

Biomonitoring of tram drivers exposed to airborne platinum, rhodium and palladium

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Abstract

Object The aim of this study was to evaluate urinary levels of Pt, Rh and Pd in occupationally exposed subjects.

Methods A total of 122 healthy male subjects of Rome (Italy) were studied; 64 were municipal tram drivers and 58 control subjects. Metal quantification in the urine samples was carried out by sector field inductively coupled plasma mass spectrometry.

Results There were statistically significant differences between urinary Pt and Rh levels of the workers and the control group (Pt median: 1.23 versus 1.03 ng/g creatinine; Rh median: 19.16 versus 11.18 ng/g creatinine), while no difference in Pd levels was observed (Pd median: 11.47 versus 8.75 ng/g creatinine).

Conclusions Urinary Pt and Rh could be useful biomarkers for monitoring population groups occupationally exposed to these elements. Urinary concentration of Pt and Rh, though still low, could be of some concern in workers heavily exposed to urban car traffic.

Keywords Platinum · Rhodium · Palladium · Urine · Occupational exposure

Introduction

Platinum (Pt), Rhodium (Rh) and Palladium (Pd) together with Iridium (Ir), Osmium and Ruthenium belong to the so-called platinum-group elements (PGEs). Although, they are among the rarest elements in the Earth's crust, their excellent catalytic properties have led to an increment in their use. In fact, they are used in modern automotive catalytic converters to reduce the emission of hazardous pollutants such as carbon monoxide, hydrocarbons and nitrogen oxides by converting them into carbon dioxide, water and nitrogen (Palacios et al. 1999).

Occupational exposure occurs primarily in the mining and processing of PGEs, mostly during refining and catalyst manufacturing and, to a lesser extent, in subjects heavily exposed to automotive traffic (Palacios et al. 1999).

Automobile catalytic converters containing PGEs were introduced in the USA in the late 1970s, and in Europe at the beginning of the 1980s. In Italy, since 1st January 1993, new cars must be equipped with catalytic converters to comply with European Directive 91/441/EEC. In Rome, the capital and the largest city of Italy, in order to reduce atmospheric pollution, the Municipality has established two traffic zones: one inside the urban 'railway ring', and the other, called 'green zone', delimited by the city ring road. Since January 2002, cars not equipped with catalytic converters are not allowed to circulate in the former area on working days, and are also banned from the 'green zone' on days when atmospheric pollution is higher than established limits. Public transport and emergency services are exempt from these regulations.

In Rome there are six tram lines with 159 tramcars. Some lines converge on the city centre from the suburbs, while others cross the city diagonally. All of these lines run through the 'green zone' and most are within the urban 'railway ring'.

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Exposure to Pt, Rh and Pd may cause acute toxicity or hypersensitivity with respiratory symptoms, urticaria and, less frequently, contact dermatitis (Begerow and Dunemann 1999; Santucci et al. 2000; Cristaudo et al. 2005). Knowledge about adverse health effects due to low-level exposure to these metals is still lacking. PGE determination in human body fluids is thus of considerable importance for individual exposure assessment which is the basis for the studies on possible health effects and risk assessment (Begerow and Dunemann 1999).

Due to the extremely low PGE concentrations (ng/l and sub-ng/l levels) generally detectable in human body fluids, only a few data are available regarding the internal PGE background exposure of humans. In biological monitoring, the choice of the medium depends on kinetic factors, convenience of sample collection and the risk of sample contamination. Urine collection is easier, less invasive and more readily accepted by workers (Bolt and Thier 2006).

To the authors' knowledge, few biomonitoring studies on subjects heavily exposed to traffic PGEs have been performed so far (Begerow and Dunemann 1999; Schierl 2000); in Italy, data are available only for traffic police officers (Iavicoli et al. 2004). The aim of this study was to assess exposure to airborne Pt, Rh and Pd of a group of 64 tram drivers of Rome through the quantification of these elements in the workers' urine by sector field inductively coupled plasma mass spectrometry (SF-ICP-MS).

