σ PCB	14	2.70	0.20-19.40	3	0.75	0.40-1.50	18	3.25	0.80-18.70	2	4.41	3.60-5.40

*$p<0.05$ Kruskall Wallis's H test

Table 7 Distributions of biomarkers in aluminum workers stratified by smoking habits.

Biomarkers	Current smokers			Not Smokers		
	N	GM	Min-Max	N	GM	Min-Max
Al-U	64	7.31	1.70-60.00	100	8.00	0.70-51.00
Cd-U*	64	0.38	0.17-2.00	99	0.29	0.05-0.99
Cr-U	64	0.16	0.02-15.00	100	0.13	0.02-0.75
Ni-U	64	0.51	0.10-4.60	100	0.42	0.10-8.00
PbB	18	6.31	1.90-11.90	19	5.79	2.50-13.00
As-U*	64	3.92	0.10-39.90	100	5.34	0.10-52.20
Be-U	64	0.005	0.005-0.01	100	0.005	0.005-0.01
1-OHP*	68	0.33	0.03-1.13	103	0.13	0.02-0.62
Σ PCB	18	2.19	0.40-18.70	19	3.37	0.20-19.40

*$p<0.05$, Mann-Whitney U test

4.2 Copper alloy foundries

4.2.1 Environmental monitoring

The general descriptive results (as μg/m^3) of airborne sampling are below reported:

	Cu	Zn	Cd	Cr-tot	Ni	As	Be	Pb	BaP**
Minimum	3.49	8.71	0.05	<0.05	<0.05	<0.05	<0.01	0.31	0.000001
Maximum	840	2446	1.50	7.94	10.90	0.25	0.62	175	0.0255
Mean	145	223.09	0.26	1.44	0.90	0.05	0.04	28.07	0.00261
Geometric mean	56.16	117.26	0.12	0.87	0.28	0.04	0.02	11.69	0.00034
***TLV-TWA (**μg/m^3**)**	n.a.	n.a.	10	500	100	10	2	75***	n.a.

*Treshold limit values according to ACGIH; **Benz[a]pyrene; n.a.: not available; ***according to the Italian Law (DLgs 81/2008).

The concentrations of the carcinogenic elements fell below the corresponding TLV-TWA values, whereas lead overwhelmed the limit value fixed by the Italian law (DLgs 81/2008) in the furnace (123 μg/m^3) and scrap (175 μg/m^3) areas.

4.2.2 Biological monitoring

Urinary samples were collected at the end of the work-shift from all workers (n=160), whereas blood samples were drawn from a fraction of workers (27%) according to the following criteria:

- employment duration in a job task;
- continuity of involvement in a job task during the week of biological sampling.

The enrolled people belonged to the following main working areas:

- scrap: 28 %;
- furnace/casting 53 %;
- maintenance: 4 %;
- jolly/heads: 4 %;

– others: 11 %.

The workers' age was mostly (69%) comprised between 40 and 60 years and the majority (94 %) was constituted by Caucasians, whereas most of the rest (n=9) included Africans (n=8). Most of workers lives in rural zones or municipalities of the Brescia province, whereas only 41 subjects live in the Brescia hinterland.

Sixty-five workers were current smokers and 74% of non smokers were former smokers.

Table 8 resumes the descriptive analysis of evaluated biomarkers on a whole sample basis. On average, the values fell below the biological limit values (BLV) indicated by either DFG or ACGIH, or, in the case of 1-OHP, by authoritative published results in this field (Jongeneelen FJ 2001).

A fair fraction of workers showed levels of Cu-U, As-U and 1-OHP higher than the corresponding upper limits of the reference values (0.6%, 4.6%, and 8.3%, respectively), but in the cases of Zn-U and PbB the corresponding figures where even higher (22.7% and 74.4%, respectively).

Detectable levels of Be-U could be determined in 23 % of workers, only.

Table 9 resumes data stratified by plants and statistical comparisons applying the Mann-Whitney U test. With the exception of ΣPCB that were dosed only in plants 2 and 3, the other investigated biomarkers showed significant differences among factories. In plant 1 we determined the lowest levels of both Be-U and As-U and the highest values of Ni-U, 1-OHP and PbB. Workers from factory 3 showed the lowest values of Cu-U, Zn-U, Ni-U and PbB and the highest levels of both Cd-U and As-U.

Table 10 resumes the distributions of results by different job tasks of the whole sample, in order to have sufficient subgroup sizes. Be-U and 1-OHP showed significant differences among the five categories. On average, Be-U prevailed among scrapping and the "others" group and 1-OHP among workers of the furnace /casting task.

Age was available only as ordinal variable (age brackets). The analysis of data by age groups (Table 11) evidenced significant differences for Cd-U, Be-U, PbB and ΣPCB values that showed a trend toward higher values with increasing age. These significant trends were confirmed for Cd-U and Be-U by the data analysis by groups of work seniority (Table 12). For PbB and ΣPCB values the trend toward higher values with increasing of

exposure duration was not significant, probably due the low number of subjects in the highest duration group.

No significant difference was apparent among biomarker values in workers classified by residence area (Table 13).

Table 14 summarizes distribution of biomarkers in workers classified by ethnic group. Apart from Caucasians, there were only 8 Africans, that excreted significantly higher Zn-U levels, as compared to Caucasians (p<0.05).

The analysis of data by smoking habits demonstrated an interference by tobacco smoke on Cd-U and 1-OHP levels (Table 15), as current smokers excreted significantly higher Cd-U and 1-OHP levels than non-smokers.

Subjects who had consumed fish/jellyfish in the days preceding the biomonitoring campaign eliminated significantly higher levels of As-U and 1-OHP, as compared to subjects who had not eaten such foods (Table 16).

Stepwise multiple regression analyses were performed for all investigated biomarkers in models including them as dependent variables and lifestyle factors (smoking and alcohol intake) as independent variables.

Cd-U, 1-OHP and PCB levels were significantly associated to the extent of smoking habits (as packyears). The respective equations were: *Cd-U=0.01(packyears)+0.389 (p<0.000); 1-OHP=0.019(packyears)+0.22 (p<0.000)* and *PCB=0.084(packyears)+3.608 (p=0.039)*.

Table 8 Distribution of biomarkers in workers of copper-alloy foundries.

Parameters	Cu-U	Zn-U	Cd-U	Cr-U	Ni-U	As-U	Be-U	1-OHP	PbB	ΣPCB
N°	150	150	150	150	150	150	150	157	43	34
GM	13.67	557.99	0.39	0.16	0.37	4.65	0.006	0.22	15.67	3.12
Mean	15.39	688.37	0.47	0.21	0.58	6.96	0.007	0.37	17.64	4.32
SD	8.28	399.66	0.29	0.21	0.60	6.28	0.004	0.97	8.75	3.08
Asymmetry	2.66	1.16	1.38	5.09	2.26	2.47	2.16	11.17	0.91	0.88
Minimum	2.00	3.83	0.0	0.02	0.10	0.10	0.005	0.01	5.60	0.20
Maximum	72.00	2042.00	1.42	2.00	3.70	43.00	0.021	12.02	41.30	12.70
Percentiles										
5th ntile	6.62	205.00	0.146	0.050	0.100	0.500	0.005	0.05	6.62	0.42
95th ntile	29.44	1519.90	1.153	0.489	1.845	20.15	0.017	0.96	35.60	10.75
Reference Values										
5th ntile	5.00	200.00	0.10	0.05	0.10	0.10	0.01	0.03	0.50	0.80
95th ntile	60.00	900.00	1.50	2.20	4.00	20.00	0.06	0.70	10.00	14.10
BLV										
ACGIH	N.A.	N.A.	5.00	25.00	N.A.	35.00	N.A.	2.7†	30.00	N.A.
DFG	N.A.	N.A.	N.A.	12-40	15-45* 25-70°	50-300	N.A.		40.00	N.A.
N°< LOD	0	0	0	2	0	0	115	0	0	0
N°> 95th of RV	1	34	0	0	0	7	0	13	32	0

*Ni metal, oxide, carbonate, sulfide, sulfidic ores; $^{\circ}$easily soluble Ni compounds, e.g. Ni acetate and similar soluble salts, Ni chloride, Ni hydroxide, Ni sulfate.
†NOAEL according to Jongeneelen et al.
N.A.: not assigned.

Table 9 Distribution of biomarkers in copper-alloy workers stratified by plants.

Biomarkers	Plant 1			Plant 2			Plant 3		
	N°	GM	Min.- Max.	N°	GM	Min.- Max.	N°	GM	Min.- Max.
Cu-U	40	14.88[b]	5.50-41.00	68	15.75[c]	3.80-72.00	42	10.03[b,c]	2.00-35.60
Zn-U	40	615.03[b]	221-2002	68	651.26[c]	55-2042	42	395.98[b,c]	3.83-1748
Cd-U	40	0.42[a]	0.14-1.16	68	0.34[a,c]	0.09-1.42	42	0.46[c]	0.07-1.38
Cr-U	40	0.15[b]	0.05-.85	68	0.16[c]	0.02-2.00	42	0.20[b,c]	0.02-1.20
Ni-U	40	0.50[a,b]	0.10-2.90	68	0.33[a]	0.10-3.70	42	0.32[b]	0.10-2.40
As-U	40	2.93[a,b]	0.10-30.50	68	5.47[a]	0.20-43.00	42	5.54[b]	2.00-21.20
Be-U	40	.005[a]	0.005-0.02	68	0.007[a]	0.005-0.02	42	0.006	0.005-.017
1-OHP	48	0.28[a]	0.03-1.51	67	0.18[a]	0.01-12.02	42	0.22	0.02-1.18
PbB	9	28.06[a,b]	17.30-41.30	21	15.88[a,c]	6.60-30.00	13	10.24[b,c]	5.60-19.70
Σ PCB	0	-	-	21	2.85	0.20-12.70	13	3.62	0.70-9.80

[a,b,c]p<0,05 Mann-Whitney U test; a = plant 1 *vs* 2; b = plant 1 *vs* 3; c = plant 2 *vs* 3.

Table 10 Distributions of biomarkers in copper alloy workers stratified by job task.

Biomarkers	Scrap			Oven/casting			Jolly, heads			Maintenance			Others		
	N	GM	Min-Max	N	GM	Min-Max	N	GM	Min-Max	N	GM	Min-Max	N	GM	Min-Max
Cu-U	42	13.74	3.80-72.00	86	13.50	2.00-32.00	7	12.62	3.80-25.00	6	12.29	6.90-21.60	9	17.29	7.30-41.00
Zn-U	42	484.44	4.50-1474	86	620.33	86-2042	7	476.89	55-1854	6	245.10	3.83-928	9	767.01	205-1748
Cd-U	42	0.38	0.14-1.38	86	0.42	0.09-1.42	7	0.37	0.21-0.82	6	0.23	0.07-0.48	9	0.40	0.14-1.03
Cr-U	42	0.14	0.05-1.20	86	0.17	0.02-2.00	7	0.15	0.025-0.400	6	0.19	0.13-0.30	9	0.20	0.10-0.85
Ni-U	42	0.38	0.10-3.70	86	0.35	0.10-2.90	7	0.32	0.10-1.10	6	0.41	0.10-2.50	9	0.46	0.10-2.40
As-U	42	6.22	1.00-30.50	86	4.26	0.10-43.00	7	5.04	1.00-9.50	6	1.79	0.10-6.50	9	4.84	0.10-30.20
Be-U*	42	.007	0.005-.02	86	0.006	0.005-0.02	7	0.006	0.005-0.01	6	0.007	0.005-0.01	9	0.007	0.005-0.01
1-OHP*	46	0.15	0.01-1.18	89	0.26	0.02-12.02	7	0.22	0.10-0.53	6	0.14	0.08-0.23	9	0.26	0.14-1.14
PbB	21	16.38	5.60-41.30	20	15.48	6.70-31.90	0	-	-	1	9.40	9.40-9.40	1	13.00	13.00-13.00
Σ PCB	14	3.54	0.70-9.80	18	2.89	0.20-12.70	0	-	-	1	4.00	4.00-4.00	1	1.80	1.80-1.80

*$p<0.05$ Kruskall Wallis's H test

Table 11 Distributions of biomarkers in copper alloy workers stratified by age brackets.

Biomarkers	**< 30 years**			**30-40 years**			**40-50 years**			**> 50 years**		
	N	GM	Min-Max	N	GM	Min-Max	N	GM	Min-Max	N	GM	Min-Max
Cu-U	5	16.03	6.90-35.60	41	14.26	2.00-41.00	57	12.60	3.80-30.60	47	14.31	6.90-72.00
Zn-U	5	317.63	3.83-1748	41	567.99	86-1854	57	501.97	4.50-1471	47	663.21	221-2042
Cd-U*	5	0.18	0.07-0.51	41	0.29	0.14-0.90	57	0.41	0.09-1.14	47	0.52	0.14-1.42
Cr-U	5	0.16	0.08-0.36	41	0.18	0.02-2.00	57	0.15	0.02-1.20	47	0.17	0.05-.70
Ni-U	5	0.34	0.10-1.80	41	0.38	0.10-3.70	57	0.37	0.10-2.90	47	0.36	0.10-2.40
As-U	5	2.72	0.50-19.20	41	4.93	0.10-30.20	57	4.25	0.10-43.00	47	5.21	0.70-30.50
Be-U*	5	0.006	0.005-0.01	41	0.006	0.005-.018	57	0.006	0.005-.021	47	0.007	0.005-.020
1-OHP	6	0.17	0.10-0.26	42	0.20	0.07-0.60	60	0.23	0.01-1.51	49	0.22	0.02-12.02
PbB*	0	-	-	10	13.05	5.60-32.00	19	14.30	7.90-41.30	14	20.22	6.60-35.70
Σ PCB*	0	-	-	9	1.17	0.20-3.70	18	3.65	0.90-12.70	7	7.38	5.10-10.10

*$p<0.05$ Kruskall Wallis's H test

Table 12 Distributions of biomarkers in copper alloy workers stratified by work seniority.

Biomarkers	**1-10 years**			**11-20 years**			**> 20 years**		
	N	GM	Min-Max	N	GM	Min-Max	N	GM	Min-Max
Cu-U	64	13.72	5.50-41.00	64	13.45	2.00-32.00	22	14.20	3.80-72.00
Zn-U	64	528.46	3.83-1748	64	604.69	86.00-2042	22	517.33	55-1474
Cd-U*	64	0.33	0.07-1.36	64	0.41	0.14-1.38	22	0.57	0.18-1.42
Cr-U	64	0.17	0.05-2.00	64	0.17	0.02-1.20	22	0.14	0.02-0.44
Ni-U	64	0.39	0.10-2.90	64	0.38	0.10-3.70	22	0.28	0.10-1.30
As-U	64	4.35	0.10-30.50	64	5.06	0.10-43.00	22	4.39	0.10-12.20
Be-U*	64	0.006	0.005-0.02	64	0.006	0.005-.020	22	0.007	0.005-0.021
1-OHP	65	0.23	0.05-1.51	70	0.22	0.02-12.02	22	0.17	0.01-0.83
PbB	14	14.89	5.60-41.30	19	15.52	6.70-35.70	10	17.12	6.60-31.90
Σ PCB	11	1.94	0.20-7.00	16	3.27	0.90-10.10	7	5.95	3.00-12.70

*$p<0.05$ Kruskall Wallis's H test

Table 13 Distributions of biomarkers in workers stratified by residence area.

Biomarkers	Country / Mountain			Brescia			Neighbouring villages			Brescia hinterland		
	N	GM	Min-Max	N	GM	Min-Max	N	GM	Min-Max	N	GM	Min-Max
Cu-U	28	15.15	5.50-35.60	19	14.88	3.80-41.00	83	13.04	2.00-72.00	20	13.32	5.50-26.70
Zn-U	28	698.36	205-2042	19	641.46	329-1576	83	492.20	3.83-2002	20	600.88	195-1410
Cd-U	28	0.39	0.15-1.36	19	0.39	0.16-1.16	83	0.41	0.07-1.42	20	0.34	0.09-1.02
Cr-U	28	0.18	0.05-2.00	19	0.16	0.05-0.85	83	0.16	0.02-1.20	20	0.17	0.07-0.30
Ni-U	28	0.40	0.10-3.70	19	0.54	0.10-2.90	83	0.31	0.10-1.90	20	0.44	0.10-2.50
As-U	28	4.66	0.20-24.00	19	5.72	0.40-30.50	83	4.54	0.10-43.00	20	4.19	0.10-30.20
Be-U	28	0.006	0.005-0.02	19	0.0052	0.005-.01	83	0.006	0.005-.02	20	0.006	0.005-.020
1-OHP	28	0.29	0.07-12.02	21	0.29	0.03-1.51	88	0.19	0.02-1.31	20	0.16	0.01-0.51
PbB	9	14.06	6.60-30.00	3	23.34	17.30-35.70	25	15.19	5.60-41.30	6	17.18	8.90-35.20
Σ PCB	9	2.80	0.50-10.10	0	-	-	20	3.21	0.20-12.70	5	3.41	1.90-9.80

Table 14 Distributions of biomarkers in copper alloy workers stratified by ethnic groups.

Biomarkers	**Caucasians**			**Africans**		
	N	GM	Min-Max	N	GM	Min-Max
Cu-U	142	13.60	2.00-72.00	8	15.11	8.00-29.00
Zn-U*	142	542.22	3.83-2042	8	928.17	495-1576
Cd-U	142	0.40	0.07-1.42	8	0.32	0.09-0.77
Cr-U	142	0.16	0.02-2.00	8	0.14	0.05-0.29
Ni-U	142	0.37	0.10-3.70	8	0.41	0.10-1.60
As-U	142	4.55	0.10-43.00	8	6.71	2.30-30.20
Be-U	142	0.006	0.005-0.02	8	0.006	0.005-0.02
1-OHP	148	0.21	0.01-12.02	9	0.29	0.12-1.51
PbB	43	15.67	5.60-41.30	0	-	-
Σ PCB	34	3.12	0.20-12.70	0	-	-

*$p<0.05$ Kruskall Wallis's H test

Table 15 Distributions of biomarkers in workers stratified by smoking habits.

Biomarkers	Current smokers			Not Smokers		
	N	GM	Min-Max	N	GM	Min-Max
Cu-U	60	13.45	3.80-35.60	90	13.82	2.00-72.00
Zn-U	60	500.38	3.83-2042	90	600.03	86-2002
Cd-U*	60	0.51	0.071-1.42	90	0.33	0.086-1.13
Cr-U	60	0.17	0.02-0.70	90	0.16	0.02-2.00
Ni-U	60	0.37	0.10-2.90	90	0.37	0.10-3.70
As-U	60	4.50	0.10-43.00	90	4.74	0.10-30.50
Be-U	60	0.006	0.005-0.02	90	0.006	0.005-.021
1-OHP*	63	0.45	0.10-12.02	94	0.13	0.01-0.47
PbB	15	17.30	9.60-31.90	28	14.86	5.60-41.30
Σ PCB	12	3.56	0.50-12.70	22	2.91	0.20-8.90

*$p<0.05$, Mann-Whitney U test

Table 16 Distributions of biomarkers in workers stratified by fish/jellyfish consumption.

Biomarkers	Fish consumption					
	Yes			No		
	N	GM	Min-Max	N	GM	Min-Max
Cu-U*	35	11.41	2.00-35.60	115	14.45	3.80-72.00
Zn-U	35	528.68	86-1748	115	567.23	3.83-2042
Cd-U	35	0.45	0.15-1.36	115	0.38	0.07-1.42
Cr-U	35	0.16	0.02-0.44	115	0.16	0.02-2.00
Ni-U	35	0.28	0.10-2.40	115	0.40	0.10-3.70
As-U*	35	6.65	1.10-30.20	115	4.17	0.10-43.00
Be-U	35	0.006	0.005-0.017	115	0.006	0.005-0.021
1-OHP*	38	0.26	0.01-1.14	119	0.20	0.02-12.02
PbB	10	12.55	6.70-19.50	33	16.76	5.60-41.30
Σ PCB	9	2.88	0.20-12.70	25	3.22	0.50-10.10

*p<0.05, Mann-Whitney U test

4.3 Electric steel plants

4.3.1 Environmental monitoring

The general descriptive results (as μg/m^3) resulting from personal sampling (n=9) in a previously investigated plant representative of the sector are below reported.

