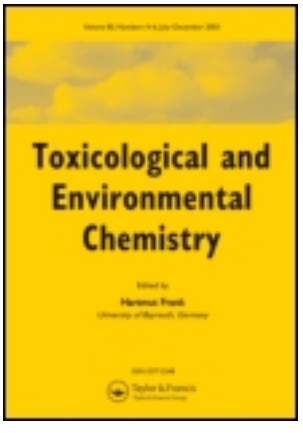

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Levels of p53 protein as biomarker in plasma of workers exposed to carcinogenic polycyclic aromatic hydrocarbons

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This study was conducted to evaluate the levels of the p53 protein as biomarker in plasma of workers of a carbon anode plant exposed to carcinogenic polycyclic aromatic hydrocarbons (cPAHs). The exposed population consisted of 42 workers in the plant and a matched control group consisting of 43 office workers. Personal air sampling was performed to assess workers' atmospheric exposure to cPAHs. Blood and urine samples were taken to determine the plasma levels of the p53 protein using an enzyme linked immunosorbent assay, and urinary 1-hydroxypyrene was determined by high performance liquid chromatography. Statistical analyses were performed. Occupational exposure to cPAHs in the exposed group ranged from 3.6 to 31.5 $\mu g\,m^{-3}$. The mean level of 1-hydroxypyrene in the exposed group was significantly higher compared to the control group (6.3 ± 4.9 and $0.5 \pm 0.5\,\mu mol\,mol^{-1}$ creatinine, respectively; $p < 0.001$). Mean plasma levels of the p53 protein in the exposed and control subjects were significantly different ($2.2 \pm 0.6\,U\,mL^{-1}$ and $1.7 \pm 0.5\,U\,mL^{-1}$, respectively; $p < 0.001$). A correlation between p53 protein plasma level and urinary 1-hydroxypyrene ($r = 0.44$, $p = 0.01$) was found. The p53 protein can serve as a biomarker of cPAHs exposure and effects; however, to evaluate the validity of this biomarker over time, long term follow up is required.

Keywords: p53 protein; carcinogenic polycyclic aromatic hydrocarbons; carbon anode plant; biomarker

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals consisting of a few hundred compounds with two or more fused benzene rings (Bosveld et al. 2002). PAHs can be generated naturally, for instance in forest fires and during volcanic eruptions, or by human activities such as combustion of fossil fuels for power generation, heating, by waste incineration, and from traffic (Simoneit 2002). Some of them are considered as human carcinogens (Teixeira et al. 2002). Epidemiological studies have demonstrated an

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association between PAHs exposure and increases in mortality and/or morbidity from respiratory diseases, cardiovascular diseases, and cancer (Taioli et al. 2007a).

Some occupational groups with PAHs exposure are coke oven workers, aluminium industry workers, roofers, iron and steel founding workers, chimney sweeps, asphalt road builders, and workers in various industries with combustion processes (Crebelli et al. 2002; Jongeneelen 2001; Petry, Schmid, and Schlatter 1996; Teixeira et al. 2002).

Carcinogenic PAHs (cPAHs) include, among others, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene, dibenz[ah]anthracene, and indeno[cd]pyrene (Taioli et al. 2007b; Topinka et al. 2009).

Atmospheric exposure to PAHs is usually monitored by personal air sampling. The internal exposure dose is usually assessed by detection of urinary 1-hydroxypyrene. Other biomarkers for exposure monitoring are PAH–DNA and PAH–protein adducts, and response markers include chromosomal aberrations, sister chromatid exchanges, and micronuclei (Jongeneelen 2001; Rossner, Binková, and Sram 2003; Siwinska, Mielzynska, and Kapka 2004; Teixeira et al. 2002).

P53 is a nuclear phosphoprotein which is considered as tumor suppressor protein and has a central role in cell cycle arrest, DNA repair, and apoptosis (Luch 2005). It can be inferred that overexpression of p53 protein is an indicator of critical events at cellular and molecular levels including those which may lead to cancer. Some studies show that over expression of p53 in tumor tissue is reflected in elevated levels of p53 protein in serum and urine (Darabi et al. 2006). To date, many studies have been dedicated to evaluate the usefulness of p53 as a marker in cancer patients as prognosis tool, for monitoring the treatment effectiveness, and for predicting recurrent tumors before they are clinically detectable (Balogh et al. 2006; Charuruks et al. 2001; Evans 2005; Luo et al. 1995; Ramet et al. 1995; Suwa, Ohshio, and Okada 1997).