Materials and methods

Subjects

The study group consisted of 122 healthy male subjects (age 45.3 ± 9.9 years, mean \pm SD; range 23–64 years): 64 municipal tram drivers [mean age \pm Standard Deviation (SD), 47.9 ± 8.1 years, range 29–59 years] who operate trams along metal tracks laid on specific routes within

Rome (Fig. 1), and 58 control subjects aged 42.3 ± 10.8 years (range 23–64 years). This study sampled 16.5% of the total number of 389 tram drivers working in Rome. All eligible subjects were interviewed and a questionnaire was filled in. Personal data included gender, age, weight, height, dental alloys and, more comprehensively, any kind of prosthesis, information on general health state, use of pharmaceuticals, dietary habits, consumption of alcohol and coffee, smoking habits, physical exercise and duration of employment as tram driver. None of the subjects in the control subgroup was occupationally exposed to Pt, Rh or Pd, and none had been engaged in activities that could have involved non-occupational exposure to these metals or their compounds. All the subjects, controls and tram workers, lived within the 'green zone'. The difference in exposure between the two groups was, therefore, that the tram drivers spent their work-shift on the tram, while the controls, which were employed in the same society, spent their work time in the offices.

The study was performed during October 2005. All subjects gave their informed consent after the objectives of the study had been illustrated.

Sample collection and treatment

Urine samples of tram drivers were collected in 50 ml polyethylene vials (Kartell, Milan, Italy) at the end of the 6-h work shift. The work shifts were four and were comprised between 4.30 a.m. and 1.30 a.m. After overnight decontamination with 10% HNO₃ (Suprapur grade, Merck, Darmstadt, Germany), the vials were rinsed several times with high purity de-ionized water (Idrolab-a-System, Idron, Rome, Italy). Samples were frozen within 2 h of collection and stored at -20°C until analysis. To preserve the integrity of the urine samples and to minimize contamination or loss of analytes, subsequent manipulation of the specimens was performed in a Class-100 clean room (Tamco, Rome, Italy). After thawing at room temperature and shaking, 1 ml

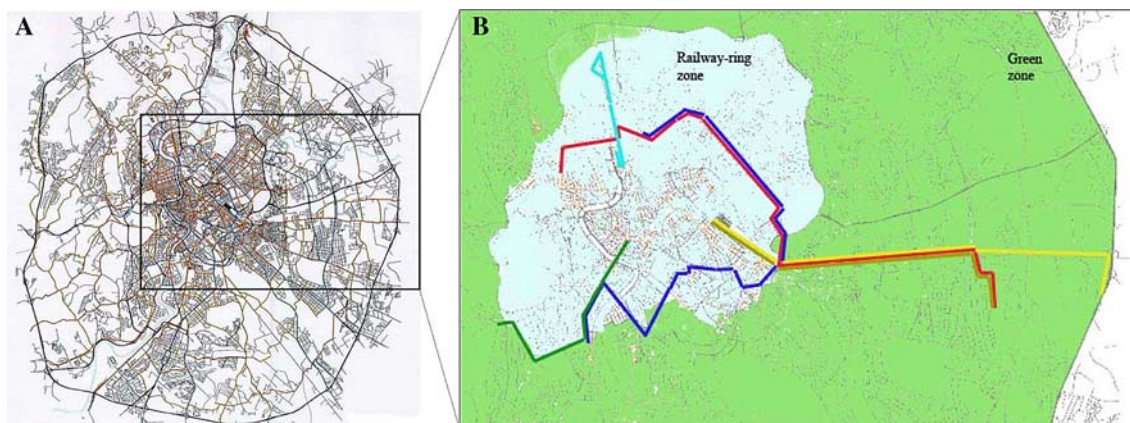


Fig. 1 a Map of the city of Rome with the "green zone" border, b the tram routes in the railway ring and in the green zone

aliquots of urine were sub-sample, acidified by addition of 20 μl of HNO_3 and diluted up to 5 ml with high purity deionized water.