	As	Be	Cd	Cr-tot	Ni	Pb
Minimum	0.03	<0.0006	0.003	0.10	0.10	0.50
Maximum	0.10	<0.0006	0.10	0.40	1.00	14.00
Mean	0.06	n.c.	0.04	0.18	0.40	7.22
Geometric mean	0.05	n.c.	0.02	0.16	0.31	4.72
***TLV-TWA (**μg/m^3**)**	10	2	10	500	100	75***

*Treshold limit values according to ACGIH; n.c..: not calculable; ***according to the Italian Law (DLgs 81/2008).

All values fell within the respective TLV-TWAs, both at individual and group level.

The median value of exposure to benz(a)pyrene was 8.05 (range 0.50-15.30) ng/m^3, from 4 area samplings

4.3.2 Biological monitoring

Urinary samples were collected at the end of the work-shift from all workers (n=340) and blood from about 17% of workers.

The enrolled people belonged to the following main working areas:

- scrap: 14 %;
- furnace/casting 60 %;
- maintenance: 13 %;
- jolly/heads: 1 %;
- others: 12 %.

The workers' age was mostly (65 %) comprised between 30 and 50 years and the majority (98 %) was constituted by Caucasians, whereas most of the rest (n=6) included Africans

(n=3). Most of workers lives in rural zones or municipalities of the Brescia province, whereas only 32% of subjects live in the Brescia hinterland.

Fourty-six percent of workers (n=157) were current smokers.

Table 17 resumes, the descriptive analysis of evaluated biomarkers on a whole sample basis.

On average, the values fell below the biological limit values (BLV) indicated by either DFG or ACGIH, or, in the case of 1-OHP, suggested by important researchers such as Joenghеleghen (Jongeneelen FJ 2001)

Some workers showed, for different analytes, levels higher than the corresponding upper limits of the reference values. In detail, this held true for Mn-U (n°=1); Cd-U (n°=2); Cr-U (n°=14), Ni-U (n°=7), As-U (n°=10), 1-OHP (n°=100) PbB (n°=16), PCB (n°=1); detectable levels of Be-U were measured in 44 workers.

One worker and 29 workers not smokers have exceeded the 95th ntile of RV for Cd-U and 1-OHP, respectively; all the workers exceeding the 95th ntile of RV for As-U (n°=10), ate fish and jellyfish in the days preceding the sampling, in 5 cases also grilled food.

Table 18 resumes data stratified by plants, for age brackets, smoking habits and alcohol consumption. The analysis of data by the chi-square test showed that differences among groups were not statistically significant.

Table 19 resumes data stratified by plants and statistical comparisons applying the Kruskal-Wallis H test. With the exception of ΣPCB, the other investigated biomarkers showed significant differences among factories. Mn-U, Cd-U and PbB resulted higher in Plant 1, whereas Cr-U and Ni-U were higher in plant 4. The 1-OHP was practically at the same level in plant 1 and 5, or 5 differently for different analytes.

In Table 20 the distributions of results by 5 different job tasks of the whole sample are reported. Cd-U, Cr-U, As-U and 1-OHP showed significant differences among the five categories. The highest values of Cd-U were determined in both scrapping and others jobs. Cr-U prevailed among oven casting, As among maintenance workers and 1-OHP among others and oven/casters. There were no data for PbB and ΣPCB in plant 2.

Age was available only as ordinal variable (age brackets). The analysis of data by age groups (table 21) evidenced significant differences for Cd-U and ΣPCB values that showed a trend toward higher values with increasing age. These significant trends were confirmed

for Cd-U, PbB, and ΣPCB by the data analysis by groups of work seniority and confirmed that these xenobiotics tend to cumulate in humans (Table 22).

Significant difference was demonstrated for Mn-U, 1-OHP and PbB values in workers classified by residence area. Mn-U and 1-OHP resulted higher for resident in Brescia town, while PbB was higher in country Mountain residents (Table 23).

Table 24 summarizes distribution of biomarkers in workers classified by ethnic group without significant differences. There were only 5 non-Caucasians and these percentages do not allow significant analysis.

The analysis of data by smoking habits demonstrated an interference by tobacco smoke on Cd-U and 1-OHP levels (Table 25), as current smokers excreted significantly higher Cd-U and 1-OHP levels than non-smokers.

Stepwise multiple regression analyses were performed for all investigated biomarkers in models including them as dependent variable "Y", and lifestyle factors (smoking and alcohol intake) as independent variables "X".

Cd-U, 1-OHP and ΣPCB levels were significantly associated to the extent of smoking habits (as packyears). The respective equations were: *Cd-U=0.008(packyears)+0286 (p<0.000), 1-OHP=0.02(packyears)+0.508 (p<0.000)* and *ΣPCB=0.089(packyears)+3.277 (p=0.027).*

The relationship between Cr-U and As-U (µg/l) and the levels of the alcohol consumption. The respective equations were: *Cr-U=0.004(gr/alcohol)+0.589 (p=0.041)* and *As-U=0.036(gr/alcohol)+6.825 (p=0.013).*

Table 17 Distribution of investigated biomarkers in electric steel workers.

Parameters	**Mn-U**	**Cd-U**	**Cr-U**	**Ni-U**	**As-U**	**Be-U**	**1-OHP**	**PbB**	**ΣPCB**
N°	333	335	335	331	335	334	339	59	59
GM	0.18	0.29	0.40	0.73	5.32	0.006	0.41	7.13	2.76
Mean	0.32	0.35	0.66	1.16	7.47	0.006	0.67	8.39	3.78
SD	0.51	0.27	0.90	1.06	6.75	0.003	0.89	4.98	2.92
Asymmetry	5.23	4.71	6.56	1.58	4.94	2.853	5.11	1.22	1.54
Minimum	0.001	0.06	0.02	0.10	0.10	0.005	0.03	2.10	0.10
Maximum	5.20	3.23	11.60	5.60	78.30	0.020	9.65	24.80	14.80
Percentiles									
5th ntile	0.10	0.13	0.06	0.10	0.70	0.005	0.08	2.60	0.40
95th ntile	1.13	0.84	2.02	3.50	16.32	0.014	1.82	19.60	10.60
Reference Values									
5th ntile	0.1	0.10	0.05	0.10	0.10	0.01	0.03	0.50	0.80
95th ntile	5.00	1.50	2.20	4.00	20.00	0.06	0.70	10.00	14.10
BLV									
ACGIH		5.00	25.00	N.A.	35.00	N.A.	2.7†	30.00	N.A.
DFG		N.A.	12-40	15-45* 25-70°	50-300	N.A.		40.00	N.A.
N°< LOD	9	0	12	0	0	290	10	0	0
N°> 95 th of RV	1	2	14	7	10	0	100	16	1

*Ni metal, oxide, carbonate, sulfide, sulfidic ores; $^{\circ}$easily soluble Ni compounds, e.g. Ni acetate and similar soluble salts, Ni chloride, Ni hydroxide, Ni sulfate.

†NOAEL according to Jongeneelen et al.

N.A.: not assigned.

Table 18 Distribution of age brackets, smoking habits and alcohol consumption in electric steel workers stratified by plants.

	Plant 1	**Plant 2**	**Plant 3**	**Plant 4**	**Plant 5**	**totals**
	N°	N°	N°	N°	N°	N°
<30 years	9	10	8	5	6	38
30-40 years	29	14	23	5	16	87
40-50 years	49	15	31	10	33	138
>50 years	24	4	25	5	21	79
totals	111	43	87	25	76	342
Current smokers	49	28	41	9	33	160
Not smokers	62	15	46	16	43	182
totals	111	43	87	25	76	342
Alcohol /yes	48	20	44	11	39	162
Alcohol /not	63	23	43	14	37	180
totals	111	43	87	25	76	342

Table 19 Distribution of biomarkers in electric steel workers stratified by plants.

Biomarkers	Plant 1				Plant 2				Plant 3				Plant 4				Plant 5			
	N°	GM	Min	Max	N°	GM	Min	Max	N°	GM	Min	Max	N°	GM	Min	Max	N°	GM	Min	Max
Mn-U*	109	0.35	0.10	3.00	39	0.23	0.10	5.20	86	0.14	0.10	3.30	25	0.27	0.10	3.50	74	0.08	0.001	0.90
Cd-U*	110	0.31	0.09	1.09	39	0.24	0.07	0.77	86	0.27	0.06	1.10	25	0.24	0.12	0.51	75	0.35	0.07	3.23
Cr-U*	110	0.57	0.13	2.70	39	0.26	0.02	5.20	86	0.18	0.02	3.90	25	1.59	0.19	11.60	75	0.47	.06	2.10
Ni-U*	110	0.89	0.10	5.60	39	0.74	0.10	3.80	86	0.51	0.10	3.90	25	2.38	0.60	5.40	71	0.54	0.10	5.00
As-U*	110	4.46	0.10	51.80	39	7.80	0.70	32.00	86	6.98	1.30	23.90	25	6.03	0.30	78.30	75	3.95	0.100	14.20
Be-U*	109	0.006	0.005	0.02	39	0.005	0.005	0.005	86	0.006	0.005	0.02	25	0.005	0.005	0.010	75	0.005	0.005	0.016
1-OHP*	111	0.57	0.03	9.65	40	0.29	0.04	2.13	87	0.30	0.03	2.38	25	0.23	0.03	0.83	76	0.54	0.07	5.99
PbB*	25	10.17	4.00	24.80	0	-	-	-	13	5.90	2.80	13.40	6	4.16	2.100	14.00	15	5.76	2.30	11.80
Σ PCB	25	2.45	0.10	14.80	0	-	-		13	3.53	1.70	10.60	6	2.47	.400	7.60	15	2.83	0.40	7.90

*$p<0.05$ Kruskal-Wallis H test

Table 20 Distributions of biomarkers in electric steel workers stratified by job tasks.

Biomarkers	Scrap				Oven/casting				Jolly, heads				Maintenance				Others			
	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max
Mn-U	46	0.18	0.001	3.30	202	0.19	0.001	5.20	20	0.21	0.10	1.80	42	0.18	0.10	0.70	23	0.14	0.001	1.40
Cd-U*	46	0.36	0.14	1.21	202	0.28	0.06	1.83	20	0.27	0.12	0.84	43	0.25	0.11	0.62	24	0.37	0.07	3.23
Cr-U*	46	.25$$6	0.02	1.40	202	0.45	0.02	11.60	20	0.32	0.02	1.36	43	0.40	0.02	2.30	24	0.37	0.07	2.00
Ni-U	46	0.74	0.10	3.90	199	0.72	0.10	5.20	20	0.74	0.10	5.40	42	0.89	0.10	5.60	24	0.55	0.10	5.00
As-U*	46	3.80	0.10	18.50	202	5.43	0.10	78.30	20	5.80	0.30	25.90	43	6.92	0.30	22.00	24	4.93	1.20	13.30
Be-U	46	0.006	0.005	0.020	201	0.006	0.005	0.020	20	0.006	0.005	0.015	43	0.006	0.005	0.015	24	0.006	0.005	0.016
1-OHP*	47	0.26	0.03	1.56	205	0.47	0.03	9.65	20	0.33	0.07	1.80	43	0.32	0.03	3.31	24	0.62	0.04	5.99
PbB	17	5.95	2.80	18.90	33	7.46	2.10	24.80	8	9.69	2.60	19.70	0	-	-	-	1	3.00	3.00	3.00
Σ PCB	17	2.95	0.50	11.80	33	2.76	0.10	14.80	8	2.20	0.40	5.50	0	-	-	-	1	5.20	5.20	5.20

*$p<0.05$ Kruskal-Wallis H test

Table 21 Distributions of biomarkers in electric steel workers stratified by age brackets.

Biomarkers	< 30 years				30-40 years				40-50 years				> 50 years			
	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max
Mn-U	37	0.19	0.10	1.80	84	0.16	0.001	3.00	134	0.20	0.001	5.20	78	0.18	0.001	2.30
Cd-U*	37	0.19	0.06	1.00	85	0.25	0.07	1.03	135	0.31	0.06	3.23	78	0.38	0.12	1.21
Cr-U	37	0.35	0.02	3.50	85	0.39	0.02	2.30	135	0.42	0.02	11.60	78	0.39	0.02	4.40
Ni-U	37	0.89	0.10	5.40	85	0.77	0.10	5.60	133	0.71	0.10	4.80	76	0.65	0.10	5.20
As-U	37	6.31	0.60	25.50	85	5.63	0.10	78.30	135	5.20	0.10	30.20	78	4.79	0.10	51.80
Be-U	36	0.006	0.005	0.016	85	0.006	0.005	0.020	135	0.006	0.005	0.017	78	0.006	0.005	0.020
1-OHP	38	0.37	0.07	4.20	85	0.49	0.08	9.65	137	0.39	0.03	4.18	79	0.41	0.03	5.33
PbB	4	3.31	2.60	4.10	17	7.52	3.30	18.20	25	7.45	2.30	19.60	13	7.74	2.10	24.80
Σ PCB*	4	0.47	0.10	2.40	17	1.80	0.40	3.40	25	3.49	0.90	11.80	13	5.33	1.40	14.80

*$p<0.05$ Kruskal-Wallis H test

Table 22 Distributions of biomarkers in electric steel workers stratified by work seniority.

Biomarkers	**1-10 years**				**11-20 years**				**> 20 years**			
	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max
Mn-U	166	0.18	0.001	5.20	99	0.20	0.001	3.50	68	0.19	0.001	1.70
Cd-U*	167	0.26	0.06	1.83	100	0.31	0.07	3.23	68	0.34	0.14	1.21
Cr-U	167	0.38	0.02	5.20	100	0.41	0.02	11.60	68	0.44	0.05	3.30
Ni-U	165	0.82	0.10	5.60	100	0.63	0.10	3.90	66	0.65	0.10	5.20
As-U*	167	6.29	0.10	78.30	100	4.82	0.10	30.20	68	4.07	0.10	51.80
Be-U	166	0.006	0.005	0.020	100	0.006	0.005	0.020	68	0.006	0.005	0.02
1-OHP	169	0.41	0.03	9.65	102	0.44	0.03	3.31	68	0.37	0.03	1.80
PbB*	20	5.52	2.10	24.80	27	7.95	3.00	19.60	12	8.55	2.30	19.70
Σ PCB*	20	1.87	0.10	7.60	27	2.84	0.90	14.80	12	4.94	2.40	10.60

*$p<0.05$ Kruskal-Wallis H test

Table 23 Distributions of biomarkers in electric steel workers stratified by residence area.

Biomarkers	Country / Mountain				Brescia				Neighbouring villages				Brescia hinterland			
	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max
Mn-U*	43	0.15	0.001	3.50	52	0.25	0.001	2.20	185	0.20	0.08	3.30	53	0.13	0.001	5.20
Cd-U	43	0.29	0.06	0.85	52	0.30	0.12	1.03	186	0.28	0.07	1.10	54	0.34	0.07	3.24
Cr-U	43	0.40	0.03	3.10	52	0.45	0.03	1.80	186	0.36	0.03	11.60	54	0.51	0.03	5.20
Ni-U	42	0.74	0.10	5.00	51	0.62	0.10	5.40	185	0.73	0.10	5.20	53	0.83	0.10	5.60
As-U	43	4.88	0.10	23.90	52	4.72	0.30	32.00	186	6.15	0.10	78.30	54	3.86	0.10	22.00
Be-U	42	0.006	0.005	0.016	52	0.005	0.005	0.020	186	0.006	0.005	0.020	54	0.006	0.005	0.020
1-OHP*	44	0.48	0.03	1.94	53	0.51	0.03	9.65	187	0.35	0.03	5.99	55	0.51	0.05	4.18
PbB*	8	13.02	5.80	24.80	9	4.94	2.30	11.80	34	7.28	2.10	19.60	8	5.40	3.70	14.60
Σ PCB	8	3.03	1.50	5.80	9	1.98	0.10	6.90	34	3.26	0.90	14.80	8	1.81	0.40	6.60

*$p<0.05$ Kruskal-Wallis H test

Table 24 Distributions of biomarkers in electric steel workers stratified by ethnic groups.

Biomarkers	Africans				Others				Arabs				Caucasians			
	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max
Mn-U*	3	0.44	0.30	0.70	1	1.80	1.80	1.80	1	2.10	2.10	2.10	328	0.18	0.001	5.20
Cd-U	3	0.28	0.24	0.37	1	0.37	0.37	0.37	1	0.15	0.15	0.15	330	0.29	0.06	3.24
Cr-U	3	0.65	0.58	0.69	1	0.61	0.61	0.61	1	4.40	4.40	4.40	330	0.39	0.03	11.60
Ni-U	3	0.63	0.40	0.90	1	1.90	1.90	1.90	1	3.70	3.70	3.70	326	0.72	0.10	5.60
As-U	3	10.12	4.70	30.20	1	18.50	18.50	18.50	1	1.20	1.20	1.20	330	5.29	0.10	78.30
Be-U	3	0.005	0.005	0.005	1	0.005	0.005	0.005	1	0.005	0.005	0.005	329	0.006	0.005	0.020
1-OHP	3	0.27	0.14	0.62	1	0.11	0.11	0.11	1	0.49	0.49	0.49	334	0.42	0.03	9.65
PbB	1	8.40	8.40	8.40	0	-	-	-	1	5.30	5.30	5.30	57	7.15	2.10	24.80
Σ PCB	1	2.80	2.80	2.80	0	-	-	-	1	1.40	1.40	1.40	57	2.79	0.10	14.80

*$p<0.05$ Kruskal-Wallis H test

Table 25 Distributions of biomarkers in workers stratified by smoking habits.

Biomarkers	Current smokers				Not Smokers			
	N	GM	Min	Max	N	GM	Min	Max
Mn-U	152	0.20	0.001	5.20	181	0.18	0.00	3.50
Cd-U*	154	0.33	0.07	3.24	181	0.26	0.06	1.83
Cr-U	154	0.40	0.03	5.20	181	0.40	0.03	11.60
Ni-U	153	0.70	0.10	5.60	178	0.75	0.10	5.20
As-U	154	5.23	0.10	78.30	181	5.39	0.10	51.80
Be-U	154	.006	.005	.020	180	.006	.005	.020
1-OHP*	157	0.66	0.12	9.65	182	0.28	0.03	4.30
PbB	24	7.21	2.60	18.20	35	7.08	2.10	24.80
Σ PCB	24	2.84	0.40	11.80	35	2.71	0.10	14.80

*p<0.05, Mann-Whitney U test

4.4 Cast iron foundries

4.4.1 Environmental monitoring

The general descriptive results (as μg/m^3) of personal airborne sampling (n=30) are below reported:

	As	Be	Cd	Cr-tot	Ni	Pb	BaP**
Minimum	<0.02	<0.02	<0.02	0.40	<0.06	0.10	<0.0005
Maximum	<3.3	<2	<3.3	7.50	<17.9	<33.1	0.0115
Mean	-	-	-	1.94	-	1.60	0.0021
Geometric mean	-	-	-	1.54	-	0.80	0.0009
***TLV-TWA (μg/m^3)**	10	2	10	500	100	75***	n.a.

*Treshold limit values according to ACGIH; **Benz[a]pyrene; n.a.: not available; ***according to the Italian Law (DLgs 81/2008)

All determined compounds fell below the corresponding TLV-TWA value.

4.4.2 Biological monitoring

Urine spot samples were collected by the RAMET Consortium from all workers (n=160) and blood from about 16% of them.

The enrolled people belonged to the following main working areas:

- scrap: 28 %;
- furnace/casting 54 %;
- maintenance: 4 %;
- jolly/heads: 4 %;
- others: 10 %.

The workers' age was mostly (64%) comprised between 30 and 50 years and the majority (94 %) was constituted by Caucasians, whereas the rest (n=9) included Africans. Most of workers lives in rural zones or municipalities of the Brescia province, whereas only 20 subjects live in the Brescia hinterland.

Sixty-five workers were current smokers and 51 % of non smokers were former smokers.

Table 26 resumes, the descriptive analysis of evaluated biomarkers on a whole sample basis.

On average, the values fell below the biological limit values (BLV) indicated by either international agencies or single expert authors (Jongeneelen FJ 2001).