As monitoring of p53 levels has been suggested as a useful marker in patients with certain types of cancer such as bladder, head, neck, esophagus, and colorectal cancers (Borska et al. 2009; Evans 2005; Shimada, Ochiai, and Nomura 2003), it is relevant to evaluate its applicability as a biomarker of effects of exposure to carcinogenic compounds.

To date, few studies have addressed the value of p53 protein as a marker of genotoxicity in workers who were exposed to cPAHs, with contradictory in results highlighting the need for more research in this area. For example, Rossner, Binková, and Sram (2003) carried out a study to evaluate the influence of occupational exposure to PAHs on the plasma levels of p53 protein. They found a negative correlation between the levels of p53 protein and occupational exposure to carcinogenic PAHs. These results did not support the expected response. Rossner et al. (2007) conducted another study to evaluate the effects of environmental exposure to carcinogenic PAHs on plasma levels of p53. Their study showed levels of cPAHs exposure ranging from 3.1 to 263 $ng\,m^{-3}$. Compared to their previous work, they concluded when exposed to low concentrations of cPAHs, the plasma level of p53 protein can serve as good biomarker of effect; however, at higher levels of exposure to cPAHs such as those of coke oven workers, a decrease in p53 levels will be observed. In a coke oven installation, Pan et al. (1998) showed higher levels of p53 protein in coke oven workers compared to the control group; no correlation was found between serum p53 protein and PAHs exposure, smoking or DNA adducts. These contradictions emphasize the need for further studies in different industries and various populations to reaching a consensus on the value of p53 protein as a biomarker in cPAHs exposed workers.

Since previous studies have indicated high levels of PAHs exposure in anode plant workers (Petry, Schmid, and Schlatter 1996) and as no study have been carried out to

evaluate the p53 plasma levels in Iranian cPAHs exposed workers, this study was conducted to evaluate the p53 plasma levels as an effect biomarker of cPAHs exposure of workers employed at the carbon anode plant of an aluminium factory in Iran.

Material and methods

Chemicals

1-Hydroxypyrene was purchased from Sigma-Aldrich (St. Louis, MO, USA); indeno[1,2,3 cd] pyrene from Supelco (Bellefonte, PA, USA); all other c-PAHs were from Dr Ehrenstorfer GmbH (Augsburg, Germany); ß-glucoronidase-arylsulphatase from Roche (Basel, Switzerland); acetonitrile, benzene, cyclohexane, methylene chloride, and toluene from Merck (Darmstadt, Germany), C18 cartridges (1 mL in volume) from Macherey-Nagel (Düren, Germany); PTFE filters and washed XAD-2 sorbent tubes from SKC (Eighty Four, PA, USA).

Experimental

The exposed population consisted of 42 male workers of a carbon anode plant in an aluminum producing factory, and the control group consisted of 43 volunteered office workers. The control group was matched with the exposed group according to age, body mass index, sex, and smoking habit. The inclusion criteria were that the subjects were not having medical treatment, radiography, or vaccination up to 3 months before sampling. Each participant completed a questionnaire on personal information and lifestyle. All participants signed an informed consent form and had the option to exit from the study at any time during the study. The study was approved by the ethical committee of Tehran University of Medical Sciences.

Blood and urine sample collection

Blood samples were taken immediately after the end of the working shift on the last working day by venipuncture into vacuettes containing ethylene diaminetetracetate (Weihai Hongyu Medical Devices Co., Weihai, China). The samples were coded and transferred to the laboratory, processed within 1 h, and kept in aliquots of 1.5 mL at -80°C until further analysis. Urine samples were collected after the workshift on the last working day and stored at -20°C until analysis.

Personal exposure monitoring

Air sampling and analysis was performed to assess the workers' atmospheric exposure to cPAHs according to NIOSH5515 (NIOSH 1994). Air samples were taken at a flow rate of $2\,\mathrm{L\,min^{-1}}$ using a personal air sampling pump, PTFE filters (2 µm pore size, 37 mm diameter), and washed XAD-2 sorbent tubes. Analysis of the air samples was performed using an Agilent model 7890A gas chromatograph (Agilent Technologies, Folsom, CA, USA) equipped with a capillary column (DB 5, 30 m × 0.32 mm, fused silica, film thickness 1 µm, Agilent Technologies, Folsom, CA, USA), split/splitless injector, and flame-ionization detector.