Analysis

Urinary PGE values were normalized to creatinine. Creatinine concentration was determined using the Jaffe method (Jaffe 1886). Quantification of PGEs was performed by means of SF-ICP-MS (see Table 1 for instrumental characteristics and settings).

The standard addition calibration approach on pooled urine was adopted in order to account for matrix-induced effects. Moreover, to correct possible instrumental drifts of the signal, a control sample (the lowest point of calibration curve) was repeatedly measured during the analytical sequence. The isotopes ^{106}Pd , ^{195}Pt and ^{103}Rh were selected for quantifications, partially modifying the method recently developed (Bocca et al. 2004). In particular, the Low Resolution (LR) setting ($m/\Delta m = 300$) was used for Pt, whilst the Medium Resolution (MR) one ($m/\Delta m = 4,000$) was adopted to reduce the number of interfering species that can affect Pd and Rh analysis. Interference contributions were calculated by adding increasing amounts of each potentially interfering element (Cd, Mo, Pb, Rb, Sr, Y and Zr) to the samples in order to exceed the range of concentration expected for each of them in urine.

Statistical evaluation

Statistical analysis was carried out using the Windows version of the statistical package SPSS - 12.0.1 (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov U test (Chakravarti et al. 1967) was performed to check the normal distribution of observations, while the Mann–Whitney test (Mann and Whitney 1947) was used to assess the significance of differences in the median values for each element determined. The Grubbs' test (Grubbs 1969) provided the criteria for the exclusion of outlier's data.

Table 1 Operative and instrumental conditions for the determination of PGEs in urine

Spectrometer	ELEMENT-2 (Thermo Fisher, Bremen, Germany)
Geometry	Double focusing reverse Nier–Johnson
Resolution ($m/\Delta m$)	Low, 300; medium, 4,000
Interface	Sampler and skimmer cones in Ni
Data acquisition	Electric scan; 5 runs; 5 passes
RF power (kW)	1.2–1.3
Argon flows (l/min)	Plasma: 14; auxiliary: 1.0; sample: 1.1
Analytical masses (amu)	^{106}Pd , ^{195}Pt , ^{103}Rh , ^{115}In (as Internal Standard)
Interfering masses (amu)	$^{106}\text{Pd}^+$: $^{40}\text{Ar}^{66}\text{Zn}^+$, $^{90}\text{Zr}^{16}\text{O}^+$, $^{88}\text{Sr}^{18}\text{O}^+$, $^{89}\text{Y}^{17}\text{O}^+$ and $^{106}\text{Cd}^+$ $^{195}\text{Pt}^+$: $^{179}\text{Hf}^{16}\text{O}^+$ $^{103}\text{Rh}^+$: $^{206}\text{Pb}^{2+}$, $^{87}\text{Rb}^{16}\text{O}^+$, $^{87}\text{Sr}^{16}\text{O}^+$, $^{40}\text{Ar}^{63}\text{Cu}^+$

Results

Analytical performances

As regards ^{106}Pd quantification, the major mass interferences were the double ions $^{40}\text{Ar}^{66}\text{Zn}^+$, $^{88}\text{Sr}^{18}\text{O}^+$, $^{90}\text{Zr}^{16}\text{O}^+$, $^{89}\text{Y}^{17}\text{O}^+$ and the one isobaric ion $^{106}\text{Cd}^+$. Operating in the MR mode, the interference arising from ArZn^+ was physically separated. The SrO^+ , ZrO^+ and YO^+ contributions were of no actual consequence thanks to the daily instrumental optimization to reduce oxide formation (the BaO^+/Ba^+ ratio constantly remained at <0.001) and because of the natural low abundance of isotope ^{18}O and the low content of Zr (10 ng/l) and Y (<5 ng/l) in urine. On the other hand, the contribution of the $^{106}\text{Cd}^+$ isotope could not be neglected and had to be taken into account by monitoring the signal of $^{111}\text{Cd}^+$, corrected in its turn for the influence of $^{95}\text{Mo}^{16}\text{O}^+$. The species $^{40}\text{Ar}^{63}\text{Cu}^+$, $^{87}\text{Rb}^{16}\text{O}^+$, $^{87}\text{Sr}^{16}\text{O}^+$ and $^{206}\text{Pb}^{2+}$ have been considered as potential interferents on mass 103. The $^{40}\text{Ar}^{63}\text{Cu}^+$ interference was circumvented by working in the MR mode, while the interference caused by RbO^+ and $^{87}\text{Sr}^{16}\text{O}^+$ was found to be negligible as a result of optimization of oxide formation. The Pb^{++} signal on mass 103 was also completely separated by the MR setting. Finally, the influence of $^{179}\text{Hf}^{16}\text{O}^+$ at the Pt mass 195 turned out to be unimportant given the scarce amount of Hf (<5 ng/l) in the matrix.