Detectable levels of Be-U were measured in 44 workers.

Some workers showed, for different analytes, levels higher than the corresponding upper limits of the reference values. This held true in more detail for Cr-U (n°=1), Ni-U (n°=4), As-U (n°=22), 1-OHP (n°=31).

One and 29 not smoker subjects presented values higher than the 95th percentile of RV for Cd-U and 1-OHP, respectively; all the workers exceeding the 95th percentile of RV for As-U (n°=10), have probable alimentary sources of t he element to be controlled.

Table 27 resumes data stratified by plants, age brackets, smoking habits and alcohol consumption.

Table 28 resumes data stratified by plants and statistical comparisons applying the Kruskal-Wallis H test. As-U, Be-U and 1-OHP showed significant differences among factories. As-U levels prevailed in plant 1, whereas 1-OHP showed the highest values in plant 4.

Table 29 shows the distributions of analytes by different job tasks. Cr-U and 1-OHP showed prevailing concentrations among casters and maintenance workers, respectively.

Age was available only as ordinal variable (age brackets). The analysis of data by age groups (table 30) evidenced significant differences for Cd-U only. The ΣPCB values tended to rise with increasing age but the trend was not significant. On the other hand, the data analysis by groups of work seniority evidenced significant differences for As-U and ΣPCB (Table 31).

Significant difference was demonstrated for As-U and Be-U values in workers classified by residence area. As-U resulted higher for resident in Brescia town, whereas Be-U was higher in country/mountain residents (Table 32).

Table 33 summarizes distribution of biomarkers in workers classified by ethnic group and shows that Africans eliminate significantly higher values of the element as compared to other ethnic groups.

As expected, we observed a significant interference by tobacco smoke on Cd-U and 1-

OHP levels (Table 34), current smokers excreting significantly higher Cd-U and 1-OHP levels than non-smokers.

Stepwise multiple regression analyses were performed for all investigated biomarkers in models including them as dependent variable "Y", and lifestyle factors (smoking and alcohol intake) as independent variables "X".

Cd-U and 1-OHP levels were significantly associated to the extent of smoking habits (as packyears). The respective equations were: *Cd-U=0.009(packyears)+0.257 (p<0.000)* and *1-OHP=0.012(packyears)+0.377 (p=0.014)*. The relationship between PbB (µg/dl) and the levels and the alcohol consumption; the equations was: *PbB=0.048(gr/alcohol)+2.879 (p<0.000).*

Table 26 Distribution of investigated biomarkers in cast iron workers.

Parameters	**Cd-U**	**Cr-U**	**Ni-U**	**As-U**	**Be-U**	**1-OHP**	**PbB**	**ΣPCB**
N°	157	157	156	157	157	167	25	16
GM	0.25	0.33	0.87	9.01	0.005	0.29	3.44	1.61
Mean	0.30	0.52	1.22	12.79	0.005	0.43	3.84	2.51
SD	0.19	0.49	1.06	12.20	0.001	0.48	2.00	2.52
Asymmetry	2.46	2.02	2.15	3.54	8.028	4.20	1.49	1.70
Minimum	0.000	0.03	0.10	0.30	0.005	0.01	1.80	0.30
Maximum	1.45	2.60	6.30	101.00	0.017	4.18	10.00	8.90
Percentiles								
5th ntile	0.11	0.03	0.10	1.77	0.005	0.06	1.83	0.30
95th ntile	0.64	1.90	3.23	36.86	0.005	1.16	9.19	8.90
Reference Values								
5th ntile	0.10	0.05	0.10	0.10	0.01	0.03	0.50	0.80
95th ntile	1.50	2.20	4.00	20.00	0.06	0.70	10.00	14.10
BLV								
ACGIH	5.00	25.00	N.A.	35.00	N.A.	2.7†	30.00	N.A.
DFG	N.A.	12-40	15-45* 25-70°	50-300	N.A.		40.00	N.A.
N°< LOD	0	10	0	0	153	3	0	0
N°> 95 th of RV	0	1	4	22	0	31	0	0

*Ni metal, oxide, carbonate, sulfide, sulfidic ores; $^{\circ}$easily soluble Ni compounds, e.g. Ni acetate and similar soluble salts, Ni chloride, Ni hydroxide, Ni sulfate.

†NOAEL according to Jongeneelen et al.

N.A.: not assigned.

Table 27 Distribution of age brackets, smoking habits and alcohol consumption in cast iron workers stratified by plants.

	Plant 1	**Plant 2**	**Plant 3**	**Plant 4**	**totals**
	N°	N°	N°	N°	N°
<30 years	9	9	1	1	20
30-40 years	26	24	9	10	69
40-50 years	33	11	7	8	59
>50 years	14	3	3	2	22
Totals	82	47	20	21	170
Current smokers	35	24	5	4	68
Not smokers	47	23	15	17	102
Totals	82	47	20	21	170
Alcohol /yes	31	16	7	6	60
Alcohol /not	51	31	13	15	110
Totals	82	47	20	21	170

Table 28 Distribution of biomarkers in cast iron workers stratified by plants.

Biomarkers	**Plant 1**				**Plant 2**				**Plant 3**				**Plant 4**			
	N°	GM	Min	Max	N°	GM	Min	Max	N°	GM	Min	Max	N°	GM	Min	Max
Cd-U	74	0.26	0.09	0.72	45	0.29	0.07	1.02	18	0.16	0.00	0.59	20	0.28	0.09	1.45
Cr-U	74	0.30	0.03	2.20	45	0.31	0.05	2.00	18	0.46	0.12	0.93	20	0.40	0.03	2.60
Ni-U	74	0.96	0.10	5.60	45	0.67	0.10	6.30	17	0.95	0.10	3.20	20	0.98	0.10	3.60
As-U*	74	11.77	2.20	101.00	45	9.18	0.30	52.00	18	6.71	0.70	53.80	20	4.18	0.30	39.20
Be-U*	74	0.005	0.005	0.005	45	0.005	0.005	0.017	18	0.006	0.005	0.010	20	0.005	0.005	0.005
1-OHP*	79	0.27	0.01	1.47	47	0.26	0.04	1.11	20	0.23	0.06	0.81	21	0.68	0.15	4.18
PbB	9	3.33	2.10	6.40	6	3.02	2.00	6.40	4	4.39	2.90	7.30	6	3.49	1.80	10.00
Σ PCB	9	1.89	0.50	8.90	0	-	-	-	1	0.30	0.30	0.30	6	1.68	0.40	7.70

*$p<0.05$ Kruskal-Wallis H test

Table 29 Distributions of biomarkers in cast iron workers stratified by job tasks.

Biomarkers	Scrap				Oven/casting				Jolly, heads				Maintenance				Others				Cores for casting production				Forming			
	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max
Cd-U	6	0.18	0.15	0.25	34	0.28	0.15	0.80	6	0.32	0.22	0.59	18	0.28	0.09	1.02	72	0.26	0.07	1.45	2	0.37	0.36	0.38	19	0.18	0.000	0.63
Cr-U*	6	0.23	0.02	0.91	34	0.60	0.08	2.20	6	0.35	0.09	0.74	18	0.48	0.10	2.60	72	0.27	0.02	2.00	2	0.16	0.08	0.32	19	0.21	0.02	1.60
Ni-U	6	1.16	0.50	5.40	34	0.74	0.10	6.30	6	0.86	0.10	2.90	18	1.26	0.10	3.60	72	0.89	0.10	5.60	2	0.46	0.30	0.70	18	0.71	0.20	2.00
As-U	6	10.92	5.30	24.90	34	9.54	0.90	48.60	6	7.31	2.30	14.20	18	7.83	1.50	53.80	72	8.91	0.30	101.00	2	9.29	8.00	10.80	19	9.68	0.70	54.30
Be-U*	6	0.007	0.005	0.010	34	0.005	0.005	0.005	6	0.005	0.005	0.005	18	0.005	0.005	0.005	72	0.005	0.005	0.017	2	0.005	0.005	0.005	19	0.005	0.005	0.005
1-OHP*	6	0.22	0.06	0.41	36	0.39	0.04	2.97	6	0.29	0.14	0.81	18	0.48	0.22	1.35	76	0.26	0.01	4.18	3	0.23	0.12	0.41	22	0.19	0.02	0.84
PbB	3	3.54	2.10	7.30	13	3.29	1.90	6.40	0	-	-	-	4	4.89	3.60	10.00	5	2.85	1.80	4.20	0	-	-	-	0	-	-	-
Σ PCB	2	0.57	0.30	1.10	6	1.01	0.50	2.30	0	-	-	-	3	4.01	2.50	8.90	5	2.47	0.40	7.70	0	-	-	-	0	-	-	-

*p<0.05 Kruskal-Wallis H test

Table 30 Distributions of biomarkers in cast iron workers stratified by age brackets.

Biomarkers	**< 30 years**				**30-40 years**				**40-50 years**				**> 50 years**			
	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max
Cd-U*	20	0.20	0.07	0.43	62	0.23	0.00	1.45	57	0.27	0.09	0.85	18	0.34	0.14	1.02
Cr-U	20	0.34	0.07	0.93	62	0.35	0.03	2.60	57	0.32	0.03	2.20	18	0.32	0.03	1.60
Ni-U	20	0.95	0.30	5.40	61	0.84	0.10	5.60	57	1.00	0.10	6.30	18	0.56	0.10	2.90
As-U	20	11.10	3.80	39.20	62	9.06	0.30	101.00	57	7.80	0.30	36.60	18	11.04	2.20	54.30
Be-U	20	0.005	0.005	0.005	62	0.005	0.005	0.010	57	0.005	0.005	0.017	18	0.005	0.005	0.005
1-OHP	20	0.27	0.04	1.47	67	0.29	0.01	2.97	58	0.31	0.05	4.18	22	0.28	0.02	0.96
PbB	2	2.83	2.10	3.80	12	3.46	1.80	7.30	9	3.66	1.90	10.00	2	3.06	2.60	3.60
Σ PCB	1	1.10	1.10	1.10	6	0.90	0.40	2.30	7	1.76	0.30	3.90	2	8.28	7.70	8.90

*$p<0.05$ Kruskal-Wallis H test

Table 31 Distributions of biomarkers in cast iron workers stratified by work seniority.

Biomarkers	**1-10 years**				**11-20 years**				**> 20 years**			
	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max
Cd-U	104	0.24	0.00	1.45	30	0.27	0.15	0.80	23	0.31	0.11	1.02
Cr-U	104	0.35	0.03	2.60	30	0.35	0.03	2.00	23	0.26	0.03	2.20
Ni-U	103	0.98	0.10	6.30	30	0.70	0.10	3.20	23	0.67	0.10	3.60
As-U*	104	9.90	0.30	101.00	30	10.94	2.60	36.60	23	4.57	0.30	15.90
Be-U	104	0.005	0.005	0.010	30	0.005	0.005	0.017	23	0.005	0.005	0.005
1-OHP	111	0.30	0.01	2.97	33	0.25	0.06	4.18	23	0.34	0.08	1.90
PbB	14	3.18	1.80	7.30	4	3.47	2.10	6.40	7	3.99	2.60	10.00
Σ PCB*	7	0.80	0.40	1.60	3	1.20	0.30	2.50	6	4.25	2.30	8.90

*$p<0.05$ Kruskal-Wallis H test

Table 32 Distributions of biomarkers in cast iron workers stratified by residence area.

Biomarkers	Country / Mountain				Brescia				Neighbouring villages				Brescia hinterland			
	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max
Cd-U	10	0.27	0.15	0.64	13	0.27	0.16	1.45	115	0.25	0.00	1.02	19	0.25	0.09	0.61
Cr-U	10	0.44	0.12	1.20	13	0.47	0.15	2.60	115	0.31	0.03	2.20	19	0.32	0.03	0.93
Ni-U	10	0.84	0.10	3.20	13	0.93	0.10	3.40	115	0.91	0.10	6.30	18	0.61	0.10	3.10
As-U*	10	8.27	2.20	14.90	13	12.88	2.60	52.00	115	9.50	0.30	101.00	19	5.33	0.30	53.80
Be-U*	10	0.006	0.005	0.010	13	0.005	0.005	0.010	115	0.005	0.005	0.017	19	0.005	0.005	0.005
1-OHP	10	0.32	0.06	1.19	15	0.29	0.02	2.97	121	0.29	0.01	4.18	21	0.28	0.09	0.86
PbB	3	3.08	1.90	7.30	2	3.73	2.90	4.80	14	3.30	2.00	6.40	6	3.89	1.80	10.00
Σ PCB	1	0.80	0.80	0.80	2	0.49	0.30	0.80	9	1.89	0.50	8.90	4	2.43	0.40	7.70

*$p<0.05$ Kruskal-Wallis H test

Table 33 Distributions of biomarkers in cast iron workers stratified by ethnic groups.

Biomarkers	Africans				Others				Arabs				Caucasians			
	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max	N	GM	Min	Max
Cd-U	33	0.26	0.11	1.45	0	-	-	-	14	0.16	0.00	0.54	110	0.27	0.07	1.02
Cr-U	33	0.37	0.03	1.90	0	-	-	-	14	0.44	0.23	0.74	110	0.31	0.03	2.60
Ni-U	33	0.89	0.10	4.80	0	-	-	-	14	0.92	0.10	2.60	109	0.86	0.10	6.30
As-U*	33	16.67	0.30	101.00	0	-	-	-	14	7.72	0.70	26.00	110	7.64	0.30	48.60
Be-U	33	0.005	0.005	0.005	0	-	-	-	14	0.005	0.005	0.005	110	0.005	0.005	0.017
1-OHP	39	0.26	0.02	4.18	1	0.12	0.12	0.12	16	0.27	0.10	1.47	111	0.31	0.01	1.90
PbB	2	3.02	1.90	4.80	0	-	-	-	2	4.18	3.80	4.60	21	3.42	1.80	10.00
Σ PCB	2	0.80	0.80	0.80	0	-	-	-	0	-	-	-	14	1.78	0.30	8.90

*$p<0.05$ Kruskal-Wallis H test

Table 34 Distributions of biomarkers in cast iron workers stratified by smoking habits.

Biomarkers	**Current smokers**				**Not Smokers**			
	N	GM	Min	Max	N	GM	Min	Max
Cd-U*	65	0.33	0.09	1.02	92	0.21	0.00	1.45
Cr-U	65	0.29	0.03	2.20	92	0.36	0.03	2.60
Ni-U	65	0.88	0.10	6.30	91	0.86	0.10	5.40
As-U	65	7.70	0.30	48.60	92	10.07	0.30	101.00
Be-U	65	0.005	0.005	0.017	92	0.005	0.005	0.010
1-OHP*	68	0.45	0.05	1.47	99	0.22	0.01	4.18
PbB	9	3.67	2.00	10.00	16	3.31	1.80	7.30
Σ PCB	5	2.41	0.50	8.90	11	1.34	0.30	7.70

*p<0.05, Mann-Whitney U test

5. DISCUSSION

5.1 Significance and usefulness of biological monitoring

The quantitative estimation of the internal dose following occupational or environmental exposure to chemicals is of fundamental importance in the protection of workers' health and safety. However, there are difficulties in the estimation of dose or "body burden" which are characterised by the fact that it often fluctuates considerably over time making any correlation with chronic disease difficult. Also, the 'target' dose, which is the concentration of chemicals at the site of action, cannot be measured directly because biological targets such as organs and tissues are generally, inaccessible in exposed people.

Surrogates of the target dose, i.e. airborne concentrations of the pollutants or biomarkers in biological matrices must therefore be used.

Air monitoring can introduce a large bias into the estimation of the true target tissue dose. This is because variation in factors such as physical workload, skin absorption, the partition coefficient of the chemical of interest, and individual anatomical, physiological and biochemical differences which determine chemical pharmacokinetics, ensure that air monitoring is, more often than not, a crude indicator of the biologically active dose (Droz et al. 1991). Wide variations in exposure concentrations due to changes in work procedures and practices occurring over periods of months to years can be taken into account during air monitoring. Short-term within-day and day to day fluctuations, however, are best captured by biological monitoring (Droz et al. 1991).

Biological monitoring is based on measurements of chemicals or their metabolites, known as 'biomarkers', in biological media such as blood, urine, breath, saliva, and sometimes tissues, as an index of absorption of chemical (Mason and Wilson 1999). Biological monitoring refers to the periodic measurement of a biomarker to assess the health risk associated with exposure to a chemical (Mutti 1999), on a regular basis. The strength of biological monitoring lies in the potential to define total absorbed dose from all routes of exposure i.e. inhalation, dermal penetration and ingestion (Mason and Wilson 1999). On the other hand, the results are affected by biological variability.

Biomarkers of exposure are exogenous substances or metabolites or the product of an interaction between a xenobiotic and a target molecule or cell measured within a

compartment of an organism (NRC 1989). The terms 'biomarker of exposure' and 'biological indicator of internal dose' have essentially the same meaning but the latter expression emphasizes its relationship with adverse effects in a critical organ or tissue. Several biomarkers may be available for the same chemical and the meaning of the marker may depend on the sampling time. For example in the case of markers with a short half-life, e.g. compounds measured in blood, the internal dose may mean the amount of chemical absorbed shortly before the sample was taken. For markers with intermediate half-life, e.g. urinary metabolites, the internal dose, which can be estimated, is that occurring during the preceding days. For markers with long half-life, e.g. adducts to DNA or haemoglobin, the internal dose is integrated over a period of months. For chemicals that accumulate, the internal dose refers to amounts stored in tissues and organs over an extended period of years.

The sampling time of a biomarker may give different meanings to the marker. Therefore, knowledge of the mechanism of the end-point to be measured is especially important in order to permit selection of a relevant marker of internal dose and appropriate sampling times.

The principal aims of biomarkers in exposure assessment are to establish unequivocally the fact of exposure in population studies, reduce or prevent misclassification in epidemiological studies, and, to estimate the internal dose occurring in a critical organ, cell or molecule. Biomarkers of exposure allow a focus on the body burden or the total absorbed dose and integrated sources and routes of exposure, patterns of exposure, and, inherent and behavioural differences between individuals.

Between individuals, the same biomarker may vary both as a function of time, and at a given time, between individuals exposed to the same air concentrations. The kinetics of the disposition of the exposure chemical is important in respect of possible biological variability relative to exposure (Droz 1993). Thus, biological monitoring is normally not feasible for biomarkers with a half-life less than 2 h. For half-lives in the range 2 10 h a sample taken at the end of a working day represents the integrated exposure during the work day. In the case of half-lives of between 10 and 100 h the optimal time for sampling is at the end of the 5-day working week and the determinations reflect exposure during the preceding few days (UK HSE 1992). For the monitoring of chemicals that have long half-lives, it is generally agreed that biomarkers of exposure have considerable advantages

due to the chemical stability and require relatively few measurements to define exposure compared with measurements based on air levels (Rappaport 1995).

Rather than being a complication, the variations seen between and within individuals are valuable in the determination of risk. A particular value of biological monitoring is that it helps to explain variance of data and the choice of a particular biomarker, instead of others. It should not be seen as a means to reduce variance, which always occurs in human populations. Obviously there is a need to standardize collection procedures and analytical methodology to maintain as low as possible variance in these components, but inter-individual differences in the rates of uptake, biotransformation and excretion need to be emphasized in order to use the data for assessing and managing the associated health risks.

A range of biomarkers may be available for a particular substance, including the parent compound or its metabolites in body fluids such as blood, serum and urine or in accessible tissues such as hair, the adducts of reactive metabolites to DNA or haemoglobin or albumin. Methods for heavy metals may employ estimates of concentrations in critical or storage tissue such as kidney cortex or bone. Selection of the most appropriate approach depends on the mechanistic basis of the adverse effect. These may be classified as acute or chronic, local or systemic, early or delayed, reversible or non-reversible, thresholded or non-thresholded.

Chronic and irreversible effects can result from either cumulative doses or cumulative effects. Similar cumulated doses may result from repeated short-term high doses or from long-term low-level exposures. Often it is not possible to predict which of these patterns is relevant for health risks. A relevant biomarker of exposure should therefore aim to link occupational or environmental exposure with a long-term health effect or a relevant intermediate end-point (Mutti 1995). In situations where the mechanism of toxicity is known, the selection of suitable biomarkers for the observed effects can be based on kinetic parameters. Knowledge of the toxicokinetics of the chemicals under consideration is an important prerequisite of biological monitoring of exposure (Bernard 1995). In general, it is assumed that biomarkers with longer half-life correlate better with effects resulting from chronic, long-term, low-level exposures. The half-life is a principal quantitative parameter derived from mathematical models describing the behaviour of xenobiotics in biological systems.