Enzyme linked immunosorbent assay assay

Plasma levels of p53 protein were measured by an enzyme-linked immunosorbent assay (ELISA) using a sandwich ELISA kit (eBioscience, San Diego, USA). According to the information in the ELISA kit manual, no interference of circulating factors of the immune system at physiologically relevant concentrations is expected.

The assay was performed according to the manufacturer's instructions. In brief, anti-human p53 precoated microwell strips were washed twice with wash buffer. Sample diluent, 100 μL each, was added to blank wells in duplicate. Sample diluent, 50 μL each, was added to the samples and the standard wells. Samples and standards, 50 μL each in duplicate, were added to the designated samples and the standard wells. Biotin-conjugate, 50 μL, was added to all wells and then microwell strips were covered and incubated for 2 h at room temperature. After incubation, the microwell strips were emptied and washed three times with wash buffer, and volumes of 100 μL diluted streptavidin-horseradish peroxidase were added to all wells. Then, the microwell strips were covered and, after 1 h of incubation at room temperature, they were washed three times with wash buffer. Then, 100 μL of tetramethyl-benzidine substrate solution at a concentration of $0.4\,g\,L^{-1}$ was added to all wells and incubated for 10 min at room temperature. Finally, 100 μL stop solution was added to each well and the absorbance was measured at 450 and 620 nm using a Biochrom Anthos 2020 microplate reader (Biochrom Ltd., Cambridge, UK).

To evaluate reproducibility, the overall interassay coefficient of variation of independent experiments was determined.

1-Hydroxypyrene analysis

Urinary 1-hydroxypyrene was determined according to Jongeneelen et al. (1985). According to this method, 10 mL urine was adjusted to pH 5 using $1\,mol\,L^{-1}$ HCl. Then, $0.1\,mol\,L^{-1}$ acetate buffer (pH = 5) was added to make a final volume of 30 mL. This solution was incubated overnight (16 h) with 15 μL glucoronidase-arylsulphatase solution in a shaking bath at 37°C. A C_{18} reversed-phase cartridge was used for the extraction of the metabolites. The cartridge was activated with 5 mL methanol followed by 10 mL distilled water. The urine samples were passed through the cartridges at a flow rate of $10\,mL\,min^{-1}$. The cartridges were washed with 3 mL distilled water followed by 3 mL 50% methanol in water. Thereafter, 1-hydroxypyrene was eluted with 8 mL methanol. The solvent was completely evaporated, and the dry sample was reconstituted with 1 mL methanol. The concentration of 1-hydroxypyrene was determined according to Jongeneelen et al. (1985) using an Agilent series 1200 high performance liquid chromatograph (Agilent Technologies, Folsom, CA, USA) equipped with a C_{18} reversed phase column (150 × 4.6 mm; Agilent Technologies, CA, USA) and a fluorescence detector (Agilent Technologies, Waldbronn, Germany) at an excitation wavelength of 242 nm and an emission wavelength of 388 nm. Urinary 1-hydroxypyrene concentrations were calculated relative to excreted urinary creatinine ($\mu mol\,mol^{-1}$ creatinine).

Determination of urinary cotinine

For determination of urinary cotinine levels, a commercially available direct ELISA kit (Abnova, Jhongli, Taiwan) was used according to the manufacturer's instructions.

Table 1. General data on the study groups.

Factors	Exposed workers	Controls
Number of subjects	42	43
Age* (years)	30.4 ± 4.5	32.5 ± 5.7
Number of smokers	12	12
Number of non smokers	30	31

Note: *Mean $\pm$ SD.

Statistical analysis

Statistical analysis was performed using SPSS ver. 16 software. The Kolmogorov–Smirnov *t*-test was used to evaluate the normality of data. An independent samples *t*-test was used for comparison of two groups and the Pearson correlation test was used to evaluate the relationship between variables.

Results and discussion

The general characteristics of the studied population are shown in Table 1. Subjects with urinary cotinine levels of more than $500\,\mathrm{ng\,mg^{-1}}$ creatinine have been considered as smokers (Taioli et al. 2007a).