The limits of detection of the method were as follows: Pt, 0.05 ng/l; Pd and Rh, 0.2 ng/l. To check for the accuracy of the method, recovery tests were performed adding a known concentration of PGEs to pooled urine. Recoveries ranged from 99.1 to 103.5% for all the elements under study.

Biomonitoring data

The main characteristics of the population groups enrolled in the study are illustrated in Table 2. The percentage of the workers aged >45 years was slightly higher than that of the controls, i.e., 61% compared to 38%. Most of the workers (about 80%) have been employed as tram drivers for more

Table 2 Characteristics of the study subjects

		Controls	Tram drivers
Age (years)	≤45	36	25
	>45	22	39
Smoke	Yes	16	20
	No	42	44
Employment (years)	<10	–	13
	≥10	–	51
Residence	Urban (within the green zone)	55	63
	Suburban	3	1
Total		58	64

than 10 years, whilst the remaining 20% had an employment duration of 4.15 ± 2.0 years. The workers and the controls had an incidence of smoking habit of 31.2 and 27.6%, respectively. Moreover, almost all the subjects were living in the urban area of Rome.

Statistical calculations were carried out on a reduced data set, after the exclusion of urine samples with creatinine concentrations lower than 0.3 g/l and higher than 3 g/l (ACGIH 2006) and with PGE levels (expressed in ng/g creatinine) identified as outliers. As a consequence of these criteria, the entire data set was reduced by two subjects because of creatinine values out of the selected range, and by 17 PGEs concentrations because outliers. In general, except for Pt in the control group, poor approximation was found between urinary PGEs and normal distribution, at a confidence limit of $\alpha = 0.05$. Thus, statistical analyses were carried out using non-parametric tests. Table 3 reports the urinary concentrations of Pt, Rh and Pd, normalised to creatinine content (ng/g creatinine) in the control group and in drivers.

Simple descriptive statistics, such as minimum and maximum levels, 25th, 50th and 75th percentiles, mean, stan-

dard deviation, geometric mean and lower and upper 95% confidence limits of arithmetic and geometric mean have been reported. Figure 2 shows the dispersion of concentration data around the means for Pt (Fig. 2a), Rh (Fig. 2b) and Pd (Fig. 2c).

When the two population groups were compared using the Mann–Whitney *U* test (see Table 4), statistically significant differences in the urinary content of Pt and Rh were found, at *P* values of 0.04 and < 0.0001, respectively.

On the contrary, Pd levels did not appear to be affected by prolonged exposure to urban airborne particulate containing PGEs. When control subjects and drivers were subgrouped according to age, i.e., <45 years and >45 years, median Rh concentration values were constantly significantly lower in controls than in drivers in both age-groups. In fact, for subjects <45 years, the Rh content in controls ($n = 36$) was 11.03 ng/g creatinine, while it was found to be 13.50 ng/g creatinine ($P = 0.0013$) in drivers ($n = 25$). On the other hand, when subjects were grouped by age, no evidence was found even of a slight difference in Pt concentrations between controls and workers. Age was found not to affect Pd urinary levels.

