

Review

Monitoring Human Occupational and Environmental Exposures to Polycyclic Aromatic Compounds

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Many research groups have been carrying out studies to develop biomarkers of exposure to polycyclic aromatic compounds (PACs) and apply these for human biomonitoring. The main objectives of the use of biomarkers are to determine specific occupational and environmental exposures to monitor the effectiveness of exposure controls and prediction of the risk of disease. This article presents a review of the literature in the field of biomarkers of human exposure to PACs and an evaluation of the relevant biomarkers for monitoring exposure to PACs in a range of exposure situations from coke ovens to bitumen handling and environmental exposures. For this evaluation, the relationships between external PAC exposures and the corresponding biomarker levels have been studied. The literature data indicate that urinary excretion of 1-hydroxypyrene correlates well with external PAC exposure and this compound appears to be a suitable marker for internal exposure to PACs. DNA adducts, mostly measured in white blood cells, do not show satisfactory correlations with exposure to PACs in a variety of workplace and exposure situations. It is not clear which factors are mainly responsible for this poor correlation. Micronuclei and sister chromatid exchanges measured in peripheral white blood cells are also unsatisfactory as biomarkers for PAC exposure. From the relatively limited data available, chromosome aberrations appear to show considerable promise as indicators of exposure to PACs. Because of their strong association with cancer, chromosome aberrations are considered suitable indicators of increased cancer risk arising from exposure to PACs.

Keywords: environment; human exposure; monitoring; occupation; polycyclic aromatic compounds

INTRODUCTION

Polycyclic aromatic compounds (PACs) are classes of compounds found in crude oils, mineral oils, bitumens and tars (Gelboin and Ts'o, 1978; IARC, 1985, 1987). They are also formed during the incomplete combustion of fossil fuels and oil products. As a result, PACs are compounds that are widely distributed in the environment, principally through combustion processes and spillages of oil-derived materials that contain these compounds. They are highly lipophilic non-polar persistent substances and have been detected in aquatic environments and in many wild organisms. Many industrial processes, such as coke oven operations, aluminium smelting, asphalt paving and roofing operations, give rise to environ-

mental releases and occupational exposures to these compounds (reviewed in Brandt *et al.*, 1985; Herbert *et al.*, 1990; Hemminki *et al.*, 1990a,b, 1994; Dor *et al.*, 1999). Oil well fires, automotive exhaust gases, tobacco smoking and dietary intake also represent sources of human exposure (Darcey *et al.*, 1992; Hemminki *et al.*, 1994). The main concern about such human exposures is that many PACs have mutagenic and carcinogenic properties (Lijinsky, 1991). Although in occupational settings people are exposed to mixtures of PACs that also contain heteroaromatic compounds, for monitoring purposes mostly one, often benzo[*a*]pyrene (BaP), or a small subset of unsubstituted polycyclic aromatic hydrocarbons (PAH) are used to represent the whole set of PACs. The term PAH refers specifically to those PACs that consist only of hydrogen and carbon, unsubstituted and substituted by alkyl (mostly methyl) side chains.

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Analytical techniques for detecting PACs continue to increase in sensitivity, thus progressively lowering the detection limits for these compounds in the environment, animals and humans. Because of the very low levels detected, it is difficult to assess with certainty the possible increased cancer and health risks associated with these low level exposures. Until recently, the detection and identification of human carcinogens were based exclusively on the analysis of tumour incidences in human epidemiological studies. However, for exposures to PACs, it is only in instances of specific and high exposures that these methods have identified the causative agents, for example, coal tar pitch volatiles (CTPV). Because of the long latency for the onset of human cancer, the occurrence of mixed exposures and the low resolving power and retrospective nature of epidemiology, alternative methods are needed to make prospective risk assessments. A key factor in the risk assessment process is the determination of the dose. Biomonitoring offers an approach to determine an individual's received dose. This article reviews the principal methods that have been used to monitoring exposures to PACs and discusses how well the measurements correlate with the external PAC exposures and the potential for estimating cancer risk.