Personal exposure monitoring

The mean concentration of cPAHs in the air of the exposed group was $11.4 \pm 7.6\,\mu\mathrm{g\,m^{-3}}$, ranging from 3.6 to $31.5\,\mu\mathrm{g\,m^{-3}}$. The mean level of occupational exposure to benzo[a]pyrene in this group was $1.4\,\mu\mathrm{g\,m^{-3}}$, ranging from 0.5 to $5.2\,\mu\mathrm{g\,m^{-3}}$ (Table 2). The mean level of urinary 1-hydroxypyrene in the exposed and the control group was $6.3 \pm 4.9\,\mu\mathrm{mol\,mol^{-1}}$ creatinine and $0.5 \pm 0.5\,\mu\mathrm{mol\,mol^{-1}}$ creatinine, respectively (Table 3). The level of 1-hydroxypyrene in the exposed group was significantly higher than the control group ($p < 0.001$). In addition, there was a significant difference in urinary 1-hydroxypyrene between smokers and non-smokers in the both exposed ($p < 0.001$) and control ($p = 0.027$) groups.

Statistical analysis of atmospheric exposure to cPAHs and excretion of 1-hydroxypyrene in the urine using Pearson correlation test showed that there is a significant correlation between total cPAHs exposure and urinary 1-hydroxypyrene ($r = 0.79$, $p < 0.001$). In addition, a positive and significant correlation was found between benzo[a]pyrene exposure and urinary 1-hydroxypyrene ($r = 0.69$, $p < 0.001$).

In a study in a carbon anode plant, Petry, Schmid, and Schlatter (1996) reported that personal exposure to PAHs and benzo[a]pyrene varies from 4 to $120\,\mu\mathrm{g\,m^{-3}}$ and from 0.2 to $4.9\,\mu\mathrm{g\,m^{-3}}$, respectively. They also reported the level of urinary 1-hydroypyrene in the range of 0.5 to $62\,\mu\mathrm{mol\,mol^{-1}}$ creatinine. These findings are comparable to our results. For example, Rossner, Binková, and Sram (2003) also reported the personal exposure to cPAHs and benzo[a]pyrene in coke oven workers to be in the range of 0.6–$547\,\mu\mathrm{g\,m^{-3}}$ and 0.002–$62.1\,\mu\mathrm{g\,m^{-3}}$, respectively. These personal exposure levels are much higher than our findings in the carbon anode plant workers.

Table 2. Mean levels of personal exposure to cPAHs in the exposed group.

cPAHs	Exposure level* ($\mu g\,m^{-3}$)
benz[a]anthracene	2.6 ± 1.7
chrysene	2.7 ± 1.7
benzo[b]fluoranthene	1.3 ± 1.1
benzo[k]fluoranthene	1.1 ± 0.9
benzo[a]pyrene	1.4 ± 1.1
benzo[ghi]perylene	1.1 ± 0.8
dibenz[ah]anthracene	0.4 ± 0.3
indeno[cd]pyrene	1.1 ± 0.8
Total cPAHs	11.4 ± 7.6

Note: *Mean ± SD.

Table 3. Mean urinary 1-hydroxypyrene levels* ($\mu mol\,mol^{-1}$ creatinine) in the exposed ($n = 42$) and control ($n = 43$) groups.

Grouping	Smoking status		*p*-value	Smokers + Non-smokers	*p*-value
Exposed group	Smokers	2.9 ± 1.7	<0.001	6.3 ± 4.9	<0.001
	Non-smokers	7.7 ± 5.1			
Control group	Smokers	0.8 ± 0.6	0.027	0.5 ± 0.5	
	Non-smokers	0.4 ± 0.4			

Note: *Mean ± SD.

Plasma p53 protein level

The interassay coefficient of variation of the used ELISA was 7.8%. The data on the mean plasma levels of p53 protein are presented in Table 4, in the exposed subjects being $2.2 \pm 0.6\,U\,mL^{-1}$ and $1.7 \pm 0.5\,U\,mL^{-1}$ in the control subjects; the difference was statistically significant ($p < 0.001$). Compared to non-smokers, smokers in the control group have higher p53 protein levels, although statistically insignificant.

There is a direct and positive correlation between p53 protein plasma level and urinary 1-hydroxypyrene ($r = 0.44$) which is statistically significant at 0.01 level.