Since almost all the tram drivers had an occupational age of over 10 years, this parameter could not be used as a grouping variable. Although, the presence of prostheses in the subjects was considered as a potential and confounding source of PGEs, the present study failed to ascertain effects on PGE content related to the occurrence of metallic parts in the body (dental crowns, bridges, amalgams or other prostheses). Their presence was therefore not used as an exclusion criterion.

Discussion and conclusions

The results of this investigation demonstrate that there is a significant difference in Pt levels between tram drivers and controls. The urinary Pt concentration range in tram drivers

Table 3 Descriptive statistics of PGE levels (ng/g creatinine) in urine of controls and tram drivers

	Pt		Rh		Pd	
	Controls ($n = 55$)	Drivers ($n = 62$)	Controls ($n = 55$)	Drivers ($n = 61$)	Controls ($n = 49$)	Drivers ($n = 55$)
Minimum	0.08	0.22	0.80	2.54	2.86	2.58
Maximum	16.39	27.61	40.83	48.81	29.05	89.71
25th percentiles	0.51	0.80	5.38	15.15	5.56	4.94
50th percentiles	1.03	1.22	11.18	19.16	11.79	8.75
75th percentiles	1.84	3.69	16.47	27.30	16.75	11.47
Mean	1.86	3.55	12.21	21.73	12.40	12.34
Standard deviation	2.68	5.42	8.84	10.73	7.42	14.81
Geometric mean (GM)	0.99	1.70	8.95	18.95	10.23	8.85
Geometric standard deviation	3.15	2.99	2.45	1.77	1.96	1.98

CI confidence limits

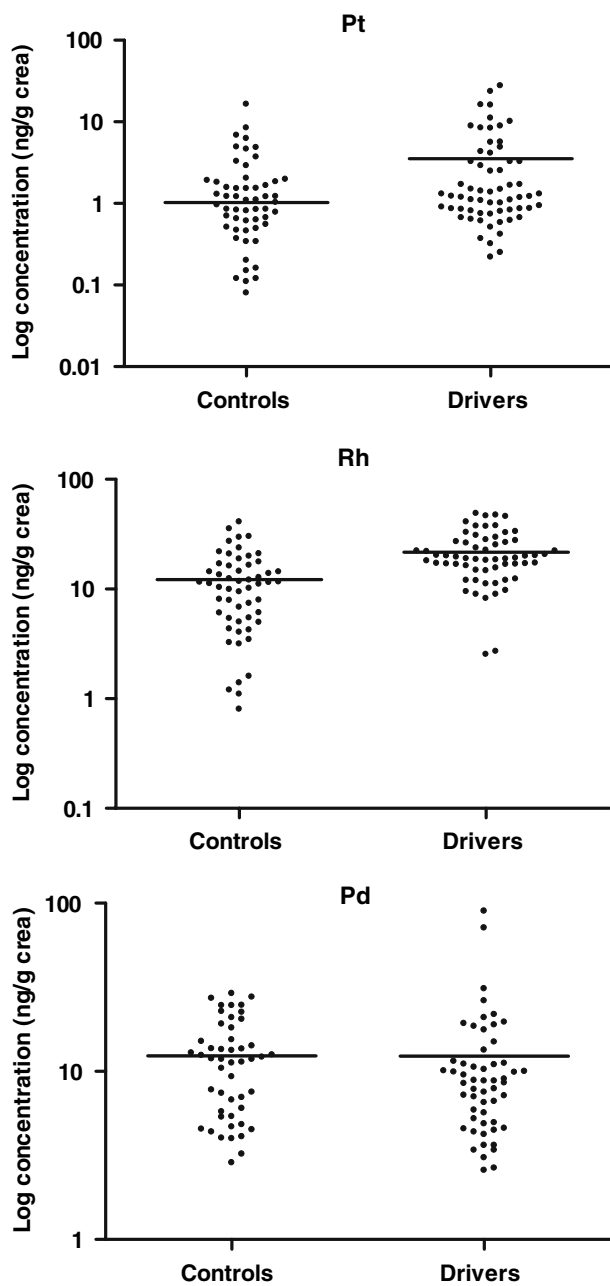


Fig. 2 Scatter plot of the urinary PGEs data around mean values (*horizontal lines*) in controls and tram drivers (**a** Pt, **b** Rh and **c** Pd)