Exposures to PACs

Human exposure to foreign compounds (xenobiotics), such as PACs in bitumen fumes, can occur via several routes, e.g. inhalation, skin deposition/contamination or orally. In general, exposures are proportional to the concentration of the compound in the working environment. The environmental concentration can be measured by taking static samples of the ambient air. Methods for measuring skin deposition involve taking skin wipes or the determination of the deposition on a skin pad, and for inhalation taking breathing air samples with a personal sampler near the breathing zone. Because numerous different sets of PAHs have been used to report external PAC exposures, which are very difficult to compare, in this article we use, whenever possible, the sum of eight (carcinogenic) 4–6 ring PAHs (Σ 8PAHs: benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*ah*]anthracene, benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene) as a way of presenting PAC exposures. In addition, the often reported exposure to benzo[*a*]pyrene (BaP) is given where possible. Σ 8PAHs is only a subset of the group of potentially carcinogenic PACs (4–6 ring PAHs, their sulfur- and nitrogen-containing analogues and their alkylated, mostly methylated, homologues). Nevertheless, Σ 8PAHs can be used as a marker for the whole set because generally the concentrations of heterocyclic PACs show proportionality to the concentrations of the corresponding PAHs (Williams *et al.*, 1986;

Grimmer *et al.*, 1987; Heinrich *et al.*, 1994; Sivak *et al.*, 1997; Binet *et al.*, 2002).

The above mentioned measurements only give estimates of the external concentration that can potentially enter the body. In order to exert an effect, the compound has to enter the body, e.g. by passage through the skin or the lung tissue. Once in the body there are several possibilities for its detection. A first possibility is to look for the unchanged compound or its metabolites in urine, blood, faeces, exfoliated cells, sweat, nails, etc. For biomonitoring in the work environment, mainly urine and blood samples are used. The concentrations of the unchanged parent PACs in urine are generally so low that a concentration determination is seldom possible (Grimmer *et al.*, 1994). Mostly, the concentration of one or more metabolites is high enough to be determined (Jongeneelen *et al.*, 1988; Grimmer *et al.*, 1994). Both phenols, dihydrodiols and tetrahydrotretols can be found in this way. These compounds are called specific biomarkers of exposure, because they record that exposure to a particular compound has taken place. Sometimes the part of the molecule to which the metabolite is conjugated, e.g. thioethers (van Doorn and Henderson, 1979) or D-glucaric acid (Pasquini *et al.*, 1989), is looked for. These conjugate fragments provide evidence of exposure to any electrophilic compound that the worker has been exposed to and are therefore called aspecific biomarkers of exposure. The most important value of these measurements is to signal that exposure to an electrophilic compound has taken place, regardless of the route(s) of exposure.

Bioactivation of PACs

Many xenobiotics are subject to mammalian metabolism (biochemical alteration), which is divided into two distinct phases.

In phase I, the major reaction is usually hydroxylation, catalysed by members of a class of enzymes referred to as monooxygenases or cytochromes P-450 (CYP). Other types of reactions in phase I include reduction and hydrolysis. In phase II, the hydroxylated or other metabolites produced in phase I are converted by specific enzymes to various polar metabolites by conjugation (binding) to, for example, glucuronic acid, sulphate, glutathione or certain amino acids (Murray, 1990). The overall purpose of these two phases of metabolism of xenobiotics is to increase their water solubility (polarity) and thus to facilitate their excretion from the body. If this did not occur, very water-insoluble (hydrophobic) compounds, such as PACs or PCBs, would remain in the tissues for very long times.

In certain cases, phase I metabolism converts chemically unreactive compounds into chemically and biologically highly reactive species. For example, PACs can be oxidized to electrophilic compounds that can react with macromolecules such as DNA or

Table 1. Biomonitoring of PAHs in four highly exposed coke plant workers by measurement of urinary phenanthrene and pyrene metabolites

	Inhaled PAH ($\mu\text{g}/8\text{ h}$)	Excreted PAH		Excreted phenols ($\mu\text{g}/24\text{ h}$)	Excreted diols ($\mu\text{g}/24\text{ h}$)	Excreted phenols + diols		Excreted PAH % of phenols + diols
		$\mu\text{g}/24\text{ h}$	% of inhaled			$\mu\text{g}/24\text{ h urine}$	% of inhaled	
Phenanthrene	485	0.285	0.06	8.3	40.7	49	10	0.6
Pyrene	108	0.055	0.05	10.5	5.5	16	15	0.3
Benzo[a]pyrene	47	0.006	0.01		0.3	0.3	0.6	2.0

proteins to give adducts, before phase II conjugation can take place. If DNA adducts are not repaired prior to cell replication or are misrepaired, a permanent gene mutation can occur, which can lead to cancer development. Those compounds that can lead to DNA adduct formation are referred to as being genotoxic.