Statistical analysis of environmental exposure to cPAHs in the exposed subjects and plasma level of p53 protein using Pearson correlation test showed that there is a positive but statistically insignificant ($r = 0.24$, $p = 0.12$) correlation between total cPAHs exposure and plasma level of p53 protein. The correlation between benzo[a]pyrene exposure and plasma level of p53 protein was also insignificant ($r = 0.11$, $p = 0.45$).

The mean level of environmental exposure to cPAHs in the exposed subjects is $11.4\,\mu g\,m^{-3}$. According to previous studies, this exposure level is high enough to have a significant increase in urinary 1-hydroxypyrene level (Jongeneelen 2001) and our results showed a significant increase in urinary 1-hydroxypyrene level of exposed subjects in comparison to controls. It can be inferred from Jongeneelen (2001) studies that, when a urinary1-hydroxypyrene level of more than $1.4\,\mu mol\,mol^{-1}$ creatinine is measured, genotoxic effects should be observed. In our study, the mean level of urinary 1-hydroxypyrene in the exposed group was found to be $6.3\,\mu mol\,mol^{-1}$ creatinine and in

Table 4. Mean plasma levels of p53 protein* (U mL) in the exposed and control groups.

Grouping	Smoking status		*p*-value	Smokers + Non-smokers	*p*-value
Exposed group	Smokers	2.2 ± 0.6	0.77	2.2 ± 0.6	<0.001
	Non-smokers	2.2 ± 0.6			
Control group	Smokers	1.8 ± 0.4	0.32	1.7 ± 0.5	
	Non-smokers	1.6 ± 0.5			

Note: *Mean ± SD.

the control group it was $0.54\,\mu mol\,mol^{-1}$ creatinine. Hence, it can be concluded that our exposed and control groups could be used to evaluate the genotoxic effects from cPAHs exposure.

In our study, plasma level of p53 protein was evaluated as a hypothesized biomarker of effect as a response to genotoxic effects from cPAHs exposure. Results of this study showed statistically significant higher plasma levels of p53 protein in cPAHs exposed workers ($2.2 \pm 0.6\,U\,mL^{-1}$ in exposed group against $1.7 \pm 0.5\,U\,mL^{-1}$ in control group). On the other hand, a significant correlation was found between plasma levels of p53 and urinary 1-hydroxypyrene which is an accepted biomarker of exposure to PAHs (Hansen et al. 2008; Lee and Byeon 2010; Strickland and Kang 1999; Tsai et al. 2002; Zhang et al. 2001). Previous studies have suggested that, accumulation of p53 protein can occur in response to signals arising from exposure to PAHs (Binkova and Sram 2004; Topinka et al. 2008). Hence, elevated plasma level of p53 protein in the exposed group is attributable to cPAHs exposure and p53 plasma level can be suggested as a biomarker of effect in the cPAHs exposed studied population. This conclusion is in agreement with the result of the studies which claim a positive relationship between exposure to genotoxic agents and expression level of p53 protein (Gao et al. 2011; Indulski, Lutz, and Krajewska 1999). In contrast to our findings, Rossner et al. reported lower levels of p53 protein in subjects with cPAHs exposure concentrations above $1\,\mu g\,m^{-3}$. As all participants in their study population had work experience of more than 10 years, they suggested long-term exposure to cPAHs as a probable factor which might have depressed a defense system resulting in lower levels of p53 protein (Rossner, Binková, and Sram 2003). However in our study all subjects had less than 4 years experience on the job which can be the reason for difference between our and Rossner, Binková, and Sram (2003) study results. This speculation is strengthened by considering the study by Borska et al. (2009) which showed a significant increase in plasma levels of p53 in subjects with high cPAHs exposure during a short period of time. They reported that a significant increase in plasma levels of p53 will occur as a result of skin exposure to cPAHs in psoriatic patients who applied coal tar to their skin for 8–30 days as a treatment. It is notable that, among coke oven workers, psoriatic patients, chimney sweep, and anode aluminum workers the highest PAH exposure levels are indicated to occur in psoriatic patients (Pavanello et al. 1999). Decreased level of p53 protein in prolonged exposures can be due to the PAHs accumulation inside the body cells which may result in cytotoxicity or saturation of metabolic activation enzymes (Binkova et al. 2000; Topinka et al. 2008). It has been revealed that, exposure to complex PAHs mixtures may induce synergistic or antagonistic effects on the genotoxic properties of PAHs and it may affect the expression level of p53 protein. Therefore, another factor which may affect the PAHs induced expression of p53 protein is the concentration of individual PAHs (Tarantini et al. 2011). Studies have shown

that, some low molecular weight PAHs like fluoranthene and benzo[c]phenanthrene inhibit enzymes which are responsible for metabolic activation of PAHs thereby decreasing cPAHs genotoxicity potential and p53 protein expression (Shimada and Guengerich 2006; Topinka et al. 2008). Therefore, it could be expected that in cPAH contaminated settings with high concentration of low molecular weight PAHs there is no increase in p53 protein expression level. It could also be another explanation for the cases showing no increase in p53 protein levels despite the high cPAHs exposure.