There are four main categories of biomarkers of exposure based on their biological half-life. These are defined as: very short, e.g. phenol for benzene exposure, short, e.g. 2,5-hexanedione for hexane exposure, long, e.g. heavy metals such lead, mercury and cadmium in blood or adducts to DNA and haemoglobin for electrophilic compounds or metabolites, and very long, e.g. heavy metals in bone. For metallic and transition elements, there are often complex metabolic pathways dependent on particle size, solubility and oxidation state and species. These characteristics imply differences in respect of local and systemic effects as well as biotransformation of the same element.

The validity of biomarkers of exposure is the degree to which the results of measurements correspond to the end-point or phenomenon being measured. Other terms such as accuracy, sensitivity and specificity need to be considered especially in the context of the application with regard to the ability to predict adverse effects reflected by numbers of the false negatives and false positives found in applications. Relevance and stability are important properties in determining whether a biomarker is suitable for applications in the field. Determinations of concentrations of the parent compound in the biological media are generally preferable to metabolites where the same metabolites may arise from different compounds. In case of solvent exposure, the parent compound correlates better with exposure compared with metabolites. However, the parent compound itself is often unrelated to the adverse effects that frequently occur due to biotransformation and metabolic activation. To gain information on dose-response relationships, urinary metabolites have often been used successfully as biomarkers of dose (Alexander et al. 2002). The analytical validity of biomarkers is determined by their selectivity, range, accuracy and precision. Although simple colorimetric assays can be used in some instances sophisticated chromatographic and analytical methods are usually necessary. Prior separation of analytes is often necessary and many of the methods rely on the use of mass spectrometric detection because of its selectivity and sensitivity.

The measurement of sub-nanomolar concentrations is routinely possible and this has led to the detection of 'backgrounds' with the necessity for stringent controls and a need for their interpretation. In some instances, well validated analytical techniques such as gas and liquid chromatography coupled with specific detection and quantification methods such as electron capture and electrochemical detection have sufficient sensitivity and specificity for occupational monitoring and sometimes even for environmental monitoring.

To monitor exposure to metals, inductively coupled mass spectrometry (ICP-MS) and atomic absorption spectrometry (AAS) is useful for the analysis of trace elements in biological fluids.

For identification of exposure, the chemical itself provides more specific evidence than any metabolite, which could originate from different sources and sometimes include confounders. The available analytical methods usually permit quantification of both organic and inorganic analytes in the nanomolar range. At such low levels, confounders are likely to occur if variables such as smoking or dietary habits are not considered in the data analysis. The local values for controls need to be defined and taken into account since variations can occur due to time and place related environmental pollution.

For quantitative exposure assessment, factors such as kinetics should be considered since these may influence the ability of the biomarker to reflect exposure during the selected exposure period. For risk characterization, factors such as toxicodynamics and, where available, the dose-response relationships need to be taken into account. So far, there are only a few accepted biomarkers of dose that can be used to predict adverse effects with a high degree of certainty. Amongst these, there are blood lead (PbB) and cadmium urinary levels (Cd-U).

Quality assurance is a key issue and a prerequisite for the validation of a biomarker of exposure (Aitio and Apostoli 1995). The assurance process entails taking measures that results are reliable, that scientifically sound criteria are used in selection of the biomarker, that technically sound practices are used in the collection, storage and analyses and also in the recording, reporting and interpretation of results. Quality control, either internal or more preferably external, is a necessary part of the quality assurance process with the aim of verifying the analytical results of the laboratory. A critical assessment of the relationships for exposure-dose, dose-response and effect-disease is necessary. For short-lived organic compounds, there are many studies confirming the validity for exposure-dose but rather fewer for dose-response relationships. For relatively long-lived metals, the opposite appears to be true. In these cases, biomarkers of dose should not necessarily relate to recent exposure but predict adverse effects. Biomonitoring of organic compounds has been used mainly to determine that the internal dose is indicative of an exposure that can be relatively easily measured. Many xenobiotics, however, are easily absorbed through the skin. Moreover, with the adoption of personal protection devices in

the workplace, ambient exposure-dose relationships may become difficult to prove and perhaps meaningless. In such situations, the lack of relationships between exposure and biomarkers of dose points to a better definition of the actual absorption judged by biomarkers and should not be used to disprove their validity. Indeed, in these situations there is no credible alternative to biological monitoring as a means to assess actual exposure.

5.2 Biomarkers of Polycyclic aromatic hydrocarbons (PAH) exposure

Measurement of the excretion of urinary 1-hydroxypyrene (1-OHP) for measurement of exposure to polycyclic aromatic hydrocarbons is a widely applied procedure. Methods for determination of 1-hydroxypyrene have been extensively validated in a broad range of industrial settings with potential exposure to polycyclic aromatic hydrocarbons and in control populations (Boogaard and Van Sittert 1994). The method has been applied to identify sources and routes of exposure (Boogaard and Van Sittert 1995b, reviewed in Dor et al. 1999). In a review of many types of occupational exposures to polycyclic aromatic hydrocarbons excreted 1-hydroxypyrene was found to correlate well with exposures to these compounds (Brandt and Watson 2003). In contrast DNA adducts mostly determined in white blood cells did not show good correlations with exposure to polycyclic aromatic hydrocarbons in a variety of workplace and exposure situations. This was in contrast to the results from controlled laboratory studies showing correlations between exposure to polycyclic aromatic hydrocarbons and the levels of adducts in tissues. A possible explanation for the lack of correlation between DNA adducts and occupational exposures to polycyclic aromatic hydrocarbons is that unquantified sources of exposure, such as from the diet or in the cooking of food, could make a large contribution to the total human exposure load compared with the quantified occupational sources.

5.3 The confounding factors

Smoking represents a prominent source of non-occupational exposure to chemicals, though diet can also contribute significantly. The oral intake of PAH from the diet may reach a level similar to those after occupational exposure (Buckley and Lioy 1992). The cooking of food also can give rise to significant exposures to carcinogens. The sum of the

eight carcinogenic polycyclic aromatic hydrocarbons found in fumes caused by frying meat was greater than the levels on coke-oven plants (Chen and Chen 2003). There is a correlation between DNA adduct levels in human lung and cigarette smoking (Phillips et al. 1988a). In a study of tissues from human autopsy samples a strong association was found between levels of adducts in lung DNA and smoking (Routledge et al. 1992). However, there was much weaker association between smoking and increased levels of adducts in bladder DNA. Levels of aromatic DNA adducts in white blood cells correlate with the degree of smoking but saturation occurs at high exposures (Van Schooten et al. 1997).

5.4 Biological monitoring evaluation

5.4.1 Reference values

In 1989, Grasbeck and Saris established the concept of reference values (Grasbeck and Saris 1989), to correctly interpret the increasing amount of data from modern clinical chemistry. Their intuition was based on the concept of a comparison between two terms: the 'unknown', which must be interpreted, and the 'reference' which can be used to interpret. Therefore, laboratory determinations had to be evaluated by means of a comparison with values derived from subjects classifiable as 'controls'. These 'controls' are not necessarily 'healthy' subjects or belonging to the entire 'general population', but subjects whose 'characteristics' must be well defined (Solberg and Grasbeck 1989). The most important difference between normal values and reference values is that by using RVs one had 'guaranteed information' regarding the subjects examined, as well as the sampling conditions, the performed analysis and the type of statistics used for the data elaboration (Grasbeck and Solberg 1981).

In the most exhaustive definition of biological monitoring was formulated during the UNEP, WHO, EEC meeting of 1984 when it was identified as the “measurement and quantification of chemical substances or their metabolites in tissue fluids, secretions excretions, expired air or in any combination, carried out to evaluate exposure and health risks, compared with an appropriate reference” (Berlin 1984).

In this definition it is explicitly affirmed that the results of monitoring practice must be compared with “appropriate references”. Reference values can be used as a means of

comparison in industrial and environmental toxicology to evaluate exposure to xenobiotics. Moreover, they can be useful to monitor subjects presenting levels of xenobiotics which are greater than established reference levels and to correlate those levels with possible effects.

When the results of biological monitoring are interpreted, appropriate references should be used. These could be more adequately defined as "operative" or "guide values" which are useful to assess the significance of the measurements. In this respect, reference values must be considered together to other currently used values , such as the action levels and the limit values (LV).

The use of a system of guide values could furnish two types of information:

– the difference between the results of biomonitoring and the reference values (ie values determined in subjects not exposed to xenobiotics) should indicate exposure levels higher than the general population;

– the differences between the results of the biomonitoring and the Limit Value should indicate possible health effects.

The various guide values are therefore conceptually related to each other, and when using action levels or limit values, reference values should not be excluded. This can be demonstrated by considering the list of BEI® published by the ACGIH. In fact, for the majority of chemicals the list reports the notation "B" which indicates that "the determinant is usually present in significant amount in biological specimens collected from subjects who have not been occupationally exposed and such background levels are included in the BEI® value". The same or other BEI® also may have the "Ns notation" which indicates that "the determinant is non specific since it is observed after exposure to some other chemicals". B notation, and also to a certain extent Ns, tries to generically express the same concept that RVs explain by a more precise and specific means. The ratios between RV and LV are greater when chemicals are also absorbed due to personal habits, such as cigarette smoking (Cd) or dietary habits (hippuric acid, phenol), or for a combination of environmental and personal habits (Pb, Hg, As).

In order to establish a valid reference value, its purpose must be precisely defined. The various phases of RVs should control the different factors of biological variability, such as the characteristics of the indicator and the population examined, as well as factors of analytical variability related to sampling, treatment, analysis of biological samples and data

elaboration. In the past, lack of control of these factors led to the production of reference values with too wide a variability in relation to the generally low concentrations of xenobiotics present in biological fluids resulting in a too high reference value. At the same time, the poor sensitivity of methods has prevented the measurement of many elements or compounds, thus limiting the number of reference values.

An improvement of information concerning the aspects of sampling, collection and analysis has given greater importance to the criteria of population selection and statistical analysis of the data. Among the factors that influence the determination of reference values is the use of accurate analytical techniques and sufficient information on toxicokinetics, the possibility to provide accurate analytical data and to interpret them correctly. In addition, pre-analytical factors, such as sampling, transport, conservation and careful preparation of samples, are other crucial aspects together with analytical factors, instruments, methods and quality control (Minoia 1992). A very important influence on the quality of reference values is represented by the selection of reference subjects (Table 4): in clinical chemistry, impaired health constitutes the main exclusion factor, whereas in environmental toxicology the primary exclusion factor should be the abnormal exposure to xenobiotics (Apostoli 1992).

As mentioned earlier, other important sources of variability are represented by food, water, tobacco smoking, alcohol consumption, other lifestyle, etc.

Many are examples of "low" quality of reference values due to the use of incorrect pre-analytical or analytical methods or individuals selection methods. This is well depicted by the wide range of reference values, such as those of Al in serum, for which 'normal values' between 800 and 40 µg/L have been reported in the past years, while the real RV is probably below 10 µg µg/L.

Attempts have been made to assure a more scientífic approach to assess RVs and programs aimed at achieving concepts and practices for reference values have been proposed, such as those of the Italian Society for Reference Values (SIVR).

5.4.2 Biological limit values

The interpretation of the results of biological monitoring is based on the comparison with appropriate references. These could be more adequately defined as "operative" or "guide" values" and include the limit values and the reference values. The difference between the

biomonitoring results and the corresponding limit values should indicate possible health effects. The limit values for chemical compounds determined in biological matrices are proposed by two main international agencies, namely the *Deutsche Forschungsgemeinschaft* (DFG, German Research Foundation) and the *American Conference of Governmental Industrial Hygienists* (ACGIH) that annually publish the BAT (and EKA) (DFG List 2007) and the BEI® lists (ACGIH 2007a), respectively.

5.4.3 BAT values

The BAT value (*Biologischer Arbeitsstoff-Toleranz-Wert*: biological tolerance value for occupational exposures) is defined as the maximum permissible quantity of a chemical substance or its metabolites or the maximum permissible deviation from the norm of biological parameters induced by these substances in exposed humans. The BAT value is established on the basis of currently available scientific data which indicate that these concentrations generally do not affect the health of the employee adversely, even when they are attained regularly under workplace conditions. BAT values are established on the assumption that persons are exposed at work for at most 8 hours daily and 40 hours weekly (Bolt and Rutenfranz 1986).

BAT values can be defined as concentrations or rates of formation or excretion (quantity per unit time). BAT values are conceived as ceiling values for healthy individuals. They are generally established for blood and/or urine and take into account the effects of the substances and an appropriate safety margin, being based on occupational medical and toxicological criteria for the prevention of adverse effects on health.

By definition, BAT values can be established only for such substances which can be taken up by the body in substantial quantities via the lungs and/or other body surfaces (skin, gastrointestinal tract) during occupational exposure. Another prerequisite for the establishment of a BAT value is that sufficient occupational medical and toxicological data are available for the substance and that these data are supported by observations in man. The data must have been obtained with reliable methods.

The derivation of a BAT value can be based on various scientific data, which reveal a quantitative relationship between exposure concentration and body burden and therefore permit the linking of MAK (Maximum Allowable Concentration) and BAT values. These include:

– studies which reveal a direct relationship between concentrations of a substance, metabolite or adduct in biological material (body burden) and adverse effects on health;
– studies which reveal a relationship between a biological indicator (effect parameter) and adverse effects on health.

The effects of changes related to sex-specific factors are limited so that for health protection at the workplace it is the effects on the embryo and foetus which are of particular importance.

BAT values are intended to protect employees from impairment of health at work. They provide a basis for deciding whether the amount of a chemical substance taken up by the organism may be harmful or not. For substances that can be absorbed through the skin, individual exposures can be determined only by biological monitoring.

Under workplace conditions, it is not necessarily possible to deduce the level of a substance to which a particular person was exposed from its specific biological parameter in that person because a series of other factors in addition to the amount of substance in the air can determine the extent of exposure of the organism. These factors include the level of physical activity (respiratory minute volume), absorption through the skin and individual variations in metabolic or excretory patterns.

In general for substances with low vapor pressure which are readily absorbed through the skin, there is no correlation between exposure concentration and body burden. For these substances a BAT value can often be established only on the basis of a relationship between body burden and effect. In addition, the concentrations of substances in the workplace air may vary with time and the biological parameters may not vary to the same extent. Therefore observance of BAT values does not make it unnecessary to monitor the concentrations of substances in the workplace air. This applies especially for local irritants and caustic substances. When evaluating macromolecular adducts of foreign substances it should be borne in mind that the persistence of such adducts can lead to discrepancies between the pattern of external exposure and the behaviors of the biological parameters. Similar considerations apply for all highly cumulative substances such as heavy metals and polyhalogenated hydrocarbons.

In spite of all these interfering factors and the consequent differences in the definitions of MAK and BAT values, the two thresholds are generally based on equivalent effects of substances on the organism. However, for substances for which the MAK value is not

established on the basis of systemic effects but because of local irritation of skin and mucous membranes, a BAT value can still be based on "critical toxicity" resulting from systemic exposure. In such exceptional cases where the MAK and BAT values are based on different end points, the two values do not necessarily correspond.

The protection of the health of the individual, which is the reason for establishing BAT values, can be monitored by periodic quantitative determination of the chemical compounds or their metabolites or of biological parameters in biological material. The methods used must be diagnostically specific and sensitive enough for the purpose, acceptable to the employee and practicable for the physician. The sampling time, that is, the measurement strategy, must take into account both the exposure conditions at the workplace and the kinetics of the substance. As a rule, especially for substances which accumulate in the organism, this may be achieved by taking samples at the end of a working day after an extended period of work (working week). During exposure to gaseous substances which are metabolized rapidly and for which the blood/air distribution coefficient is larger than 10, it must be taken into account that the concentrations of the substances in blood and tissues are positively correlated with the level of physical activity. Likewise, the concentrations of inhaled gaseous substances in blood and tissues of persons working under hyperbaric pressure have been shown to correlate positively with the pressure. In such cases, the observance of the BAT value must be monitored more frequently as the BAT value is attained in such workers at lower exposure concentrations than in persons working at normal pressures.

Whole blood, serum and urine samples are used as assay materials, occasionally and under certain conditions, also samples of alveolar air. Saliva and hair samples are not suitable assay materials for occupational medical biomonitoring. The analytical methods must yield reliable results and meet the requirements of statistical quality control.

For workers who come into direct skin contact with substances which are designated with an "H", it is necessary to check that the BAT value has not been exceeded or, in the case of carcinogenic substances, to assess the systemic dose in terms of the EKA (TRGS 150).

Like any results of laboratory investigations, toxicological analytical data can only be evaluated given knowledge of the whole situation. As well as the other medical findings, especially

- the dynamics of patho-physiological processes

- the short-term effects of exposure-free periods
- the long-term effects of ageing
- the specific workplace conditions
- intensive physical activity and unusual conditions of atmospheric pressure and any individual background exposures must be taken into account.

In occupational-medical health practice analyses of urine specimens for the purpose of biological monitoring are carried out using spontaneously voided urine samples (spot samples). These are not suitable for an analysis if they have been highly concentrated or highly diluted through diuresis. In occupational medical practice the creatinine content of the urine specimen is used as a criterion for the specimen's acceptance while the specific weight or the osmolality is of little importance as a basis for evaluation. Creatinine concentrations <0.5 g/l or >2.5 g/l are criteria which would exclude the usability of the spot sample (Biological Exposure Values for Occupational Toxicants and Carcinogens, 1999).

Results from analyses of substances in biological material are subject to medical discretion. Only the physician who is responsible may interpret the results. BAT values are established on the basis of the results of scientific studies and practical medical experience.

Depending on individual disposition, allergic reactions can be induced by various kinds of substance, more or less rapidly and in differing degrees of severity after sensitization of, for example, the skin or respiratory passages. The observance of BAT values cannot provide a guarantee that such reactions will not occur.

5.4.4 EKA values

Chemical substances which, by their own action or by that of their reactive intermediates or metabolites, are known to cause cancer in man or for which there is good evidence of a human cancer risk (Carcinogen categories 1 and 2) or which cause concern because they are or could be carcinogenic (Carcinogen categories 3A and 3B) and for which no MAK value can be derived are not given BAT values because at present it is not possible to specify safe levels of such substances in biological materials.

Therefore, the handling of such substances must take place under the conditions described in Section III of the List of MAK and BAT Values. The analysis of carcinogenic substances in biological material is not carried out for the application of BAT values in the

strict sense but rather for the occupational medical detection and quantification of the individual exposure to the substances. Concentrations of a substance or its metabolites in biological material which are higher than those known to correspond to the concentration of the substance in the workplace air are indicative of additional exposure by other routes, usually percutaneous.

For this reason for substances in Carcinogen categories 1 to 3, the DFG Commission investigates the relationships between the concentration of the carcinogen in the workplace air and that of the substance or its metabolites in biological material (*Expositionsäquivalente für krebserzeugende Arbeitsstoffe*, EKA: exposure equivalents for carcinogenic substances). From these relationships, the body burden which results from uptake of the substance exclusively by inhalation may be determined.

5.4.5 BEI® values

The BEI® are reference values intended as guidelines for the evaluation of potential health hazards in the practice of industrial hygiene (ACGIH 2007a). The mission of industrial hygiene is the anticipation, recognition, evaluation, and control of exposure to health hazards in the workplace, with the overall aim of preventing or minimizing adverse health effects of exposure. Thus, when the BEI® are used by physicians, nurses, engineers, or industrial hygienists, their principal application should be to support prevention of injurious exposures. These reference values are the recommendations of a professional society, the ACGIH, which also establishes reference values for airborne chemical concentrations in the workplace. The latter are called Threshold Limit Values (TLV) and represent conditions under which nearly all workers may be exposed repeatedly over a working lifetime without adverse health effects. It should be noted that the ACGIH is a private organization without regulatory authority and its reference values are offered as recommendations for good practice without guarantee that they are a clear demarcation between safe and unsafe conditions. As presented by the ACGIH, industry compliance with the BEI® and the TLV is voluntary. Despite this disclaimer, the TLV and to a lesser extent the BEI® have been used by government agencies around the world as the basis for workplace environmental regulations.