Biomarkers of exposure and of effect

Some biomarkers are indicative of the internal dose received, but do not persist in the body. These are, for example, hydroxylated metabolites in urine or faeces and those that indicate an effect in a specific target organ, e.g. DNA adducts or sister chromatid exchanges (SCE). Other biomarkers are indicative of irreversible effects, for example chromosomal aberrations (CA), micronuclei (MN) and mutations (van Delft *et al.*, 1998).

Hydroxylated metabolites

These are compounds that are formed in phase I metabolism and are excreted, mainly as conjugates, in urine. They can be analysed after hydrolysis of the conjugates. Currently, 1-hydroxypyrene (1OHPy) is the metabolite that is used most frequently. Other metabolites that are occasionally used are the hydroxylated metabolites of phenanthrene, benz[a]anthracene and BaP. The ratio of phenanthrene metabolites to 1OHPy might be a useful indicator of the relative contribution of smoking in the presence of other PAH sources (Jacob *et al.*, 1999). To monitor smoking itself, urinary cotinine is, however, a reliable and specific biomarker of exposure (Yang *et al.*, 2001). Linear correlations have been found between the total amount of PAHs inhaled during a working day and the amount of the main metabolite(s) for phenanthrene, pyrene and BaP excreted in the corresponding 24 h urine (Grimmer *et al.*, 1994). Correlations between atmospheric pyrene and urinary 1OHPy concentrations improved greatly ($r = 0.74$) if the amount of pyrene inhaled over the shift and the corresponding amount of urinary 1OHPy excreted were considered (Lafontaine *et al.*, 2000). The fraction of unchanged PAH excreted was found to be much lower than the amount inhaled (Grimmer *et al.*, 1994). For phenanthrene and pyrene the total amount of hydroxylated metabolites was between 10 and 15% of the amount inhaled, but for BaP this amount

was much lower (0.6%) (see Table 1). In a study where hydroxylated metabolites in urine were used to monitor personal exposures to phenanthrene, pyrene, benz[a]anthracene and BaP, a correlation was not observed between the external exposure to PAHs and the internal exposure (Gundel *et al.*, 2000).

1-Hydroxypyrene. Jongeneelen *et al.* (1987) introduced 1OHPy as a biomarker of internal dose arising from exposures to PACs. Subsequently Jongeneelen (2001) proposed three benchmark levels for measurements based on 1OHPy. These are as follows.

- A no observed effect level equivalent to a measurement of 1.4 μmol 1OHPy/mol creatinine. This is the level below which non-smokers showed no increased level of high frequency (HF)-SCEs (Buchet *et al.*, 1995).
- The lowest observed level of genotoxic effects indicated by 1.9 μmol 1OHPy/mol creatinine for coke oven workers and 3.8 $\mu\text{mol}/\text{mol}$ for aluminium plant workers.
- A level that equates to the present occupational exposure limits for PACs (0.2 mg/m^3 benzene soluble matter and/or 2 $\mu\text{g}/\text{m}^3$ BaP). The value used is dependent on industry type and pyrene content of the exposure and is equivalent to 2.3 μmol 1OHPy/mol for coke oven workers and 4.9 μmol 1OHPy/mol for aluminium industry workers.

Further to this, Jongeneelen defined the different benchmark levels for coke oven workers and workers in the aluminium industry on the basis of the occurrence of different PAC profiles in fumes from the two industries. The average PAC exposure profile in the aluminium industry has more of the lower molecular weight PACs, including pyrene, than that in coke oven emissions (Dr F. J. Jongeneelen, personal communication, 2001).

In actual human monitoring studies, half-lives for urinary 1OHPy elimination of 4–48 h have been reported (Buckley and Liroy, 1992; Boogaard and van Sittert, 1994, 1995; Jongeneelen *et al.*, 1988; Vu Duc and Lafontaine, 1999). In rodent studies, however, Jacob *et al.* (1989) showed that the elimination of 1OHPy is dependent upon the mode of administration. These factors suggest that when spot samples are taken, the time of sampling relative to the

exposure time is important. Grimmer's (Grimmer *et al.*, 1994) and Lafontaine's data (Lafontaine *et al.*, 2000) suggest that a sampling strategy designed to collect 24 h urine samples gives the best relation between external PAC dose and IOHPy excretion.

Urinary thioethers or D-glucuronic acid. These polar fragments, to which hydroxylated metabolites may be conjugated, are aspecific biomarkers that provide evidence of exposure to all electrophilic compounds, not only PACs. They have been reported in only a few studies on occupational exposure to PACs, possibly because these are very difficult to interpret due to confounding factors.