Conclusion

Results obtained from this study showed a significant difference between p53 plasma levels of cPAHs exposed aluminum workers and controls. In addition, a positive and significant correlation was found between the cPAHs internal exposure (as indicated by urinary 1-hydroxypyrene) and p53 plasma levels. Hence, it can be concluded that plasma level of p53 protein can serve as a biomarker of effect in the studied population. Nevertheless, to generalize this conclusion to other high-level cPAHs exposure industries and populations, there is a need for long term follow up for evaluating the validity of this biomarker over time.

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References

Balogh, G.A., D.A. Mailo, M.M. Corte, P. Roncoroni, H. Nardi, E. Vincent, D. Martinez, et al. 2006. ''Mutant p53 Protein in Serum Could be Used as a Molecular Marker in Human Breast Cancer.'' *International Journal of Oncology* 28: 995–1002.

Binkova, B., and R.J. Sram. 2004. ''The Genotoxic Effect of Carcinogenic PAHs, their Artificial and Environmental Mixtures (EOM) on Human Diploid Lung Fibroblasts.'' *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 547: 109–21.

Binkova, B., Y. Giguère, P. Rossner Jr., D. Miroslav, and R.J. Sram. 2000. ''The Effect of Dibenzo[a,l]pyrene and Benzo[a]pyrene on Human Diploid Lung Fibroblasts: The Induction of DNA Adducts, Expression of p53 and p21WAF1 Proteins and Cell Cycle Distribution.'' *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 471 (1–2): 57–70.

Borska, L., C. Andrys, J. Krejsek, K. Hamakova, J. Kremlacek, D. Ranna, L. Kotingova, and Z. Fiala. 2009. ''Plasma Levels of p53 Protein and Chromosomal Aberrations in Patients with Psoriasis Treated with the Goeckerman Regimen.'' *Clinical and Experimental Dermatology* 34: 881–8.

Bosveld, A.T.C., P.A.F. de Bie, N.W. van den Brink, H. Jongepier, and A.V. Klomp. 2002. ''In Vitro EROD Induction Equivalency Factors for the 10 PAHs Generally Monitored in Risk Assessment Studies in The Netherlands.'' *Chemosphere* 49: 75–83.

Charuruks, N., P. Tangkijvanich, N. Voravud, R. Chatsantikul, A. Theamboonlers, and Y. Poovorawan. 2001. ''Clinical Significance of p53 Antigen and Anti-p53 Antibodies in the Sera of Hepatocellular Carcinoma Patients.'' *Journal of Gastroenterology* 36: 830–6.

Crebelli, R., P. Carta, C. Andreoli, G. Aru, G. Dobrowoln, S. Rossi, and A. Zijno. 2002. ''Biomonitoring of Primary Aluminium Industry Workers: Detection of Micronuclei and Repairable DNA Lesions by Alkaline SCGE.'' *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 516 (1–2): 63–70.

Darabi, M., N. Tayebi Meibodi, R. Mahdavi, and D. Arab. 2006. "p53 Protein in Serum and Urine Samples of Patients with Bladder Transitional Cell Carcinoma and its Overexpression in Tumoral Tissue." *Urology Journal* 4: 216–19.

Evans, C.P. 2005. "p53 Immunodetection in Urine Samples Successful and Predictive of Bladder Tumor Progression." *British Journal of Cancer* 93: 242–7.

Gao, M., Y. Li, Y. Sun, J. Long, Y. Kong, S. Yang, and Y. Wang. 2011. "A Common Carcinogen Benzo[a]pyrene Causes p53 Overexpression in Mouse Cervix via DNA Damage." *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 724 (1–2): 69–75.