Table 4 Differences in urinary PGE concentration of controls and tram drivers (Mann–Whitney test)

	Pt		Rh		Pd	
	Controls	Drivers	Controls	Drivers	Controls	Drivers
Median (ng/g creatinine)	1.03	1.22	11.18	19.16	11.79	8.75
Sum of ranks	2,875	4,028	2,299	4,488	2,640	2,820
<i>P</i> (two-tailed)	0.044		<0.0001		0.1	

(0.22–27.61 ng/g creatinine or, if not normalized to creatinine, 0.35–40.9 ng/l) was wider and shifted to higher values than those found in the relevant control group and those previously reported (Iavicoli et al. 2004) in police officers occupationally exposed to car traffic in Rome (0.28–13.7 ng/l). The present Pt range was found to show a tendency towards higher concentrations also in comparison with the range reported by Begerow and Dunemann (1999) for the urban population (0.3–2.2 ng/l) and for road workers (0.1–4.4 ng/l), whilst, in the studies conducted by Herr et al. (2003) and Becker et al. (2003) similar ranges of urinary Pt concentrations were reported in the general population (<0.9–65.5 ng/l and <0.1–185 ng/l, respectively). Furthermore, the Pt median value (1.22 ng/g creatinine) of tram drivers is in the same order of magnitude as other population groups exposed to traffic such as police officers (4.51 ng/l), bus drivers (2.8 ng/l) and taxi drivers (1.3 ng/l) (Schierl 2000; Iavicoli et al. 2004). On the other hand, the Pt mean level observed for tram workers (3.55 ng/g of creatinine, i.e., 4.78 ng/l) is much lower than that observed in a worker population exposed to Pt dust in a catalyst production plant of the same urban setting (1.86 µg/l) (Petrucci et al. 2005).

As regards Rh, levels differ significantly between tram workers and the control group. To the authors' knowledge, no previous study explored Rh urinary levels in a population occupationally exposed to PGEs emitted from automotive catalysers. Once more, urinary Rh concentration in tram drivers was lower than that found in industrial workers exposed to PGE dust (range 90–270 ng/l) (Petrucci et al. 2005). On the contrary, higher Rh levels were observed in the controls of the present study than in the control group used in that study (Petrucci et al. 2005), probably because the latter were recruited from rural areas.

Palladium concentrations were equal in the two population groups investigated, thus demonstrating that traffic did not change the background concentration of this metal. Ranges and GMs for Pd observed for drivers and controls in the present study were slightly lower than those given by Begerow et al. (1999) for road-construction workers (range 9.5–133.7 ng/l; GM, 52.2 ng/l), and the corresponding control group of school-leavers (range 13.1–48.3 ng/l; GM, 31.0 ng/l). The lower levels can be explained by the fact that external exposure is more limited in tram drivers than road construction workers, since working activity is performed exclusively inside the public transport vehicle. As for Pt and Rh, Pd levels (range 20–3,020 ng/l) in workers from the catalyst production plant (Petrucci et al. 2005) were higher than those obtained in the present study, while more similar data were found for the relevant control group (20 ± 10 ng/l).

In light of these findings, urinary Pt and Rh concentrations could be proposed as appropriate biomarkers for

occupational monitoring of population groups exposed to traffic-related Pt and Rh, as they allow basal and occupational levels to be easily distinguished. Urinary concentrations of Pt and Rh, though still low might well be of some future concern in workers heavily exposed to urban car traffic. While Pd in urine cannot be used as biomarker, as it does not seem to be affected by its actual levels in the fumes deriving from car catalytic systems.

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