DNA adducts

PAC-DNA adducts arise from the reactions of reactive oxidation products of PACs with DNA in various target organs, such as skin, lungs and liver. During occupational monitoring of workers only blood and exfoliated cells (cells cast off from the tissue surface) are normally available as surrogate tissues. DNA adducts can be determined by various methods, amongst which the ^{32}P -post-labelling assay is most sensitive. Details of this method of analysis, which has found widespread use for PAC-DNA adduct determination, can be found in the Appendix (for a review see Beach and Gupta, 1992). Immunochemical methods for detecting DNA-carcinogen adducts are generally less sensitive than ^{32}P -post-labelling, but are simple and are relatively inexpensive (Appendix). Both polyclonal and monoclonal antibodies have been generated against a variety of PAH carcinogen adducts, although some show considerable cross-reactivity (Booth *et al.*, 1997). The most common procedures include enzyme-linked immunoabsorbent assay (ELISA), competitive radioimmunoassay (RIA) and ultra-sensitive enzymatic radioimmunoassay (USERIA). Details of the use of immunoassays and ^{32}P -post-labelling methods for detecting carcinogen-DNA adducts have been reviewed by Poirier (1991) and Poirier and Weston (1991). Because the immunochemical methods often show cross-reactivity for a range of PAHs and related compounds, the end points measured are generally described as 'PAH-DNA adducts'. In studies with ^{32}P -post-labelling, however, which measures a much wider range of adducts, these are normally simply referred to as 'aromatic DNA adducts'. The cross-reactivity of immunoassays reduces their value for the quantitative measurement of adducts, but nevertheless antibody based immuno-enrichment devices are useful in the pre-concentration of adducts (Booth *et al.*, 1997; Bartsch, 1996). The post-labelling methodology provides more reliable quantitative information when used with reference standards. Phillips *et al.* (2000) have reviewed the strengths, limitations and potential for inter-laboratory vari-

ation of the various assays for DNA adducts. Inter-laboratory variation in the results from the post-labelling assay can occur due to differences in reagents, enzymes and the particular protocol used.

The implications of DNA adduct formation and detection. The reactions of chemicals with DNA in cells can cause the formation of DNA adducts, which can give rise to mutation. Mutated cells can sometimes lead to cancer. The number of adducts formed depends on the dose of the chemical and its reactivity or the reactivity of its metabolite(s). The formation of DNA adducts is the earliest critical event that can be observed in the multistage process of carcinogenesis caused by chemicals such as PACs. Single doses of potent chemical carcinogens when tested in animal studies typically give rise to levels of adducts in the range 1 adduct/ 10^3 – 10^5 normal DNA bases (see Steiner *et al.*, 1992). This range corresponds to about 10000–1000000 modified bases in the DNA per cell. The number of critical changes in a single gene required to fully initiate a cell is not known with certainty, but is considered to be in the range three to six, and these can occur in any sequence (McCormick and Maher, 1994; Hanahan and Weinberg, 2000). A level of DNA adduct formation of ~ 1 adduct/ 10^5 DNA bases corresponds to about 10 000 DNA base modifications per cell, as each cell contains about 10^9 DNA bases. At this level of adduct formation there is thus a reasonable statistical probability that mutation will be initiated. A level of 1 adduct/ 10^9 DNA bases, the typical detection limit of ^{32}P -post-labelling, corresponds to only about one DNA base modification per cell. The statistical probability of this base modification occurring at critical sites in a single gene is clearly very low and therefore it is doubtful that this level of adduct formation has great significance in terms of cancer risk. Although it is doubtful that levels of DNA adducts below 1 adduct/ 10^8 DNA bases are significant, it is not possible to generalize (Mulholland *et al.*, 1997). Different adducts may have different mutagenic and carcinogenic responses. Based on the premise that several DNA lesions are needed for the formation of a malignant cell, the linearity of the dose-risk relationship can be explained by the finding that it is unlikely that more than one mutation is induced by a specific agent, at the low dose rates that occur in human exposure environments. Therefore, the qualitative detection of low levels of adducts is viewed primarily as a positive indication of exposure.

Cytogenetic biomarkers

Sister chromatid exchanges (SCE). SCE result from the interchange of DNA replication products within chromosomes between two sister chromatids at apparently identical loci, which are visualized in metaphase. The mechanism of SCE formation has not

been established, but SCE seem to be a consequence of errors of DNA replication. The biomarker HFC (or HF-SCE) is defined as the percentage of cells with a high frequency of SCE. In most cases, the value of SCE/cell corresponding to the 95th percentile (according to Bender *et al.*, 1992) of the pooled data from the control population is used as the threshold value for the definition of HFC.