Hansen, A.M., L. Mathiesen, M. Pedersen, and L.E. Knudsen. 2008. "Urinary 1-hydroxypyrene (1-HP) in Environmental and Occupational Studies – A Review." *International Journal of Hygiene and Environmental Health* 211 (5–6): 471–503.

Indulski, J.A., W. Lutz, and B.A. Krajewska. 1999. "Serum and Urine p53 Protein in Bladder Cancer Patients and in Workers Occupationally Exposed to Genotoxic and Mutagenic Dyes." *Central European Journal of Occupational and Environmental Medicine* 5 (1): 17–25.

Jongeneelen, F.J. 2001. "Benchmark Guideline for Urinary 1-hydroxypyrene as Biomarker of Occupational Exposure to Polycyclic Aromatic Hydrocarbons." *Annals of Occupational Hygiene* 45 (1): 3–13.

Jongeneelen, F.J., R.B. Anzion, C.M. Leijdekkers, R.P. Bos, and P.T. Henderson. 1985. "1-Hydroxypyrene in Human Urine After Exposure to Coal Tar and a Coal Tar Derived Product." *International Archives of Occupational and Environmental Health* 57 (1): 47–55.

Lee, K.H., and S.H. Byeon. 2010. "The Biological Monitoring of Urinary 1-hydroxypyrene by PAH Exposure Among Smokers." *International Journal of Environmental Research* 4 (3): 439–42.

Luch, A. 2005. *The Carcinogenic Effects of Polycyclic Aromatic Hydrocarbons*. London: Imperial College Press.

Luo, J.C., A.I. Neugut, G. Garbowski, K.A. Forde, M. Treat, S. Smith, W.P. Carney, and P.W. Brandt Rauf. 1995. "Levels of p53 Antigen in the Plasma of Patients with Adenomas and Carcinomas of the Colon." *Cancer Letters* 91: 235–40.

NIOSH 1994. *NIOSH Manual of Analytical Methods (NMAM): Polynuclear Aromatic Hydrocarbons by GC*. Washington, DC: US Department of Health and Human Services, National Institute for Occupational Safety and Health.

Pan, G., T. Hanaoka, Y. Yamano, K. Hara, M. Ichiba, Y. Wang, J. Zhang, et al. 1998. "A Study of Multiple Biomarkers in Coke Oven Workers – A Cross Sectional Study in China." *Carcinogenesis* 19 (11): 1963–8.

Pavanello, S., D. Favretto, F. Brugnone, G. Mastrangelo, G. Dal Pra, and E. Clonfero. 1999. "HPLC/fluorescence Determination of Anti-BPDE–DNA Adducts in Mononuclear White Blood Cells from PAH-exposed Humans." *Carcinogenesis* 20 (3): 431–5.

Petry, T., P. Schmid, and C. Schlatter. 1996. "Airborne Exposure to Polycyclic Aromatic Hydrocarbons (PAHs) and Urinary Excretion of 1-hydroxypyrene of Carbon Anode Plant Workers." *Annuals of Occupational Hygiene* 40 (3): 345–57.

Ramet, M., K. Castren, K. Jarvinen, K. Pekkala, T. Turpeenniemi-Hujanen, Y. Soini, P. Paakko, and K. Vahakangas. 1995. "p53 Protein Expression is Correlated with Benzo[a]pyrene-DNA Adducts in Carcinoma Cell Lines." *Carcinogenesis* 16: 2117–24.

Rossner Jr, P., B. Binková, I. Chvátalová, and R.J. Sram. 2002. "Acrylonitrile Exposure: The Effect on p53 and p21WAF1 Protein Levels in the Blood Plasma of Occupationally Exposed Workers and In Vitro in Human Diploid Lung Fibroblasts." *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 517 (1–2): 239–50.

Rossner Jr, P., B. Binková, A. Milcova, I. Solansky, J. Zidzik, K.D. Lyubomirova, P.B. Farmer, and R.J. Sram. 2007. "Air Pollution by Carcinogenic PAHs and Plasma Levels of p53 and p21WAF1 Proteins." *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 620 (1–2): 34–40.

Rossner Jr, P., B. Binková, and R.J. Sram. 2003. "The Influence of Occupational Exposure to PAHs on the Blood Plasma Levels of p53 and p21WAF1 Proteins." *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 535 (1): 87–94.