The BEI® are developed by the BEI® Committee of the ACGIH, which consists of volunteer scientists and practicing professionals with expertise in occupational medicine, toxicology,

industrial hygiene, analytical chemistry, biostatistics, and epidemiology. The present committee members include specialists from the United States, Japan, Germany, Switzerland, and the United Kingdom employed in academia, government, or private industry (the last category of members does not have voting privileges but otherwise participates fully in the process.) The committee meets twice a year to develop new reference values and to conduct a regular review of existing BEI® as new data emerge. Several values have undergone significant revision as a result of such review.

The BEI® are intended for use in biological monitoring where the goal is the determination of the worker's internal, or biologically effective, dose of a chemical. The determinant may be the parent compound itself, metabolite(s), or a characteristic reversible biochemical change induced upon absorption. The index values represent the level of the determinant most likely to be observed in specimens collected from a worker with an internal dose equivalent to that arising solely from inhalation exposure at the TLV concentration (ACGIH 2007a). Thus, most of the BEI® are closely linked to the corresponding TLV and are based on preventing the same health effect addressed by the TLV. This does not imply, however, that airborne concentrations and biological levels must always be correlated in exposed workers, since routes of absorption in addition to inhalation are possible. Where this occurs, comparison of biological levels to the BEI® takes on special importance, since the BEI® represents the acceptable internal exposure regardless of the route(s) of entry. One of the critical decisions in establishing a BEI® is the initial one regarding the feasibility analysis. In the course of making this decision, the committee considers several criteria, listed below.

Substances must be absorbed into the circulation to the extent that target tissues remote from the site of entry are affected and so that accessible biological fluids or tissues contain the chemical or its metabolite in detectable concentration. An industrial chemical with potent toxic properties that exerts its effect only topically or only at the site of absorption is not a candidate for setting a BEI®, since biological monitoring is unlikely to generate information useful for preventing or minimizing exposure.

In general, exposures to workers should occur in more than a single industrial facility and preferably in the workplaces of more than one company. Equally important is the recent trend in these data, as a substance whose use in industry is decreasing may be of much less interest than one whose production and use are growing. The influence of this factor

may be diminished for a substance with very potent toxicity for which other feasibility criteria are particularly compelling, such as the glycol ethers, which may penetrate the skin in significant amounts.

The great majority of BEI® are directly related to the corresponding TLV. They address the same health outcome and represent the expected internal dose corresponding to inhalation at the TLV. The present exceptions are BEI® for classes of compounds inducing methemoglobinemia and for those inhibiting acetyl cholinesterase.

There should be sufficient data of high quality that describe the absorption, systemic distribution, metabolism, storage, and excretion of the compound or its metabolites. These are necessary to support the selection of the appropriate analyte, the tissue or fluid to be sampled, and the timing of the sample. The committee requires that the toxicokinetic studies be published in the peer-reviewed scientific literature so their quality can be assessed by all interested parties. In some instances, validated toxicokinetic models have been used where experimental human data were not sufficient. Further, toxicodynamic data may also be appropriate in instances where the anticipated BEI® would be directly related to health effect rather than to an airborne concentration. This particular means of developing a BEI® has been used only rarely to date.

Data in the peer-reviewed literature must demonstrate that a method exists for assay of the determinant with acceptable accuracy, precision, and sensitivity. These performance characteristics must permit analysis of the determinant in the recommended tissue or fluid sampled at levels both below and above the anticipated level of the BEI®. Inadequate analytical methodology will preclude the development of a reference value.

A proposed BEI® is supported by a document that reviews the scientific data used in developing the reference value and that contains a synoptic rationale for the recommendation. All literature used in the preparation of the documentation is cited and a copy of each item must be provided for archiving. The BEI® Committee then conducts a thorough review of the proposed BEI® and its documentation. A member not involved in the preparation of the proposal is assigned to lead this review, during which special attention is paid to the correspondence of the BEI® to the TLV if that approach has been used, or to the relationship to health effects data if not. The review process does not include a quantitative risk assessment. The approach in most cases has been to select the level of each determinant that is most likely to result from inhalation exposure at the TLV.

The decision takes account of typical workers' physical activities during exposure and pays particular attention to experimental or epidemiologic data on the toxicokinetics of the compound.

Documentation of the proposed BEI® is available from the ACGIH by request. The proposed BEI® must remain on the "Notice of Intent to Establish or Change" list for at least 1 year, after which the committee reviews all comments received as well as any new scientific data to emerge. Revisions may be made to the BEI® and its documentation, after which another consensus is reached regarding adoption. If a proposed BEI® is revised at this point, the new proposal must spend another year on the Notice of Intent list. After the required notice period, a recommendation for adoption is again reviewed by the Board of Directors and the ACGIH membership. Final adoption results in publication of the BEI® in the booklet as an established reference value and incorporation of the new documentation into the set published by the ACGIH (ACGIH 2007b), which contains all the documentation for the TLV and BEI®.

A significant aspect of the TLV and BEI® procedure is the periodic reexamination of the adopted reference values. The annual publication of the TLV/ BEI® booklet reveals numerous revisions as a consequence of this continued surveillance. The frequent updating of the ACGIH reference values stands in marked contrast to the much slower, legislatively mandated, process of the U.S. Occupational Safety and Health Administration (OSHA) in revising its regulations governing the same substances.

In establishing its reference values for biological monitoring, it is clear that the ACGIH has chosen to limit application of reference values to the early stages in the induction of occupational disease: internal dose, biologically effective dose, and in a few cases, early biological effect. The indicators used in the BEI® are therefore best considered biological indicators of exposure, with the acknowledgment that their level in an individual reflects the influence of susceptibility. In addition, since a few of the BEI® are consistent with monitoring early biological effects, their application may be thought of as biological monitoring of response.

Using the BEI® as reference values, its most important characteristic is that this type of monitoring is part of the strategy to prevent exposures that might otherwise lead to occupational disease. Conversely, the BEI® are not appropriate for use in identifying susceptible individuals or in demonstrating the presence of preclinical or clinical disease.

Since the principal function of industrial hygiene is the prevention or minimization of occupational disease through control of chemical exposures, the limitation used by the ACGIH for its BEI® is appropriate. Further, biological monitoring of exposure using these reference values is complementary to ambient environmental monitoring performed by industrial hygienists and other occupational health specialists. The results of biological monitoring will provide additional information regarding the conditions of occupational exposure, which cannot be obtained by sampling the air of the workplace: the consequences of skin absorption, the effects of physical workload on chemical uptake via inhalation, and the effectiveness of personal protective equipment in reducing worker exposure are three important examples. It must also be emphasized that biological monitoring cannot substitute for air monitoring in the context of exposure prevention: a high biological result alone will indicate possible excessive exposure but will provide no information on the likely source or mechanism of over-exposure. Choosing an appropriate course of action for control of the hazard requires information beyond that obtained from biological monitoring alone (Lowry 1988).

Based on the premise that exposure prevention will continue to be the major application of the BEI® in industrial hygiene, the following attributes can be identified as critically important in the development of new methods and reference values.

The level of the biological indicator must be related clearly to the intensity and the duration of exposure in humans (Aitio 1992). This relationship must be demonstrable at levels of exposure found or expected in industrial settings. The analytical limits of quantitation of the biological determinants must span the range of occupational exposures.

Because the actual target tissue is seldom accessible for sampling, surrogate fluids or tissue must be used for biological monitoring. The most common samples are peripheral blood, voided urine, and exhaled air. Depending on the target tissues, these samples will reflect with variable accuracy the biologically effective dose (Aitio 1992; Bernard and Lauwerys 1986; Greim et al. 1995; Vainio 1985). Reliable information on the relationship between biological indicator level in peripheral blood or urine and in the target tissue is highly desirable.

The reversibility of the biological indicator may be most important in distinguishing industrial hygiene applications from other uses of biological monitoring (Bernard and Lauwerys 1986; Aitio 1994; Lehnert and Schaller 1995). Since the goal is prevention, the

industrial hygienist will be most interested in a biological indicator that reveals the effectiveness of control measures adopted after a judgment of hazardous exposure has been reached. If the indicator is irreversible, its appearance will alert the hygienist to a potentially hazardous situation but will not provide evidence that mitigating measures are working.

To interpret the results of biological monitoring correctly, the influence of modifying and confounding factors must be understood. These factors include the effect of non-occupational exposure to the agent of interest, prior or simultaneous exposure to other agents, host factors modifying the response, and the general influence of variation in environmental and individual response (Vainio 1985).

Some types of biological sampling are too invasive or risky for use in the workplace and others may not be acceptable to workers for a variety of reasons. Adipose tissue, liver, or bone marrow sampling are examples of the former type. In any successful application of biological monitoring, the workers' cooperation must be secured. For example, when samples must be collected several hours after the end of a work shift to interpret the results properly (Sherwood 1972), it is clear that the individuals must take major responsibility for valid sampling. Any procedure perceived by the workers to be unpleasant or unnecessary likely will not be followed (Friberg and Elinder 1993; Biological Exposure Values for Occupational Toxicants and Carcinogens 1995).

6. CONCLUSIONS

6.1 Secondary aluminum foundries

The present study shows that biological exposure levels of toxic/carcinogenetic compounds in the investigated aluminum foundries fell, on average, well within the respective limit values fixed by International Agencies, such as DFG and ACGIH.

No relationship among airborne and biological values of investigated pollutants could be demonstrated by regression analysis but they were apparent if airborne and biological concentration ranges more than individual values were compared.

The Al-U values resulted, on average, lower than previously assessed by a study performed on the same productive sector and from the same area about twenty years ago (Apostoli et al. 1992). In this study, mean Al-U values ranging from 16.9±6.9 to 42.6±20.5 µg/l, were determined. More recently, a mean Al-U level of 43.6 µg/l (Halatek T et al. 2005) and a median Al-U value of 4 µg/l (Iregren et al. 2001) have been reported in a Polish primary Al foundry and in a Swedish Al smelter group, respectively. In our case, the highest recorded Al-U value was less than one third of the BAT value and only 18% of workers excreted Al-U levels higher than the reference values. We observed the highest values in plants 1 and 3 and among oven/casting workers. We observed a positive relationship between Al-U levels and alcohol intake that could be explained by the Al content of alcoholic beverages, ranging from 0.15 to 3.20 mg/l (Schenk et al 1989).

In Al production plants, pyrene is the most abundant PAH present in environmental samples (Kriek et al 1993; van Schooten et al. 1995). Thus, in this context urinary 1-OHP is a reliable biomarker of PAH exposure (Jongeneelen et al. 1988; Jongeneelen et al. 1990). Our investigation showed that, on average, the exposure to PAH was lower than previously reported in a study on a primary Al foundry group, in which mean 1-OHP post-shift values of 0.91±0.42 and 1.43±0.73 µg/g creat were determined for non smokers and smokers, respectively (van Schooten et al. 1995). In the same study, the *lowest observed effect level* for genotoxic damage (assessed by PAH-DNA adducts in white blood cells) was 7.33 µg/g creat. Another investigation on a primary Al foundry found an end-of-shift limit value (equivalent to a TLV concentration of coal tar pitch volatiles) of 9.46 µg/g creat (Tjoe Ny et al. 1993). In our sample, the mean 1-OHP value was lower than the lowest

level target benchmark value recently proposed by Jongeneelen (2004), corresponding to the 95th percentile of non occupationally exposed local controls. As for Al-U, workers from plant 2 showed the the lowest levels. As expected, significantly higher 1-OHP levels were observed among smokers, than non smokers and a significant relationship with the extent of smoking habits (as packyears) was also found.

Lead is a toxic heavy metal for which the Italian Law (D.Lgs 25/2002) fixes an Action Level of 40 µg/dL. Several evidence lines are, however, available demonstrating that a lower biological limit, similar to that proposed by ACGIH (30 µg/dL) would be proper (Apostoli and Alessio 2002). In a previously performed study on subjects with past occupational exposure to Al dust as workers of an Al remelting plant in the province of Turin (Polizzi et al 2002), mean PbB values of 10.20±4.60 µg/dL, not significantly different from the control group, were observed. In our sample, we observed on average lower values, being about one fifth of the BEI® value. About 19% of workers showed values exceeding the upper limit of the RV. Values were not influenced by age, lifestyle factors, or job tasks.

Average As-U levels were confined into the limit values established by both ACGIH and DFG. About 8% of workers showed values higher than RV. As can be absorbed through inhaled air, water and food. Of these, food is usually the largest source of arsenic (Brima et al 2006). The predominant dietary source of arsenic is seafood, followed by rice/rice cereal, mushrooms, and poultry. While seafood contains the greatest amounts of arsenic, for fish and shellfish, this is mostly in an organic form of arsenic called arsenobetaine that is much less harmful. Some seaweeds may contain arsenic in inorganic forms that may be more harmful. In our sample, the highest As-U values were found in plant 2, significantly different from both plant 1 and 3. The values were significantly influenced by the ethnic group as they were higher among Africans and "Else", the latter including Asians. This results was confirmed by the *chi square* test on workers classified by ethnic group and by a dichotomous As-U variable (values higher or lower then the RV). Subjects with values exceeding the RV were overrepresented in the Africans and Else groups (respectively 60% and 50%), as compared to the others groups (p<0.0001). In our sample, the As-U values were seemingly not influenced by fish consumption, that was investigated by the questionnaire. We hypothesize that some unassessed environmental factor, associated to the ethnic groups, such as rice consumption, could have contributed to the observed differences in As-U values.

The levels of other carcinogenic elements were well below the biological limit values, as well as of the RV. Be-U was undetectable in the majority (88 %) of samples; Cr-U in about 5% of samples. The proportions of subjects showing values higher than the RV were negligible, ranging from 0% (Be-U), to 0.6% (Cd-U and Cr-U) to 1.8% (Ni-U). The Cd-U, Cr-U and Ni-U values were significantly influenced by the job tasks (Table 3). Our data confirmed the relationship tobacco smoke and Cd-U, already signaled by other authors (for review, see Satarug and Moore 2004).

The PCB values fell below the RV, with the exception of four subjects (about 11% of examined workers). The values showed a positive relationship with age, as expected by the long half-life of these compounds, even confirming our previous results in the general population from Brescia (Apostoli et al. 2005).

The results of the biological monitoring of toxic and carcinogenic elements/compounds led us to conclude the in the examined plants the exposure levels to chemical risk factors were very low. In this situation, we judged irrelevant for the observed situation the setting up of complex risk assessment methodologies that are deferred to the analysis of other metallurgic sections in which heavier exposure levels will be apparent. The carcinogenic risk assessment will be based either on the linear extrapolation of exposure-dose relationships for carcinogenic elements/substances for which WHO (WHO air quality guidelines for Europe 2000) or EPA (http://cfpub.epa.gov/ncea/iris/index.cfm) risk units are available (PAH, Be, As, Cr) or applying risk estimates arisen from prospective population-based studies (e.g. for Cd; Nawrot T 2006).

6.2 Copper alloy foundries

The present study shows that biological exposure levels of toxic/ carcinogenetic compounds in the investigated copper alloy plants fell, on average, well within the respective limit values fixed by International Agencies, such as DFG and ACGIH. Such values are at this moment unavailable for Cu, Zn, Be and ΣPCB.

No relationship among airborne and biological values of investigated pollutants could be demonstrated by regression analysis but they were apparent if airborne and biological concentration ranges more than individual values were compared.

Lead is a toxic heavy metal for which the Italian Law (D.Lgs 25/2002) fixes an Action Level of 40 µg/dL. Several evidence lines are, however, available demonstrating that a lower biological limit, similar to that proposed by ACGIH (30 µg/dL) would be proper (Apostoli and Alessio 2002). Six workers exceeded the BEI value for PbB (30 µg/dl), and among these, a worker showed a higher than the BAT value, corresponding to the action level for the Italian law. They all worked in plant 1 and the relative job tasks were scrap screening (n=5) and oven/casting (n=1). Such high PbB values were found in a similar survey conducted on three plants of the same productive sector, in the area of Brescia more than a decade ago (Crippa et al. 1991). In that study, the average PbB values exceeded the action level. The Cu-U values ranged, on average, from 34±14.7 to 46.4±20.3 µg/l, e.g. two to three times higher than assessed in the present study.

Urinary 1-OHP is a reliable biomarker of PAH exposure (Jongeneelen et al. 1988; Jongeneelen et al. 1990). In our sample, the mean 1-OHP value was lower than the lowest level target benchmark value recently proposed by Jongeneelen (2004), corresponding to the 95th percentile of non occupationally exposed local controls. The thirteen subjects showing values higher than the 95th ile of RV were all current smokers (chi square test, $p<0.0001$), and 11 of them were assigned to oven/casting job task. As expected, significantly higher 1-OHP levels were observed among smokers, than non smokers and a significant relationship with the extent of smoking habits (as pack-years) was also found.

Average As-U levels were confined into the limit values established by both ACGIH and DFG. Less than 5% of workers showed values higher than the 95th ntile of RV. As can be absorbed through inhaled air, water, and food. Of these, food is usually the largest source of arsenic (Brima et al 2006). The predominant dietary source of arsenic is seafood, followed by rice/rice cereal, mushrooms, and poultry. While seafood contains the greatest amounts of arsenic, for fish and shellfish, this is mostly in an organic form that is called arsenobetaine that is much less harmful for human health. Inorganic forms of arsenic that may be more harmful, are contained in some seaweeds. In our sample, As-U values were influenced by fish consumption, as subjects who had eaten fish/jellyfish in the days preceding the biological sampling eliminated significantly higher As-U levels than non fish-consumers.

The levels of other carcinogenic elements were well below the biological limit values, as well as of the RV. Be-U was undetectable in the majority (76.6 %) of samples; Cr-U in

about 1 % of samples. We confirmed the association between tobacco smoke and Cd-U levels, already signalled by other authors (for review, see Satarug and Moore 2004).
The PCB values fell below the 95th ile of RV and showed a positive relationship with age, as expected by the long half-life of these compounds, even confirming our previous results in the general population from Brescia (Apostoli et al. 2005). Also Cd-U, and PbB were significantly associated to age (Spearman's rho 0.41 and 0.36, $p<0.0001$ and $p<0.05$, respectively), as expected by their tendency to bioaccumulate over prolonged exposures (Lauwerys et al. 1984; Apostoli 1998).

6.3 Electric steel plants

The present study shows that biological exposure levels of toxic/carcinogenetic compounds in the investigated steel plants fell, on average, well within the respective limit values fixed by International Agencies, such as DFG and ACGIH, or single researchers.
No relationship among airborne and biological values of investigated pollutants could be demonstrated by regression analysis but they were apparent if airborne and biological concentration ranges more than individual values were compared.
The highest proportion of people exceeding the upper limits of the reference values was found for 1-OHP (about 29% of the sample). Jongeneelen suggested a reference values of 0.24 µmoles /mole creatinine (around 0.43 µg/g creatinine) as 95th ntile of values in not smokers not occupationally subjects and a value of 0.76 µmole/mole of creatinine (around 1.43 µg/g creatinine) as 95th ntile of values among smokers .
Epidemiological evidences show that 1.4 µmoles/mole of creatinine (around 2.7 µg/g creatinine) represent the lowest value at which genotoxic effects, such as sister chromatid exchanges have been demonstrated in not smoking people. This is therefore a precautionary limit (NOEL) below which is advisable to stay (Buchet *et al*.).
For this biomarker BEI was established only for particular groups of workers such as coke oven workers. In this case to an environmental limit of PAH expressed as *benzene soluble matter of* 0.2 mg/cm the ACGIH stated a 1 OH p urinary level of 4.4 µg/g creatinine. This level is associated to a relative risk of lung cancer of 1.3 (30% higher than expected).

The measured level are similar to those measured in previous investigation carried out in electric steel plants (Bergamaschi et al 2006), in which the 1-OH-P urinary excretion resulted well related to PAH exposure .

Plant 4 shows for Cr-U, a value as 9.44 (95th ntile) against 2.20 as RV, and Ni-U a value as 5.22 against 4.0 as RV. It is therefore necessary to consider specific technological aspects and or environmental data to interpret these biological data. It would be in addition interesting to verify the Cr and Ni species in order to qualify the carcinogenic risk for exposed workers.