Chromosome aberrations (CA). CA are abnormalities of chromosome number or structure, visible in metaphase cells during cell division, and are, in general, a result of breakage of a chromosome or breakage and rejoining within or between chromosomes (also called exchanges). Because of their strong association with cancer, CA are viewed as indicators of increased cancer risk (see below).

Micronuclei (MN). MN are small nuclear-like bodies in cells that have become separated from the main nucleus during cell division. In general, they contain a chromosomal fragment, due to breakage of a chromosome, or a complete chromosome that is left behind due to toxic effects on the spindle proteins involved in chromatid separation.

The European Study Group on Cytogenetic Biomarkers and Health has reported that CA have predictive value for cancer risk. People with high numbers of CA (a high CA frequency) appeared to be at elevated risk. No such elevated risk was observed for people with a low CA frequency. No association was seen between SCE or MN frequencies and subsequent cancer incidence/mortality (Bonassi *et al.*, 1995; Hagmar *et al.*, 1998). Typical values for the CA frequencies involved were: low, $\leq 1\%$; high, > 2.2 (median of values for the 10 laboratories in the Nordic countries) (Hagmar *et al.*, 1994). For monitoring human exposure generally SCE, CA, MN and often also DNA adducts are determined in lymphocytes.

PAC ADDUCT DETERMINATIONS IN ANIMAL MODELS

From controlled animal studies one generally sees much better correlations between adduct levels and dose than in human exposure studies. Results from skin painting studies in rats with bitumen fume condensates (Genevois *et al.*, 1996; Booth *et al.*, 1997) show a positive dose–response effect (Fig. 1). However, the efficiency of DNA adduct formation (expressed as adducts per unit dose = adducts/ 10^8 nucleotides/mg PAC) decreases with increasing PAC dose (Fig. 2). The PAC fraction was expressed as the total 3–6 ring PAC determined by FIA-DMSO extraction (extraction with DMSO using flow injection analysis, coupled with LC/GC with FID detec-

tion for quantification) (Brandt *et al.*, 1999). It has been shown for a wide range of oil products that the PAC fraction (for bitumen fume condensates 1 mg total 3–6 ring PACs roughly corresponds to 40 μg Σ 8PAHs) is correlated with mutagenicity and carcinogenicity in rodent skin painting studies (Brandt *et al.*, 1999).

In studies with bitumen fume condensates Booth *et al.* (1997) found that adduct levels in the lungs were five to seven times lower than those in the epidermis. Furthermore, the decrease in adduct formation efficiency with increasing dose was much less in the lungs than in the epidermis. An explanation for this effect may be that in the skin, the site of application, almost the full PAC dose is effective, whereas only a small fraction of the PACs reaches the lungs and is metabolized there.

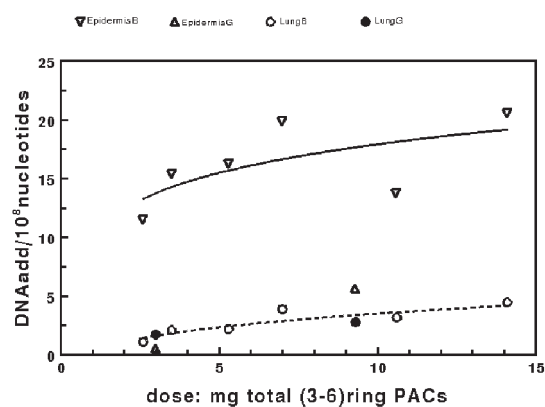


Fig. 1. Relationships between DNA adduct levels in skin and lungs of rats following skin painting of undiluted bitumen fume condensates and total 3–6 ring PACs dosed. G, data from Genevois *et al.* (1996); B, data from Booth *et al.* (1997).

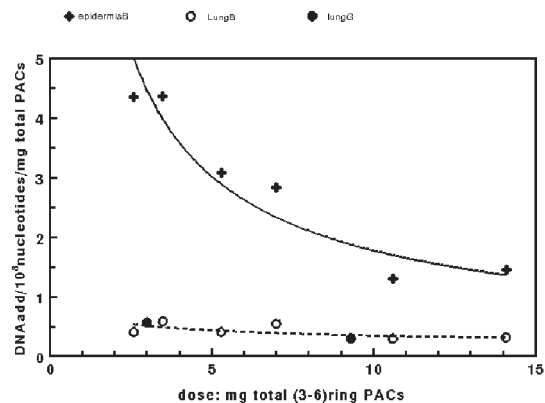


Fig. 2. Relationships between DNA adduct levels in skin and lungs of rats following skin painting of undiluted bitumen fume condensates per mg of PAC dosed and total 3–6 ring PACs.