Shimada, T., and F.P. Guengerich. 2006. ''Inhibition of Human Cytochrome P450 1A1-, 1A2-, and 1B1-mediated Activation of Procarcinogens to Genotoxic Metabolites by Polycyclic Aromatic Hydrocarbons.'' *Chemical Research in Toxicology* 19: 288–94.

Shimada, H., T. Ochiai, and F. Nomura. 2003. ''Titration of Serum p53 Antibodies in 1085 Patients with Various Types of Malignant Tumors.'' *Cancer* 97 (3): 682–9.

Simoneit, B.R.T. 2002. ''Biomass Burnings – A Review of Organic Tracers for Smoke from Incomplete Combustion.'' *Applicable Geochemistry* 17: 129–62.

Siwinska, E., D. Mielzynska, and L. Kapka. 2004. ''Association Between Urinary 1-hydroxypyrene and Genotoxic Effects in Coke Oven Workers.'' *Occupational and Environmental Medicine* 61 (3): 1–7.

Strickland, P., and D. Kang. 1999. ''Urinary 1-hydroxypyrene and other PAH Metabolites as Biomarkers of Exposure to Environmental PAH in Air Particulate Matter.'' *Toxicology Letters* 108 (2–3): 191–9.

Suwa, H., G. Ohshio, and N. Okada. 1997. ''Clinical Significance of Serum p53 Antigen in Patients with Pancreatic Carcinomas.'' *International Journal of Gastroenterology & Hepatology* 40: 647–53.

Taioli, E., R.J. Sram, B. Binkova, I. Kalina, T.A. Popov, S. Garte, and P.B. Farmer. 2007a. ''Biomarkers of Exposure to Carcinogenic PAHs and their Relationship with Environmental Factors.'' *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 620 (1–2): 16–21.

Taioli, E., R.J. Sram, S. Garte, I. Kalina, T.A. Popov, and P.B. Farmer. 2007b. ''Effects of Polycyclic Aromatic Hydrocarbons (PAHs) in Environmental Pollution on Exogenous and Oxidative DNA Damage (EXPAH project): Description of the Population Under Study.'' *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 620 (1–2): 10–16.

Tarantini, A., A. Maitre, E. Lefèbvre, M. Marques, A. Rajhi, and T. Douki. 2011. ''Polycyclic Aromatic Hydrocarbons in Binary Mixtures Modulate the Efficiency of Benzo[a]pyrene to Form DNA Adducts in Human Cells.'' *Toxicology* 279 (1–3): 36–44.

Teixeira, J.P., J. Gaspar, G. Martinho, S. Silva, S. Rodrigues, O. Mayan, E. Martin, P.B. Farmer, and J. Rueff. 2002. ''Aromatic DNA Adduct Levels in Coke-oven Workers: Correlation with Polymorphism in Genes GSTP1, GSTM1, GSTT1 and CYP1A1.'' *Mutation Research* 517: 147–55.

Topinka, J., S. Marvanová, J. Vondráček, O. Sevastyanova, Z. Nováková, P. Krčmář, K. Pensicova, and M. Machala. 2008. ''DNA Adducts Formation and Induction of Apoptosis in Rat Liver Epithelial 'Stem-like' Cells Exposed to Carcinogenic Polycyclic Aromatic Hydrocarbons.'' *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 638 (1–2): 122–32.

Topinka, J., A. Milcova, H. Libalova, Z. Novakova, P. Rossner Jr., I. Balascak, and R.J. Sram. 2009. ''Biomarkers of Exposure to Tobacco Smoke and Environmental Pollutants in Mothers and their Transplacental Transfer to the Fetus. Part I: Bulky DNA Adducts.'' *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 669 (1–2): 13–19.

Tsai, P.J., H.Y. Shieh, W.J. Lee, H.L. Chen, and T.S. Shih. 2002. ''Urinary 1-hydroxypyrene as a Biomarker of Internal Dose of Polycyclic Aromatic Hydrocarbons in Carbon Black Workers.'' *Annuals of Occupational Hygiene* 46 (2): 229–35.

Zhang, J., M. Ichiba, K. Hara, S. Zhang, T. Hanaoka, G. Pan, Y. Yamano, K. Takahashi, and K. Tomokuni. 2001. ''Urinary 1-hydroxypyrene in Coke Oven Workers Relative to Exposure, Alcohol Consumption, and Metabolic Enzymes.'' *Occupational and Environmental Medicine* 58 (11): 716–21.