6.4 Cast iron foundries

The present study shows that biological exposure levels of toxic/carcinogenetic compounds in the investigated steel plants fell, on average, well within the respective limit values fixed by International Agencies, such as DFG and ACGIH, or single researchers.

No relationship among airborne and biological values of investigated pollutants could be demonstrated by regression analysis but they were apparent if airborne and biological concentration ranges more than individual values were compared.

The highest proportions of subjects exceeding the upper limits of reference values were found for 1-OHP (about 19% of the sample) and As-U (about 14% of the sample).

For 1-OHP Jongeneelen suggested a reference values of 0.24 moles/mole creatinine (around 0.43 µg/g creatinine) as 95th ntile of values in not smokers not occupationally subjects and a value of 0.76 µmole/mole of creatinine (around 1.43 µg/g creatinine) as 95th ntile in smokers. Epidemiological evidences show that 1,4 µmoles/mole of creatinine (around 2,7 µg/g creatinine) represent the lowest value at which genotoxic effects, such as sister chromatid exchanges have been demonstrated in not smoking people. This is therefore a precautionary limit (NOEL) below which is recommended to stay (Buchet *et al*.).

For this biomarker BEI was established only for particular groups of workers such as coke oven workers. In this case to an environmental limit of PAH expressed as *benzene soluble matter of* 0.2 mg/cm the ACGIH stated a 1-OHP urinary level of 4.4 µg/g creatinine. This level is associated to a relative-risk of lung cancer of 1.3 (30% higher

than expected). The values of PbB would be examined considering technological and environmental data.

6.5 Comparison among investigated metallurgical sectors

As next step in evaluation, we looked for differences in biomarker levels in the investigated sectors.

Table 35 Descriptive data of Cd-U (µg/gcreat) values.

	Aluminum (n=163)	Copper alloys (n=150)	Electric steel (n=335)	Cast-iron (n=157)
Mean	0.378	0.470	0.350	0.302
Median	0.331	0.388	0.282	0.252
Std.Dev.	0.246	0.294	0.274	0.185
Asymmetry	2.675	1.381	4.707	2.462
Std. Err.Asymmetry	0.190	0.198	0.133	0.194
Min	0.050	0.071	0.064	0.000
Max	2.000	1.416	3.235	1.445
Percentiles 5^{th}	0.124	0.146	0.128	0.105
95^{th}	0.842	1.153	0.842	0.638

Table 36 Descriptive data of Cr-U (µg/l) values.

	Aluminum (n=164)	Copper alloys (n=150)	Electric steel (n=335)	Cast-iron (n=157)
Mean	0.284	0.212	0.660	0.515
Median	0.145	0.180	0.450	0.390
Std.Dev.	1.168	0.212	0.903	0.493
Asymmetry	12.407	5.091	6.563	2.017
Std. Err.Asymmetry	0.190	0.198	0.133	0.194
Min	0.025	0.025	0.025	0.025
Max	15.000	2.000	11.600	2.600
Percentiles 5^{th}	0.031	0.050	0.060	0.025
95^{th}	0.605	0.489	2.020	1.900

Table 37 Descriptive data of Ni-U (µg/l) values

	Aluminum (n=164)	Copper alloys (n=150)	Electric steel (n=331)	Cast-iron (n=156)
Mean	0.790	0.581	1.162	1.224
Median	0.500	0.400	0.900	0.900
Std.Dev.	0.981	0.600	1.059	1.063
Asymmetry	3.625	2.262	1.579	2.154
Std. Err.Asymmetry	0.190	0.198	0.134	0.194
Min	0.100	0.100	0.100	0.100
Max	8.000	3.700	5.600	6.300
Percentiles 5^{th}	0.100	0.100	0.100	0.100
95^{th}	2.375	1.845	3.500	3.230

Table 38 Descriptive data of As-U (µg/l) values in the four investigated sectors

	Aluminum (n=164)	Copper alloys (n=150)	Steel plants (n=335)	Cast-iron (n=157)
Mean	8.327	6.956	7.466	12.790
Median	5.550	5.300	6.400	9.900
Std.Dev.	8.937	6.284	6.747	12.200
Asymmetry	2.315	2.472	4.936	3.542
Std. Err.Asymmetry	0.190	0.198	0.133	0.194
Min	0.100	0.100	0.100	0.300
Max	52.200	43.000	78.300	101.000
Percentiles 5^{th}	0.150	0.500	0.700	1.770
95^{th}	32.300	20.155	16.320	36.860

Table 39 Descriptive data of As-U (µg/l) values.

	Aluminum (n=164)	Copper alloys (n=150)	Steel plants (n=334)	Cast-iron (n=157)
Mean	0.005	0.007	0.006	0.005
Median	0.005	0.005	0.005	0.005
Std.Dev.	0.001	0.004	0.003	0.001
Asymmetry	3.516	2.156	2.853	8.028
Std. Err.Asymmetry	0.190	0.198	0.133	0.194
Min	0.005	0.005	0.005	0.005
Max	0.011	0.021	0.020	0.017
Percentiles 5^{th}	0.005	0.005	0.005	0.005
95^{th}	0.010	0.017	0.014	0.005

Table 40 Descriptive data of 1-OHP (µg/gcreat) values.

	Aluminum (n=171)	Copper alloys (n=157)	Electric steel (n=339)	Cast-iron (n=167)
Mean	0.245	0.372	0.666	0.434
Median	0.184	0.189	0.431	0.289
Std.Dev.	0.189	0.973	0.886	0.480
Asymmetry	1.629	11.175	5.106	4.195
Std. Err.Asymmetry	0.186	0.194	0.132	0.188
Min	0.019	0.014	0.031	0.013
Max	1.135	12.022	9.647	4.180
Percentiles 5^{th}	0.053	0.049	0.079	0.058
95^{th}	0.633	0.960	1.818	1.155

Table 41 Descriptive data of PbB (µg/dl) values.

		Aluminum (n=37)	Copper alloys (n=43)	Electric steel (n=59)	Cast-iron (n=25)
Mean		6.808	17.637	8.395	3.840
Median		6.200	16.300	7.200	3.300
Std.Dev.		3.163	8.752	4.978	2.002
Asymmetry		0.268	0.907	1.217	1.489
Std. Err.Asymmetry		0.388	0.361	0.311	0.464
Min		1.900	5.600	2.100	1.800
Max		13.000	41.300	24.800	10.000
Percentiles	5^{th}	2.170	6.620	2.600	1.830
	95^{th}	12.010	35.600	19.600	9.190

Table 42 Descriptive data of PCB (ng/ml) values.

		Aluminium (n=37)	Copper alloys (n=34)	Steel plants (n=59)	Cast-iron (n=16)
Mean		4.605	4.321	3.785	2.512
Median		2.900	3.800	3.000	1.950
Std.Dev.		5.258	3.077	2.922	2.519
Asymmetry		2.070	0.881	1.543	1.702
Std. Err.Asymmetry		0.388	0.403	0.311	0.564
Min		0.200	0.200	0.100	0.300
Max		19.400	12.700	14.800	8.900
Percentiles	5^{th}	0.380	0.425	0.400	0.300
	95^{th}	18.770	10.750	10.600	8.900

The distributions of values of the biomarkers in the four metallurgical sectors are graphically resumed below. The workers exceeding the 5th and the 95th percentiles of the respective reference values (broken-lines) are showed.

Figure 1 Boxplots of Cd-U values.

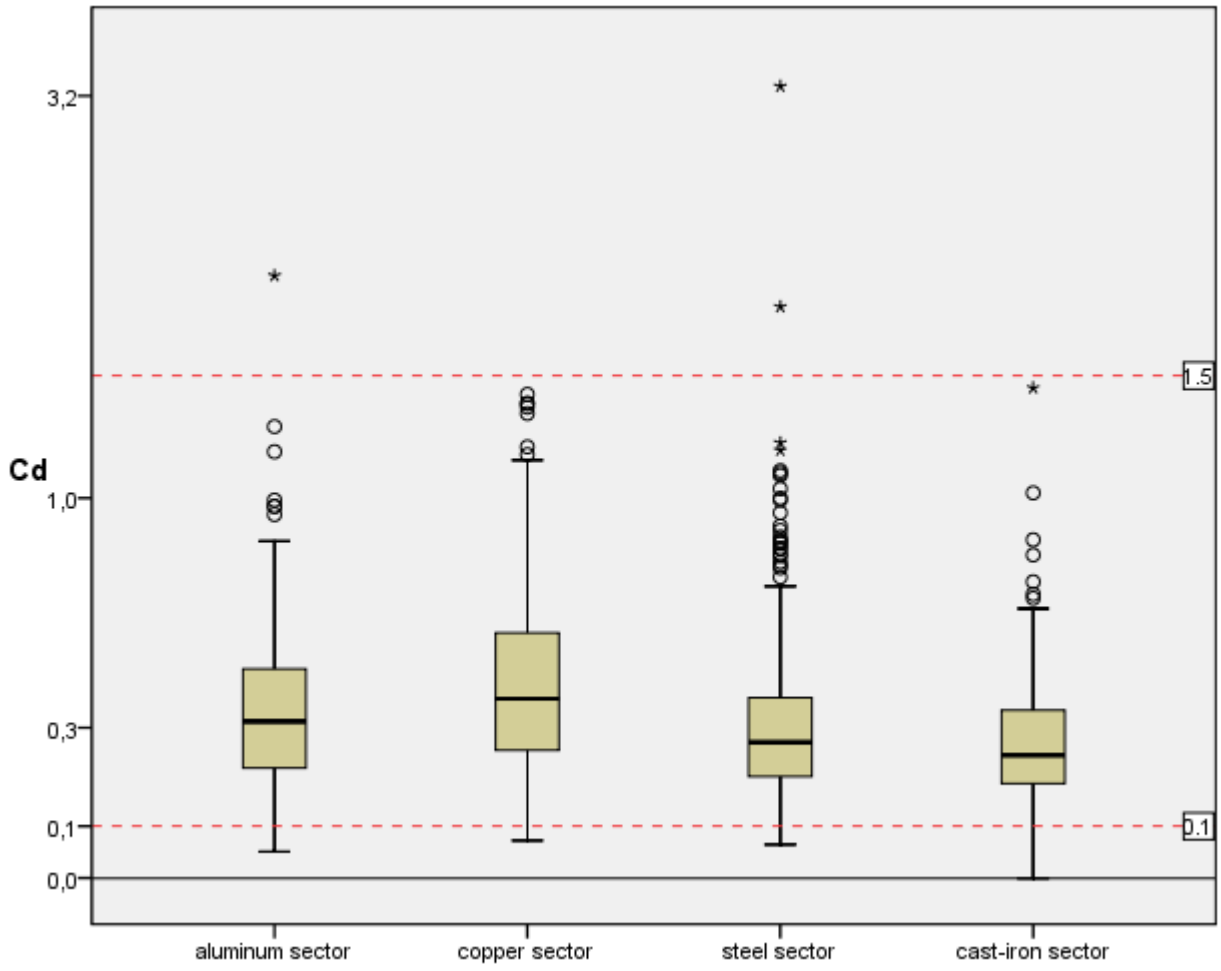


Figure 2 Boxplots of Cr-U values.

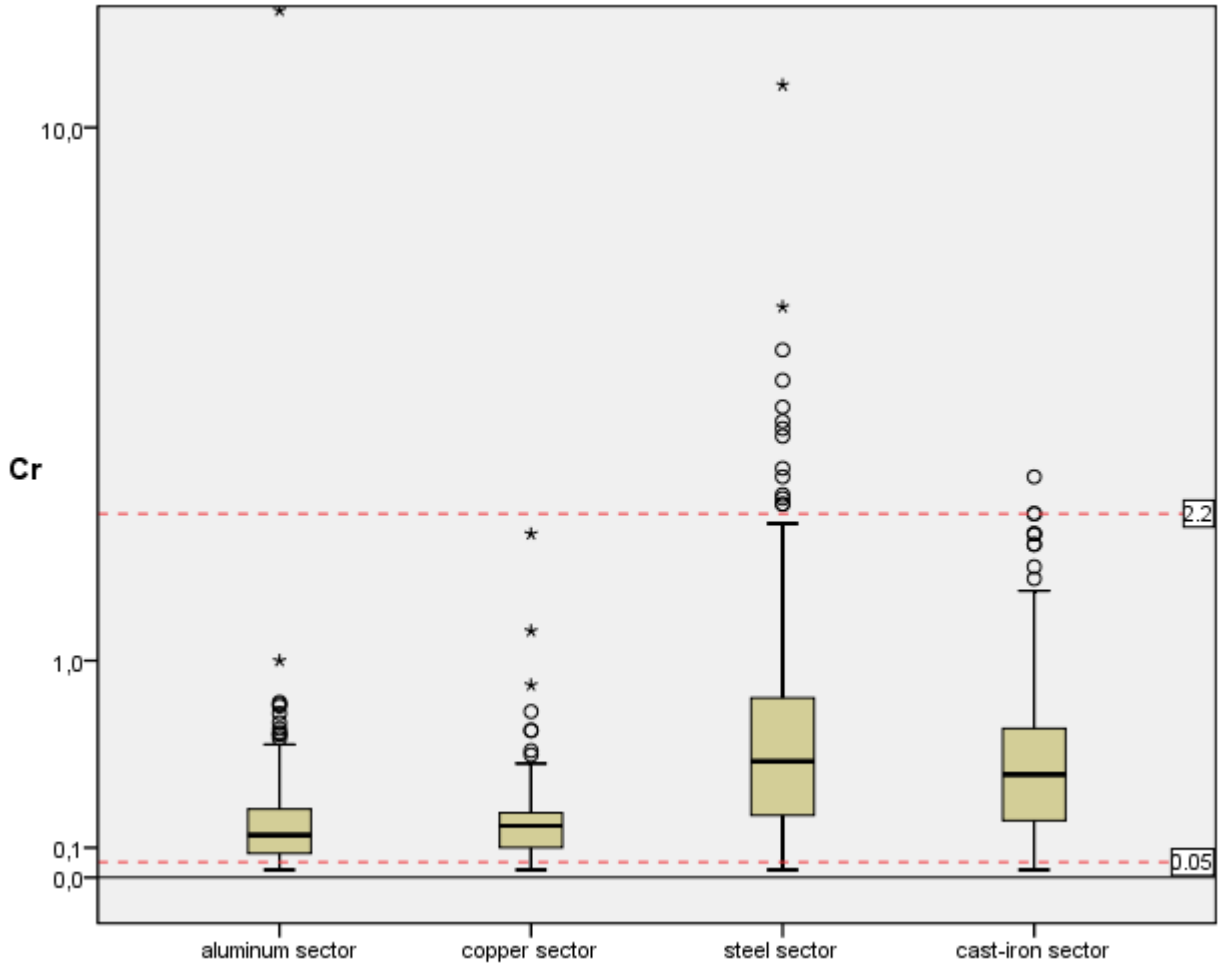


Figure 3 Boxplots of Ni-U values.

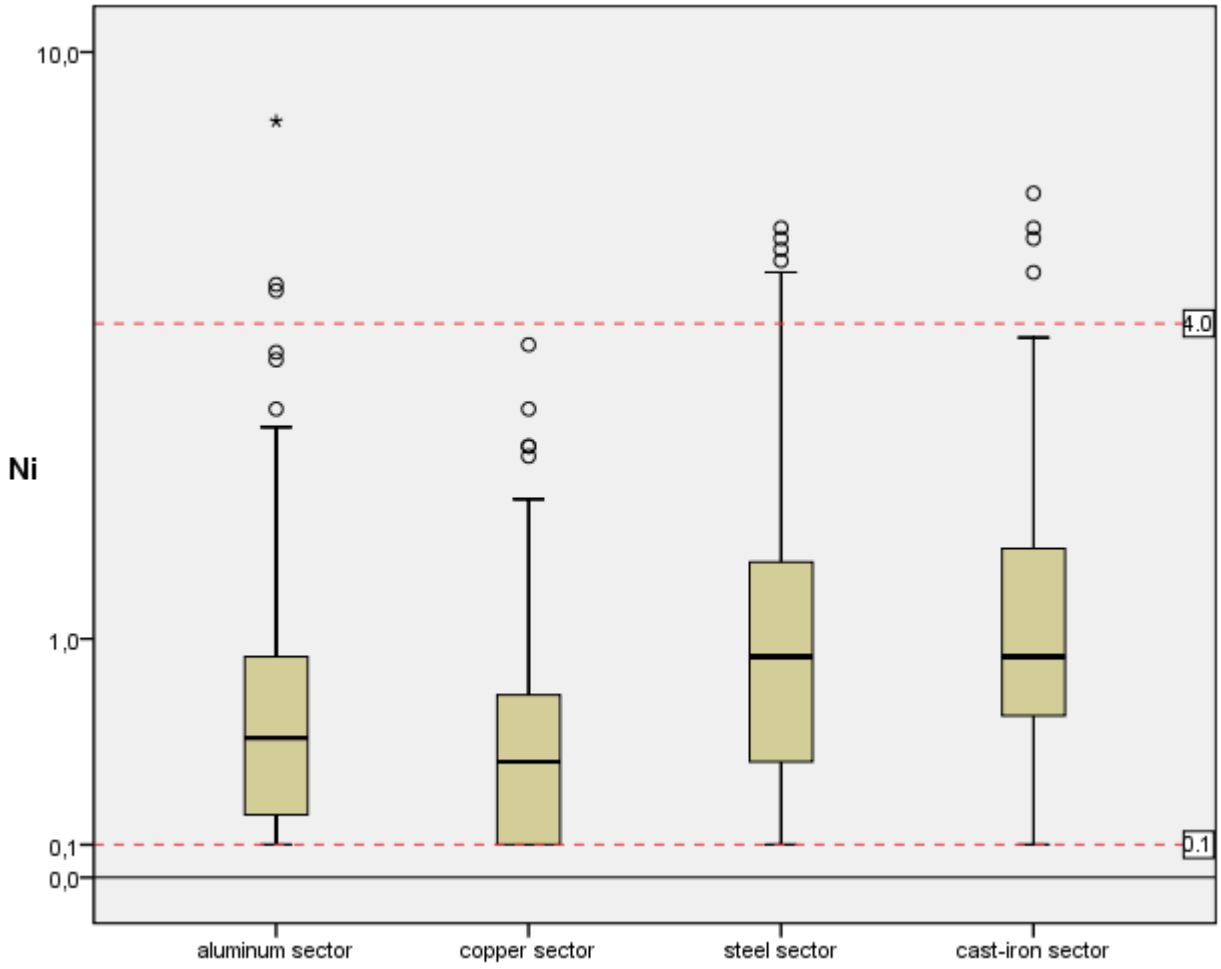


Figure 4 Boxplots of As-U values.