G, data from Genevois *et al.* (1996); B, data from Booth *et al.* (1997).

In inhalation experiments with rats exposed by inhalation to coal tar aerosols, Lewtas *et al.* (1997) also found a decreasing tendency for lung DNA adduct formation with increasing PAC dose. In contrast, for some individual compounds (e.g. 2-acetylaminofluorene) linear correlations have been observed between chronically administered doses and the steady-state levels of DNA adducts in target organs. The steady-state levels of DNA adducts in such cases have been shown to be linearly related to the tumorigenic response (Beland and Poirier, 1993; Poirier and Beland, 1994). Because DNA adducts found in experimental animals can be detected in exposed humans, there is still the prospect of using DNA adduct data to estimate human cancer risk.

EXAMPLES OF MONITORING HUMAN PAC EXPOSURES

The main exposure groups

The main human exposure groups are as follows.

- Workers having high exposure to coal tar fumes (CTPV) in coke ovens, the aluminium industry and foundries [average values for Σ 8PAHs between 5 and 50 $\mu\text{g}/\text{m}^3$, with high values up to 500 $\mu\text{g}/\text{m}^3$; average values for BaP exposure 4 $\mu\text{g}/\text{m}^3$ (1–50 $\mu\text{g}/\text{m}^3$) with high values up to 90 $\mu\text{g}/\text{m}^3$].
- Workers exposed to diesel exhaust and bitumen fumes in road paving, roofing and bitumen manufacture, with exposure levels ranging from 3.5 to 397 ng/m^3 Σ 8PAHs, 0.2–15 ng/m^3 BaP. These levels are approximately three orders of magnitude lower than the high exposure group working in coke ovens and the aluminium industry.
- Environmentally exposed persons. Here exposures can vary widely depending on the nature of the area (rural or urban, etc.).

The significance of airborne PAH concentration in exposure studies

In our opinion, the use of the concentration in air of one PAH or the sum of a limited number of individual PAHs as a marker of exposure should be treated with care. For example, when coke oven workers are compared with asphalt pavers, Σ 8PAH and BaP exposures represent completely different burdens of exposure to potentially carcinogenic compounds. Coal tar fumes consist mainly of unsubstituted PAHs, and to a minor extent monomethyl-substituted PAHs. Tjoe Ny *et al.* (1993) showed that the 16 marker PAHs recommended by the US EPA (1986) constitute between 40 and 90% of the CTPV fraction. A similar observation has been made by Brandt (1994). In bitumen fumes, on the other hand, the Σ 8PAHs constitute only a small fraction (<1%) of the total potentially carcinogenic PACs (Brandt *et*

al., 1999). Hence, the Σ 8PAH exposure represents ~50 times higher potency for bitumen fume exposures than for exposures to CTPVs. For environmental exposures to PACs the contribution of combustion sources and engine exhaust can be appreciable (Halsall *et al.*, 1994; Schauer *et al.*, 1996).

Occupational exposures to PAH

Coke oven workers. van Schooten *et al.* (1990) studied a group of 56 coke oven workers and 44 non-exposed controls for PAH–DNA adducts using an ELISA procedure. The average exposure to CTPV was 0.45 mg/m^3 , while the exposure to BaP was 0.98 $\mu\text{g}/\text{m}^3$. The average levels of PAH–DNA adducts measured were 5.1 adducts/ 10^8 nucleotides for the exposed group and 2.7 adducts/ 10^8 nucleotides for the controls. No significant correlations were found between PAH–DNA adducts in blood and the air concentrations of total PAHs or BaP. In both groups smokers had significantly higher levels of adducts than non-smokers.

The specificity of biological monitoring probes, e.g. excretion of phenanthrene and pyrene metabolites in urine, and the usefulness of some other biomarker measurement techniques, e.g. alkaline filter elution, the ^{32}P -post-labelling assay and measurement of SCE, in coke oven workers exposed to PACs were investigated by Popp *et al.* (1997). Only a weak correlation between urinary excretion of PAH metabolites and personal exposure (mean Σ 8PAHs 15 $\mu\text{g}/\text{m}^3$) was found. There were indications of metabolic differences between individuals and these were more pronounced for phenanthrene than for pyrene. Smoking also had an important influence. There was a significant difference in results of alkaline filter assays, indicating significantly more DNA single-strand breaks and also DNA protein cross-links in coke oven workers compared with controls. However, a significant difference in SCE between coke oven workers (6.2 SCE/cell) and controls (6.7 SCE/cell) was not observed. Smokers in both groups showed higher values than non-smokers. In addition, there was no difference in levels of PAH–DNA adducts in lymphocytes between smokers and non-smokers and these only correlated with exposure to PAHs in a semi-quantitative manner.

Hemminki *et al.* (1990a,b) measured DNA adducts in white blood cells of Polish coke oven workers and controls. Local controls living near the cokeries showed similar adduct profiles to the coke oven workers. The ‘topside’ workers had the highest BaP exposures. Values for rural control subjects were 2–3 times lower. In these studies there was a high variation in the estimated BaP between different plants. When DNA adduct measurements were made these were related to broad exposure groups, without precise definition of the actual personal exposure

levels. For our estimate of Σ 8PAH exposure we took an average of the reported estimates of BaP exposure.

Lewtas *et al.* (1997) found no statistically significant differences in DNA adduct levels in white blood cells between exposed coke oven workers and environmental controls (2.5–5.7 adducts/ 10^8 nucleotides for Σ 8PAH exposures of 1.2–17.4 $\mu\text{g}/\text{m}^3$ in the exposed group and 4.6–4.8 adducts/ 10^8 nucleotides for Σ 8PAH exposures of 0.007–0.013 $\mu\text{g}/\text{m}^3$ in the controls). This was attributed to a non-linear dose–response behaviour in adduct formation at the higher exposure levels. The ‘DNA adduct formation efficiency’ expressed as DNA adducts (relative adduct labelling)/PAH exposure ranged from 0.3 to 3.0 for the exposed groups and 368 to 700 for the control groups. A similar effect was found for the DNA binding potency in human bronchoalveolar lavage lung cells of another group of highly exposed subjects compared with controls. This was also reflected in values for cytogenetic markers.

Kalina *et al.* (1998) monitored cytogenetic markers in coke oven workers and found an increase in the percentages of aberrant cells (CA) and SCE/cell and the Σ 8PAH exposure levels. Their data indicated a decreased formation efficiency of CA and SCE with increasing exposure. A 44 times higher average Σ 8PAH exposure (18 versus 0.4 $\mu\text{g}/\text{m}^3$) resulted in a 2 times higher CA (2.3 versus 1.1%) and in a 1.4 times higher SCE value (7.5 versus 5.5 SCE/cell). These observations correlate with the results found by Lewtas *et al.* (1997) for DNA adduct formation in white blood cells. Hemminki *et al.* (1997), however, reported, based on results of three studies, an indication of consistency in dose–response relationships for DNA adduct formation in foundry workers with BaP exposures ranging from 5 to 50–200 ng/m^3 . These authors note that others (Kriek *et al.*, 1993; Ovrebø *et al.*, 1994, 1995) reported only a small or no increase in DNA adducts in white blood cells and lymphocyte DNA for coke and aluminium workers. They suggest as an explanation that when the occupational exposure decreases, other sources, such as food, smoking, environmental air pollution and drinking water, become relatively more important. In our opinion, possibly at least a part of the difference in DNA adduct (or CA or SCE) formation efficiency is due to the unsuitability of Σ 8PAH as a marker for potential carcinogenicity (see above), when comparing different exposure types.

Zhang *et al.* (2000) reported studies on aromatic DNA adducts in white blood cells of coke oven workers, in relation to exposure, lifestyle and genetic polymorphism of metabolic enzymes. The exposed workers were divided into high (estimated Σ 8PAH 51 $\mu\text{g}/\text{m}^3$) and low (estimated Σ 8PAH 22 $\mu\text{g}/\text{m}^3$) exposure groups. The DNA adduct level of the high exposure group (1.59 adducts/ 10^8 nucleotides) was not significantly higher than that of the low exposure

group (1.33 adducts/ 10^8 nucleotides). Effects of variables, including smoking, genetic polymorphism of CYP1A1, NAT2 and the *p53* gene and a family history of cancer, on DNA adduct levels were found, suggesting that these should be considered when evaluating the genotoxic effect of occupational exposure to PACs using white blood cell DNA adducts.

In a study by Reuterwall *et al.* (1991), in which the estimated exposure to Σ 8PAHs (17 $\mu\text{g}/\text{m}^3$) was similar to that measured in the studies by Kalina *et al.* (1998), there was no significant difference between exposed and control subjects for each of CA, SCE, frequency of MN, urinary thioether excretion or mutagenicity in urine. At this level of PAH exposure a factor of more than 10-fold difference in Σ 8PAH exposure between exposed and controls did not give a significant difference in thioether excretion, CA, MN or SCE.