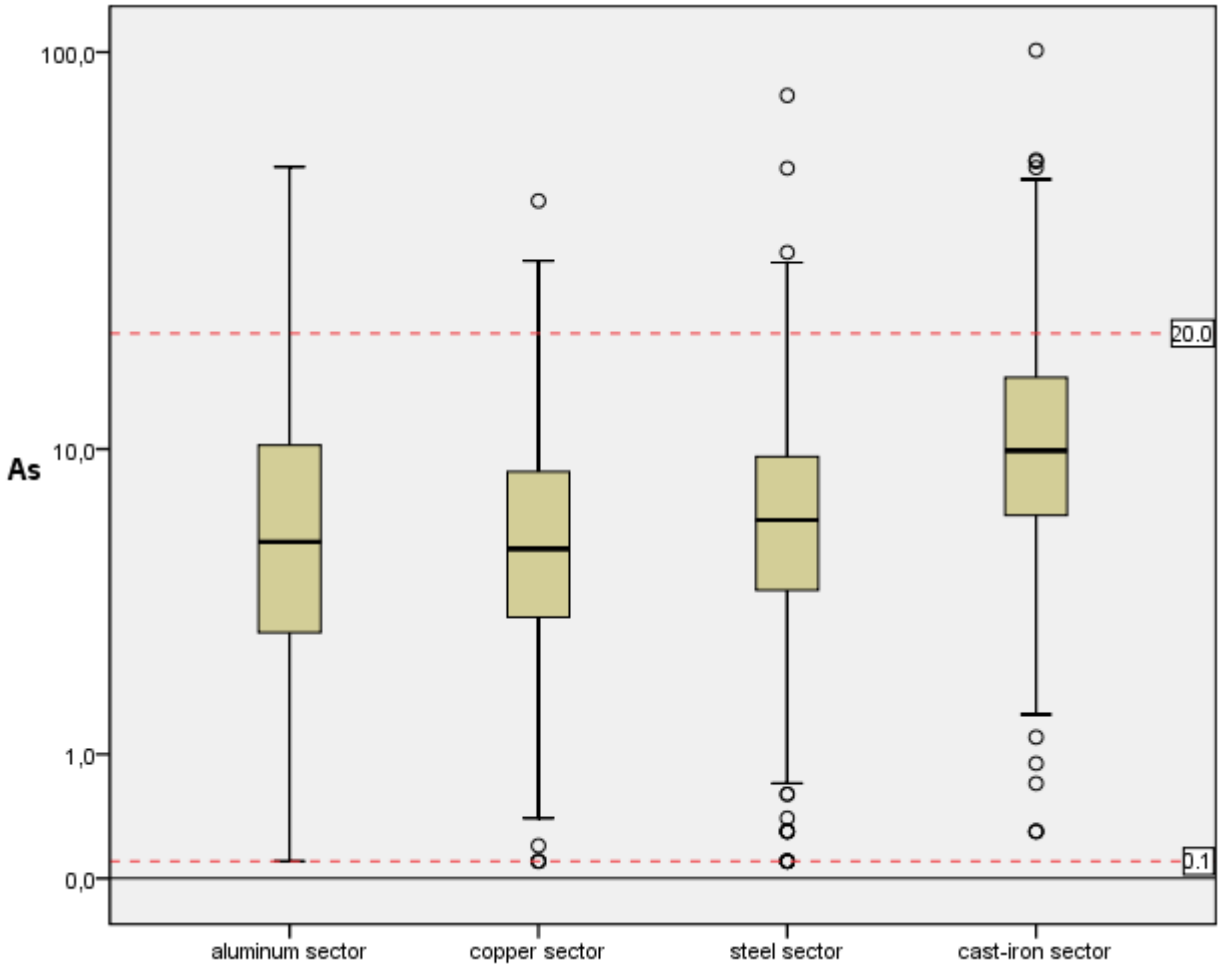


Figure 5 Boxplots of 1-OHP values.

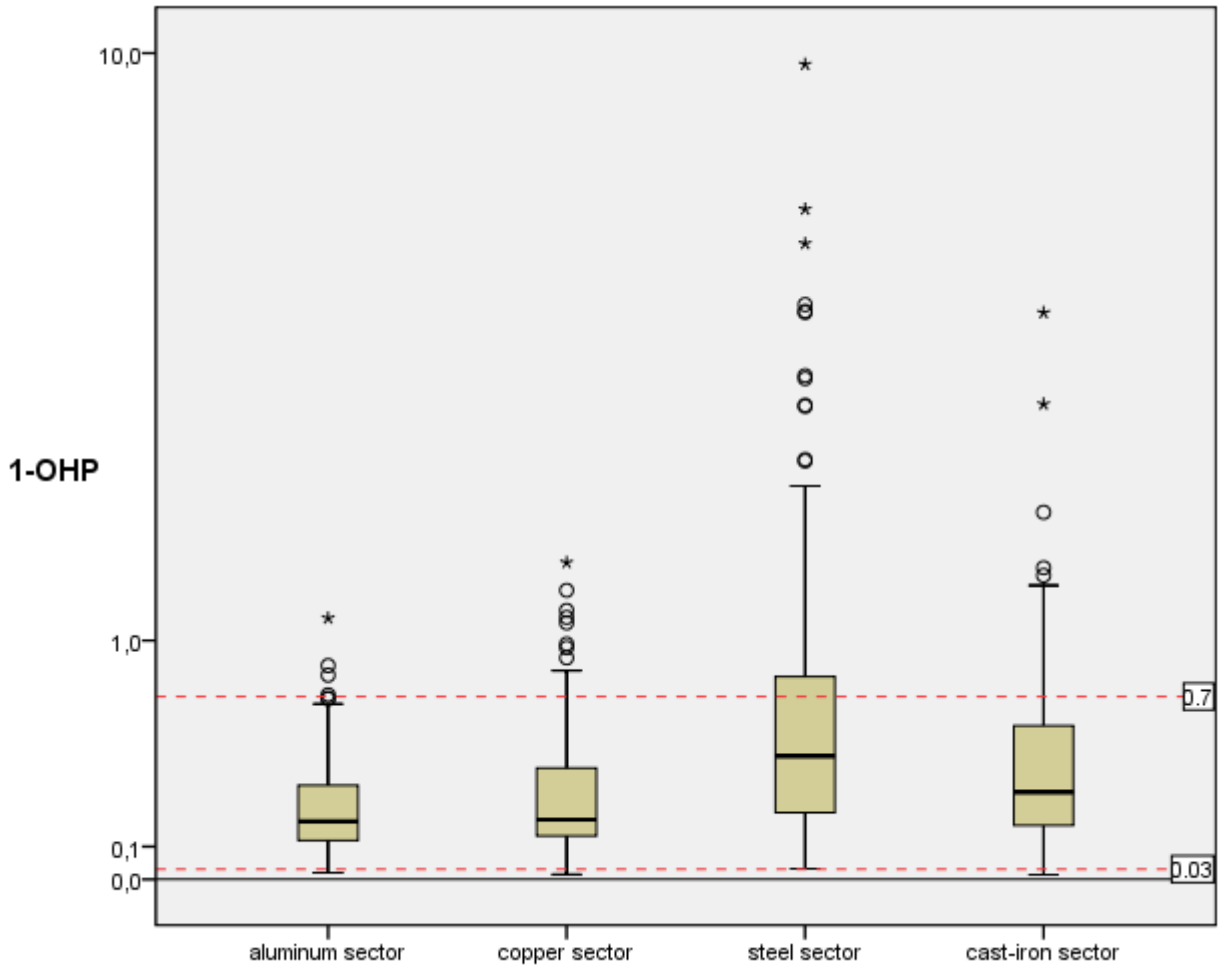


Figure 6 Boxplot of PbB values.

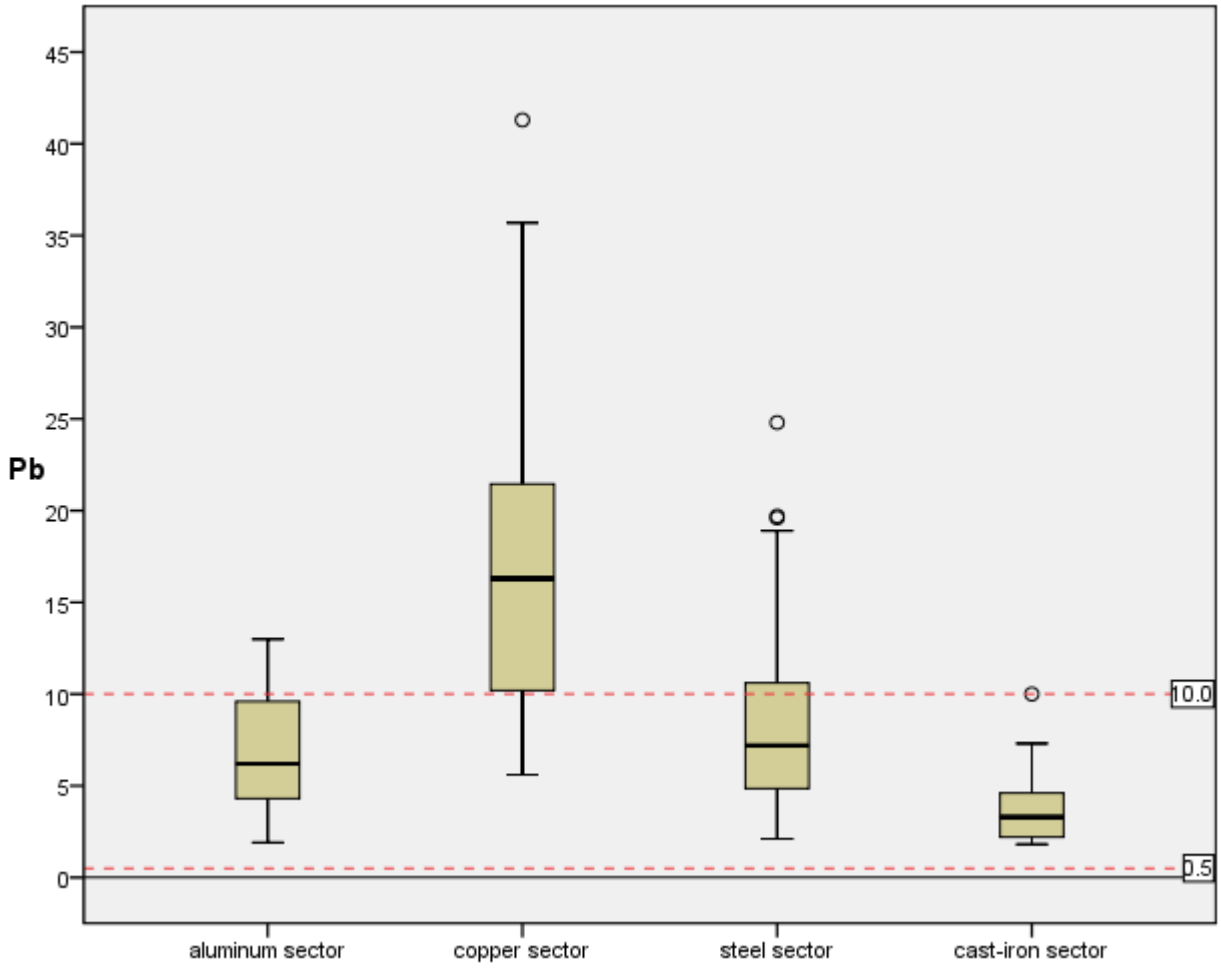
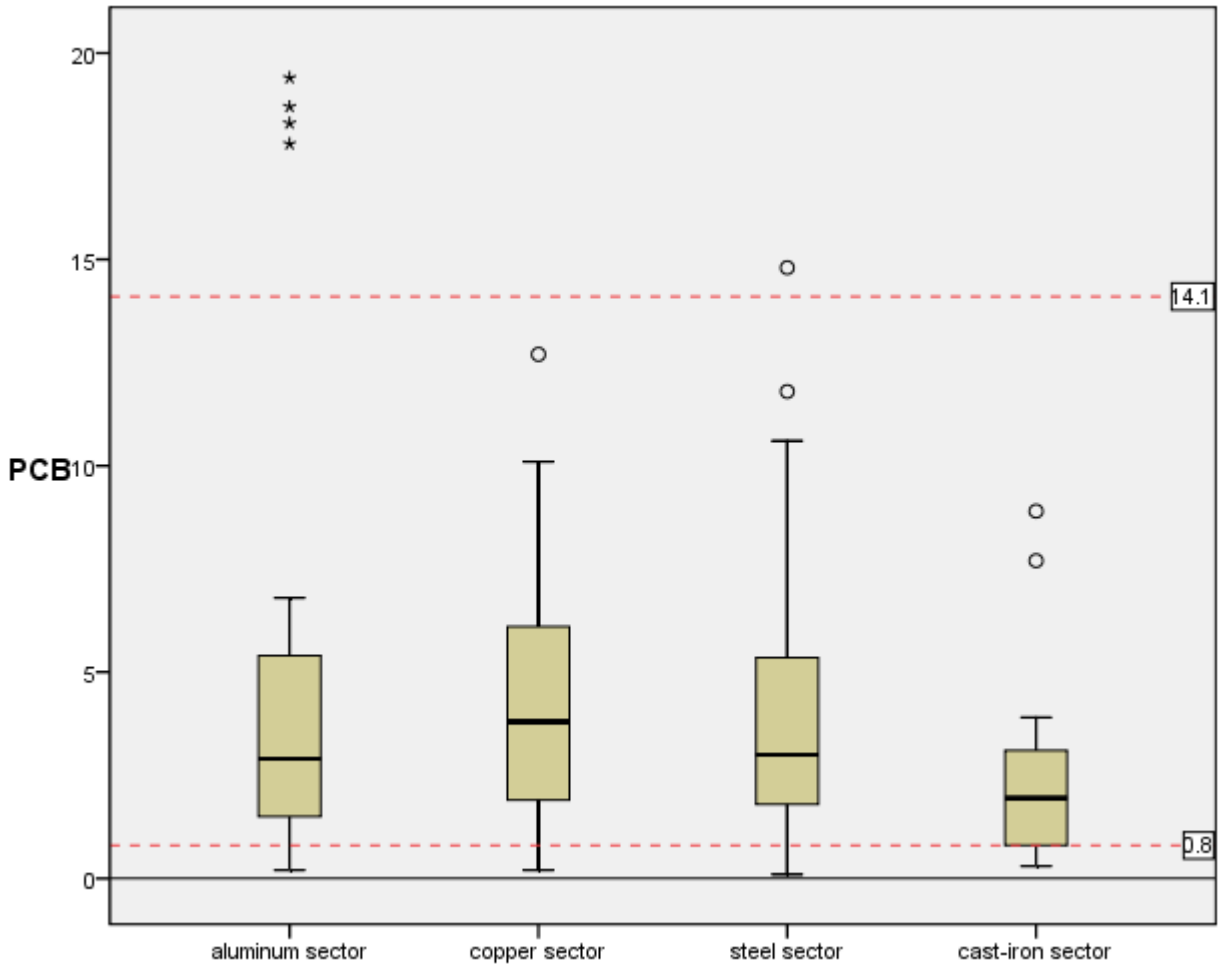

Pb
45
40
35
30
25
20
15
10
5
0
10.0
0.5
aluminum sector
copper sector
steel sector
cast-iron sector

Figure 7 Boxplot of ΣPCB-S values.



Then, we studied the relationships of Cd-U and 1-OHP with the smoking habits (as number of pack-years) on the whole dataset, pulling together the data of the four investigated sectors. As expected, both the biomarkers were significantly associated to the extent of smoking habits. The respective equations were: *Cd-U=0.009(packyears)+ 0.304* and *1-OHP=0.015(packyears)+0.349 (p<0.000 for both)*.

Figure 8 Relationship between Cd-U (µg/g creat) values and lifetime smoking habits (packyears). The broken line represents the 95th ntileof the reference values for Cd-U.

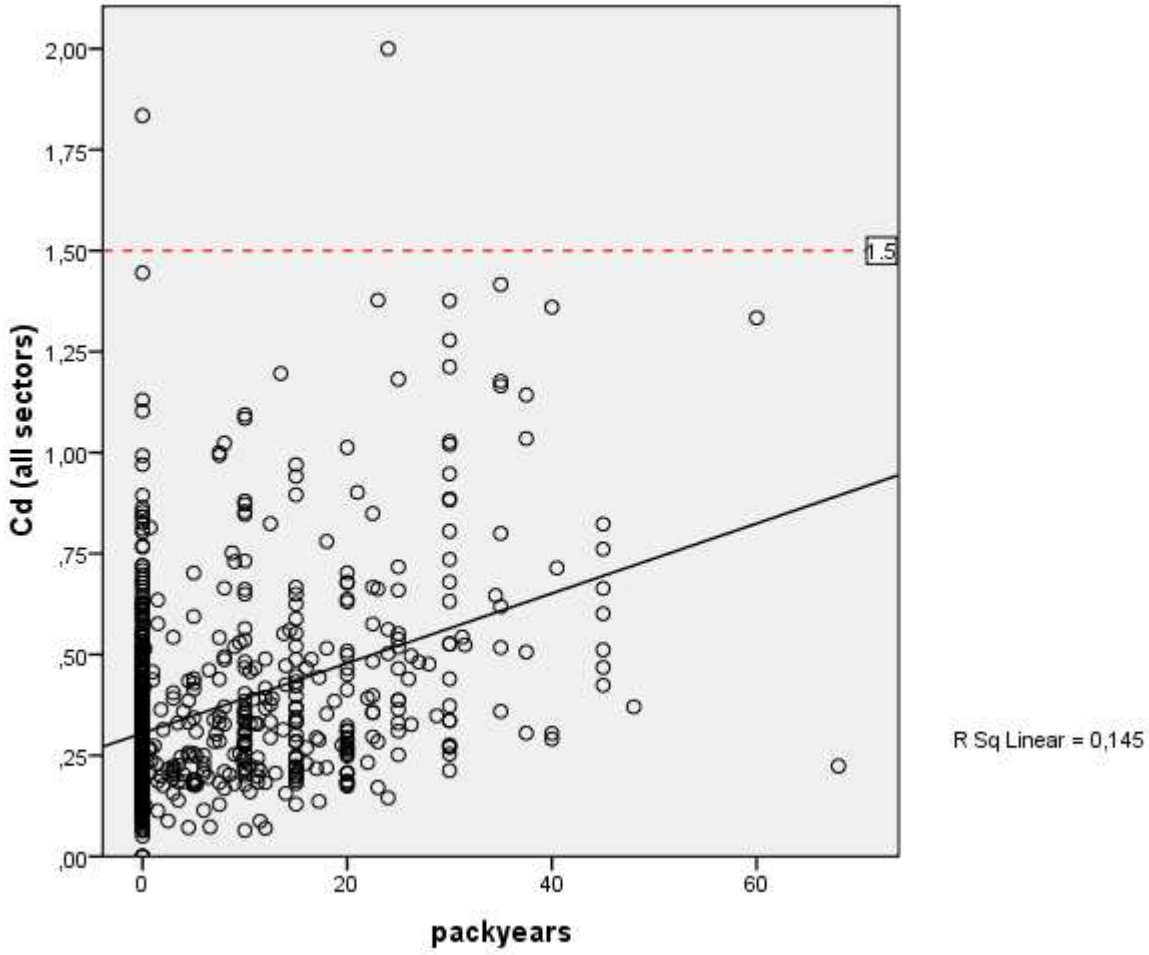
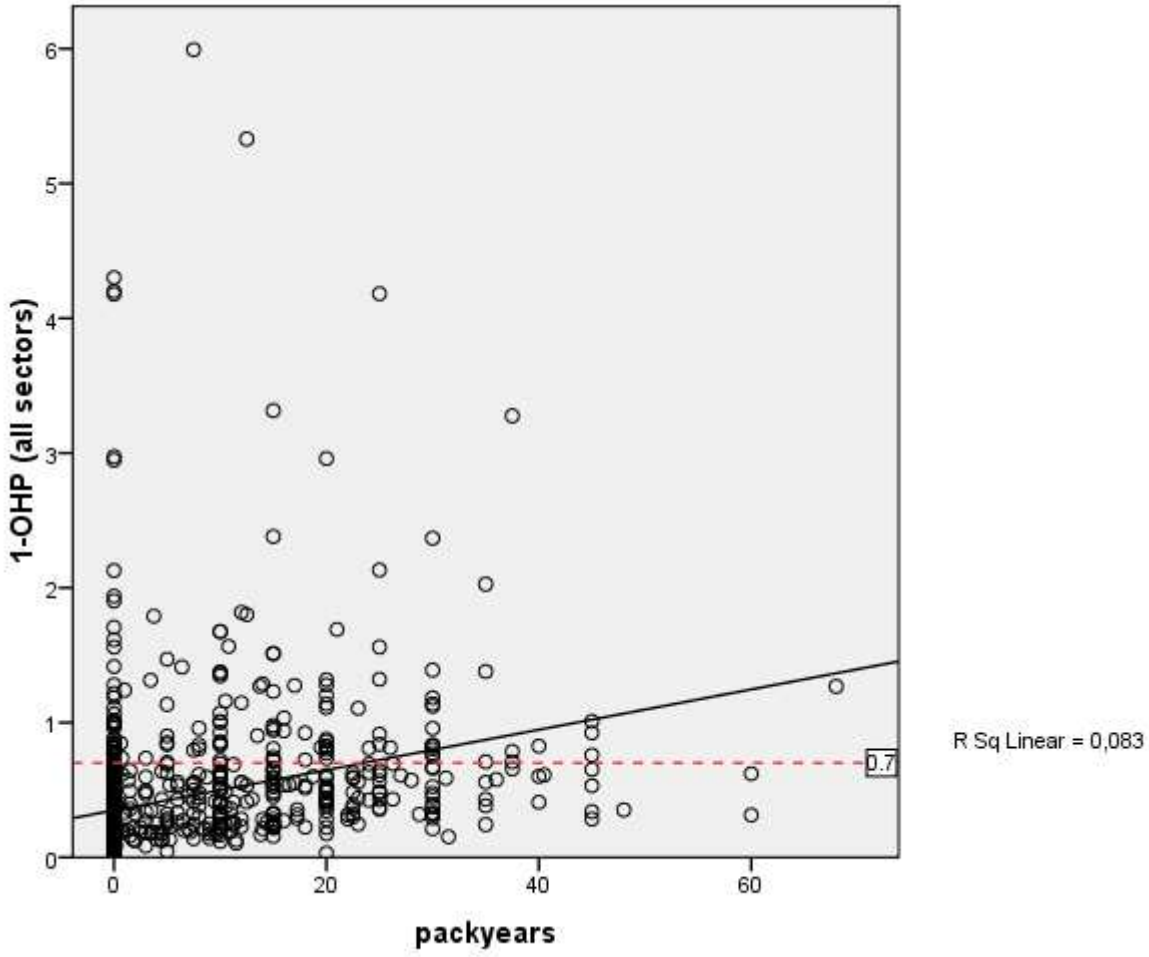


Figure 9 Relationship between 1-OHP (µg/g creat) values and lifetime smoking habits (packyears). The broken line represents the 95th ntile of the reference values for 1-OHP.



Some considerations may be suggested about the risk of lung cancer and the values of 1-OHP determined in the electric steel plants.

The application of a linear regression model for assessing the relationship between the dependent variable Y (1-OHP) and the independent X (factor "smoke" as pack-years) in all the 339 people working in sector steel, shows a "p" significant at α = 0.05 to 0.000 composing the equation *y=0.02x+0.508* (confidence interval at the same level α: *0.013-0.028*). When the scope of analysis is restricted to 157 smokers, the probability resulting from the regression becomes non-significant.

The 95th ntile of 1-OHP values was 2.44 µg/g creatinine among smokers (upper limit of the RV=1.43) and 1.27 µg/g creatinine among non-smokers (upper limit of the RV=0.43).

The following table resumes the distributions 1-OHP values determined among electric steel workers, stratified according to smoking habits, and the prevalences of workers exceeding or not the reference values for 1-OHP.

Table 43 Distribution of 1-OHP values among electric steel workers classified by smoking habits and by values exceeding or not the upper reference value (R.V.) for the analyte.

Smoking habits	1-OHP		1-OHP		
		N	Median	Min	Max
Yes	> R.V.	20	2.08	1.51	9.65
	< R.V.	137	0.57	0.12	1.41
	Total	157	0.63	0.12	9.65
No	> R.V.	55	0.71	0.43	4.30
	< R.V.	127	0.20	0.03	0.43
	Total	182	0.26	0.03	4.30
Total	>V.R.	75	0.96	0.43	9.65
	<V.R.	264	0.32	0.03	1.41
	Total	339	0.43	0.03	9.65

The table shows that among non smokers, the subgroup of *subjects exceeding the upper limit of the RV* for *1-OHP* have median values of 1-OHP 3.58 times higher than subjects *with values of 1-OHP within the RV.* Among smokers, the corresponding figures give rise to median 1-OHP values that are on average 3.64 times higher in the subgroup of subjects *exceeding the upper limit of the RV* for *1-OHP.*

Even taking into account that different RV are available for 1-OHP, depending on the smoking habits, the table shows that non smoking workers have a higher risk [odds ratio 2.97 (95% CI 1.68-5.22); p<0.0001] to exceed their RV as compared to smoking workers.

In developed countries, the percentage of deaths from all cancers attributable to tobacco smoke varies between 25% and 30%. In the case of lung cancer, the percentage of deaths due to smoking is about 90% in men and 70% in women.

The mortality rate for middle aged (50-55 years) persons, with a past cigarette smoking history of at least 15-20 years, is 3 times higher than that of never smokers. About half of

usual smokers that have started smoking at a young age, died because of their habit. Overall, a smoker loses about 15 years of life. Deciding to quit smoking at any time of his life greatly reduces the risk of cancer or of other diseases (R. Talamini and GO Risk Factors).

During the period 1998-2002, lung (including trachea and bronchi) cancer was at the 3rd and at the 4th cancer in terms of occurrence among males (14.2% of all cancers) and females (4.6% of all cancers), respectively. As cause of death for cancer, it was at the 1st and the 2nd place among in terms of frequency among males and females, respectively. According to the Italian Network of Cancer Registries (AIRT) 111.5 cases per 100.000 men and 27.9 per 100.000 women were diagnosed each year. Estimates for Italy indicate 30,384 and 6,784 new incidents cases each year among males and females, respectively. With regard to mortality, in 2002 there were 25,639 deaths among males and 6,495 among females.

The risk of having a diagnosis of lung cancer during their lifetimes (up to 74 years) is 6.77% among males (1 case every 15 men) and 1.41% among females (1 case each 71 women), while the death risk is 5.64% among males and 1.04% among females. There is a certain geographical variability in the incidence of lung cancer in our country with a ratio between areas with the highest rates and those with the lowest of about 2. In general, the highest rates are in regions of northern Italy and the lowest in the South Italy. Over time, lung cancer has shown a tendency to reduce both the incidence and mortality in males, whereas there is a steady growth in women (Epidemiology & Prevention - Report 2006).

The table below shows the standardized mortality rate (standard on Italy) and the standardized rate of malignant tumours of trachea / bronchi / lungs of males in Brescia (source: Health for all 2008 - Istat rates cast, to 10.000 inhabitants).

Table 44 Standardized mortality rates for all causes (Italy) and for tumors of the respiratory apparatus (Brescia, males).

Years	Std. mortality rate	Std. mortality rate for malignancies
1991	166.78	15.36
1992	162.87	15.02
1993	159.51	15.29
1994	161.55	15.33
1995	157.31	14.79
1996	154.71	14.29
1997	151.5	14.23
1998	147.06	13.94
1999	141.52	13.54
2000	136.19	12.9
2001	132.21	12.43

Lung cancer is at 9.4%, as percentage of total mortality on the last year available, even in sensitive steady decline over the years.

Table 45 Epidemiology of lung cancer among males in the Lombardia Region.

Year	Incidence	Incidence rate	Prevalence	Prevalence rate
1980	4,453	104.07	3,482	81.38
1981	4,581	107.14	3,726	87.14
1982	4,699	110.03	3,982	93.25
1983	4,804	112.72	4,248	99.68
1984	4,875	114.64	4,505	105.94
1985	4,932	116.18	4,762	112.17
1986	4,973	117.28	5,012	118.21
1987	5,000	118.01	5,259	124.11
1988	5,026	118.57	5,510	129.98
1989	5,041	118.79	5,756	135.65
1990	5,051	118.87	6,002	141.27
1991	5,054	118.77	6,252	146.9
1992	5,049	118.41	6,503	152.52
1993	5,039	117.97	6,763	158.33
1994	5,020	117.57	7,023	164.46
1995	4,987	116.66	7,266	169.98
1996	4,953	115.39	7,507	174.89
1997	4,915	114.04	7,742	179.64
1998	4,877	112.71	7,980	184.41
1999	4,835	111.26	8,213	188.98
2000	4,793	110.34	8,447	194.47
2001	4,750	109.4	8,684	200
2002	4,709	108.47	8,927	205.62
2003	4,664	107.43	9,166	211.15
2004	4,602	106.08	9,379	216.17
2005	4,515	104.22	9,539	220.19
2006	4,408	101.98	9,654	223.33
2007	4,296	99.61	9,750	226.08

Table 43 presents an update for the region Lombardia on the incidence and prevalence of lung malignancies among males (0-84 years), as rates per 100,000 inhabitants. The incidence rate is the number of newly diagnosed cases for a specific tumor during a

calendar year, compared to the population. The prevalence rate is the number of individuals living in a population at a certain date, who have previously had a diagnosis of cancer.

REFERENCES

ACGIH. 2007a. Threshold Limit Values (TLVs) for Chemical Substances and Physical Agents and Biological Exposure Indices (BEIs). Cincinnati, OH. American Conference of Governmental Industrial Hygienists.

ACGIH. 2007b. Documentation of the Threshold Limit Values and Biological Exposure Indices. Cincinnati, OH. American Conference of Governmental Industrial Hygienists.

Aitio A. Biological monitoring today and tomorrow. Scand J Work Environ Health 1994; 20:46-58.

Aitio A. Biological monitoring at the Institute of Occupational Health. Scand J Work Environ Health 1992; 18: 69-71.

Aitio A. Apostoli P. Quality assurance in biomarker measurement. Toxicol Lett 1995; 77: 195-204.

Apostoli P, Lucchini R, Maccarrone R, Alessio L. Biological monitoring of occupational exposure to aluminum. Med Lav. 1992; 83: 475-483.

Apostoli P. Criteria for the definition of reference values for toxic metals. Sci Total Environ. 1992; 120: 23-37.

Apostoli P, Alessio L. Does the decree 25/2002 still guarantee the health care of workers exposed to lead? G Ital Med Lav Ergon. 2002; 24: 355-60.

Apostoli P, Magoni M, Bergonzi R, Carasi S, Indelicato A, Scarcella C, Donato F. Assessment of reference values for polychlorinated biphenyl concentration in human blood. Chemosphere. 2005 Oct;61(3):413-21.

Apostoli P. Update on the subject of lead toxicology Ann Ist Super Sanita. 1998; 34(1): 5-15.

Alexander J, Reistad R, Hegstad S, Frandsen H, Ingebrigtsen K, Paulsen J E and Becher G., Biomarkers of exposure to heterocyclic amines: approaches to improve the exposure assessment. Food Chem Toxicol 2002; 40: 1131-1137.

Berlin A, Yodaiken RR, Henman BA. Assessment of toxic agents at the workplace. Roles of ambient and biological monitoring. Boston: Hague Nijhoft, 1984.

Bernard A, Lauwerys R. Present status and trends in biological monitoring of exposure to industrial chemicals. J Occup Med 1986; 28: 558-562

Bernard AM Biokinetic and stability aspects of biomarkers: recommendations for application in population studies. Toxicology 1995; 101: 65-71.

Biological Exposure Values for Occupational Toxicants and Carcinogens, Volume 3: 35–44, Weinheim, Germany:VCH Verlagsgesellschaft, 1999.

Biological Exposure Values for Occupational Toxicants and Carcinogens, Vol 2: 221. Weinheim, Germany:VCH Verlagsgesellschaft, 1995.

Bolt HM, Rutenfranz J in: Health Surveillance of Individual Workers Exposed to Chemical Agents, Notten WRF et al. (Eds), Int Arch Occup Environ Hlth, Suppl: 113 – 120 (1986);

Boogard PJ, Van Sittert NJ. Exposure to polycyclic aromatic hydrocarbons. Occup Environ Med 1994; 51: 250-258.

Boogard PJ, Van Sittert NJ Urinary 1-hydroxypyrene as a biomarker of exposure to polycyclic aromatic hydrocarbons in workers in petrochemical industries: baseline values and dermal uptake. Sci Tot Environ 1995; 163: 203-209.

Brandt HCA, Watson W P. Monitoring human occupational and environmental exposures to polycyclic aromatic hydrocarbons. Ann Occup Hyg 2003; 47: 349-378.

Brima EI, Haris PI, Jenkins RO, Polya DA, Gault AG, Harrington CF. Understanding arsenic metabolism through a comparative study of arsenic levels in the urine, hair and fingernails of healthy volunteers from three unexposed ethnic groups in the United Kingdom. Toxicol Appl Pharmacol. 2006; 216: 122-30.

Buckley TJ, Lioy P J. An examination of the time course from human dietary exposure to polycyclic aromatic hydrocarbons to urinary elimination of 1-hydroxypyrene. Br J Ind Med 1992; 49: 113-124.

Chen YC, Chen BH. Determination of polycyclic aromatic hydrocarbons in fumes from fried chicken legs. J Agric Food Chem 2003; 51: 4162-4167.

Crippa M, Apostoli P, Quarta C, Alessio L. Risk of exposure to metals in the production and processing of copper alloys. Med Lav. 1991; 82(3): 261-9.

DFG List of MAK and BAT Values 1997. Commission for the investigation of health hazards of chemical compounds in the work area. Wiley - VCH Verlag, Weinheim, 2007.

Dor F, Dab W, Empereur-Bissonet P, Zmirou D. Validity of biomarkers in environmental health studies: the case of PAHs and benzene. Crit Rev Toxicol 1999; 29: 129-168.

Droz PO, Berode M, Wu MM. Evaluation of concomitant biological and air monitoring. Appl Occup Environ Hyg 1991; 6: 465-474.

Droz PO. Pharmacokinetic modelling as a tool for biological monitoring. Int Arch Occup Environ Health 1993; 65: 553-559.

Friberg L, Elinder C-G. Biological monitoring of toxic metals. Scand J Work Environ Health 1993; 19 (S1): 7-13.

Grasbeck R, Solberg HE. Can reference values be properly used. Clin Chem 1981; 27:1795-1796

Grasbeck R, Saris ME. Establishment and use of reference values. Scand J Clin Lab Invest 1989; 26: 62-63.

Greim H, Csanady G, Filser J, Kreuzer P, Schwarz L, Wolff T, Werner S. Biomarkers as tools in human health risk assessment. Clin Chem 1995; 4: 1804-1808.

Halatek T, Sinczuk-Walczak H, Rydzynski K. Prognostic significance of low serum levels of Clara cell phospholipid-binding protein in occupational aluminium neurotoxicity. J Inorg Biochem. 2005; 99(9): 1904-1911.

Helsel DR. Less than obvious-statistical treatment of data below the detection limit. Environ Sci Technol 1990; 24: 1768-1774.

Iregren A, Sjögren B, Gustafsson K, Hagman M, Nylén L, Frech W, Andersson M, Ljunggren KG, Wennberg A. Effects on the nervous system in different groups of workers exposed to aluminium. Occup Environ Med. 2001; 58: 453-60.

Jongeneelen FJ, Anzion RB, Scheepers PT, Bos RP, Henderson PT, Nijenhuis EH, Veenstra SJ, Brouns RM, Winkes A. 1-Hydroxypyrene in urine as a biological indicator of exposure to polycyclic aromatic hydrocarbons in several work environments. Ann Occup Hyg. 1988;32(1):35-43.

Jongeneelen FJ, van Leeuwen FE, Oosterink S, Anzion RB, van der Loop F, Bos RP, van Veen HG. Ambient and biological monitoring of cokeoven workers: determinants of the internal dose of polycyclic aromatic hydrocarbons. Br J Ind Med. 1990; 47: 454-61.

Jongeneelen FJ. Benchmark guideline for urinary 1-hydroxypyrene as biomarker of occupational exposure to polycyclic aromatic hydrocarbons. Ann Occup Hyg. 2001; 45: 3-13.

Jongeneelen FJ. Guidelines for biological monitoring of workers in aluminium production facilities for urinary 1-hydroxypyrene (1-pyrenol). J Environ Monit 2004; 61N-65N.

Kriek E, Van Schooten FJ, Hillebrand MJ, Van Leeuwen FE, Den Engelse L, De Looff AJ, Dijkmans AP. DNA adducts as a measure of lung cancer risk in humans exposed to polycyclic aromatic hydrocarbons. Environ Health Perspect. 1993; 99: 71-5.

Kroll MH, Chesler R, Hagengruber C, Blank DW, Kestner J, Rawe M. Automated determination of urinary creatinine without sample dilution: theory and practice. Clin Chem 1986; 32: 446-52.

Lauwerys R, Hardy R, Job M, Buchet JP, Roels H, Bruaux P, Rondia D. Environmental pollution by cadmium and cadmium body burden: An autopsy study. Toxicol Lett 1984; 23:287-289.

Lehnert G, Schaller K-H. Strategy of biological monitoring and setting of biological threshold limits (BAT values) in Germany. Isr J Med Sci 1995; 31: 549-557.

Lowry L. Biological limit values. In: Methods for Biological Monitoring (Kneip T, Crable J, eds). Washington:American Public Health Association, 1988;109-119.

Mason H, Wilson K. Biological monitoring: the role of toxicokinetics and physiologically based pharmacokinetic modeling. Am Ind Hyg Assoc J. 1999 Mar-Apr;60(2):237-42.

Minoia C, Pietra R, Sabbioni E, Ronchi A. The control of preanalytical factors in the monitoring of trace elements in biological fluids. Sci Total Environ 1992; 120: 63-79.

Mutti A. Use of intermediate end-points to prevent long-term outcomes. Toxicol Lett 1995; 77: 121-125.

Mutti A. Biological monitoring in occupational and environmental toxicology. Toxicol Lett 1999; 108: 77-89.

NRC (NATIONAL RESEARCH COUNCIL) 1989 Biologic Markers in Reproductive Toxicology (Washington, DC: National Academy Press).

Nawrot T, Plusquin M, Hogervorst J, Roels HA, Celis H, Thijs L, Vangrosveld J, Van Hecke E, Staessen J. Environmental exposure to cadmium and risk of cancer: a prospective population-based study. Lancet Oncol 2006; 7: 119-126.

Tjoe Ny E, Heederik D, Kromhout H, Jongeneelen F. The relationship between polycyclic aromatic hydrocarbons in air and in urine of workers in a Söderberg potroom. Am Ind Hyg Assoc J. 1993; 54: 277-84.

Phillips DH, Hewer A, Martin CN, Garner RC, King MM. Correlation of DNA adduct levels in human lung with cigarette smoking. Nature 1988; 336: 790-792.

Polizzi S, Pira E, Ferrara M, Bugiani M, Papaleo A, Albera R, Palmi S. Neurotoxic effects of aluminium among foundry workers and Alzheimer's disease. Neurotoxicology. 2002; 23: 761-74.

Rappaport SM. Biological monitoring and standard setting in the USA: a critical appraisal. Toxicol Lett 1995; 77: 171-182.

Routledge M N,Garner RC, Jenkins D, Cuzick J. 32P-postlabelling analysis of DNA from human tissues. Mut Res 1992; 282: 139-145.

Satarug S, Moore MR. Adverse health effects of chronic exposure to low-level cadmium in foodstuffs and cigarette smoke. Environ Health Perspect. 2004 Jul;112(10):1099-103.

Schenk RU, Bjorksten J, Yeager L. 1989. Composition and consequences of aluminum in water, beverages and other ingestibles. In: Lewis TE, ed. Environmental chemistry and toxicology of aluminum. Chelsea, MI: Lewis Publishers, Inc., 247-269.

Sherwood R. Benzene: the interpretation of monitoring results. Ann Occup Hyg 1972; 15: 409-421.

Solberg HE, Gräsbeck R. Reference values. Adv Clin Chem 1989; 27: 1-79.

UK Health and Safety Executive 1992. Biological Monitoring for Chemical Exposures in the Workplace . Guidance Note EH 56 (London: HMSO).

Vainio H. Current trends in the biological monitoring of exposure to carcinogens. Scand J Work Environ Health 1985; 11: 1-6.

van Schooten FJ, Jongeneelen FJ, Hillebrand MJ, van Leeuwen FE, de Looff AJ, Dijkmans AP, van Rooij JG, den Engelse L, Kriek E. Polycyclic aromatic hydrocarbon-DNA adducts in white blood cell DNA and 1-hydroxypyrene in the urine from aluminum workers: relation with job category and synergistic effect of smoking. Cancer Epidemiol Biomarkers Prev. 1995; 4: 69-77.

Van Schooten FJ, Godschalk RWL, Breedijk A, Maas LM, Kriek E, Saka H, Wigbout G, Baas, P, Van'T Veer L, Van Zandwijk N. 32P-postlabelling of aromatic DNA adducts in white blood cells and alveolar macrophages of smokers: saturation at high exposures. Mut Res 1997; 378: 65-75.

WHO air quality guidelines for Europe, 2nd edition. Copenhagen, WHO Regional Office for Europe, 2000