Binkova *et al.* (1998) evaluated the DNA adduct levels in total white blood cells and lymphocytes from the blood of the same individuals using the ^{32}P -post-labelling assay. The DNA adduct pattern in lymphocytes and white blood cells were qualitatively the same and there was a reasonable quantitative relation between the two. A positive significant correlation between PAH (or BaP) exposure and DNA adduct level was observed. An order of magnitude difference in PAH exposure resulted in only about a 1.5-fold higher level of DNA adducts.

Kuljukka *et al.* (1996) studied the relation between airborne pyrene and BaP (mean 5.7 $\mu\text{g}/\text{m}^3$, range 0.02–40) exposure and urinary 1OHPy excretion (mean 6 $\mu\text{mol}/\text{mol}$ creatinine, range 0.2–70). The 1OHPy was linearly correlated with the airborne pyrene and BaP concentrations and was significantly higher than that of the controls. Skin wipe analyses also indicated a considerable skin contamination with pyrene and BaP.

van Delft *et al.* (2001) used urinary 1OHPy excretion as a biomarker of exposure for a group of coke oven workers. The urinary 1OHPy excretion of the coke oven workers was significantly increased relative to the controls. However, the mean value of 1.24 $\mu\text{mol}/\text{mol}$ creatinine was below the no effect benchmark level of 1.4 $\mu\text{mol}/\text{mol}$ creatinine proposed by Jongeneelen (2001). No significant induction of DNA adducts or cytogenetic markers (SCE, HFC and MN) was found. Smoking caused a significant increase in many of the biomarkers studied.

Wu *et al.* (2002) monitored coke oven workers for urinary 1OHPy and *trans-anti*-BaP-tetraol as biomarkers of exposure and benzene soluble matter (BSM, i.e. the fraction of the particulates collected on a filter that is soluble in benzene) as a surrogate for external PAH exposure. A significant correlation between external exposures and urinary excretion of the PAH metabolites was found.

Aluminium production workers. In studies on aluminium production workers, Carstensen *et al.* (1999a,b) measured urinary 1OHPy, DNA adducts and cytogenetic markers in peripheral lymphocytes in pot room workers exposed to 16 $\mu\text{g}/\text{m}^3$ $\Sigma 10$ 4–6 ring PAH (estimated 9.8 $\mu\text{g}/\text{m}^3$ $\Sigma 8$ PAHs). A 30 times higher level of 1OHPy excretion was found in pot room workers compared with unexposed controls. In contrast, no significant difference in DNA adduct levels, MN or DNA strand breaks were found. No correlations between any of the cytogenetic markers and 1OHPy in urine or PAH–DNA adducts were found. Age and smoking habits did not correlate with the occurrence of strand breaks.

Tjoe Ny *et al.* (1993) studied workers in an aluminium production plant reporting some very high personal exposures to BaP (0.9–48 $\mu\text{g}/\text{m}^3$) and pyrene (3.5–130 $\mu\text{g}/\text{m}^3$) and urinary 1OHPy at the beginning and the end of the working week (end of week concentration 1–43 $\mu\text{mol}/\text{mol}$ creatinine). The airborne pyrene concentration during the working week was linearly related to the increase in urinary 1OHPy. An 8-fold increase in pyrene exposure gave rise to a doubling of the change in 1OHPy.

Heussner *et al.* (1985) studied workers in an anode pre-baking plant (BaP exposure > 0.2 $\mu\text{g}/\text{m}^3$). A significant effect of CTPV exposure was only found for urine mutagenicity, in both smokers and non-smokers. No significant effect of exposure to CTPV on chromosome aberration rates or semen analysis was found.

van Hummelen *et al.* (1993) compared workers employed in a graphite electrode producing plant and a coke oven with a control population of maintenance workers in a blast furnace. Although the mean airborne PAH concentrations for the workers [$\Sigma 8$ PAH estimated from total PAHs reported ≈ 4 $\mu\text{g}/\text{m}^3$, based on reported PAH profiles (Popp *et al.*, 1997; Carstensen *et al.*, 1999a,b; Zhang *et al.*, 2000)] was significantly higher than that of the controls (~ 0.25 $\mu\text{g}/\text{m}^3$), statistically significant differences in SCE and HFC and positive correlations between the cytogenetic markers and airborne PAH levels or urinary hydroxypyrene concentrations were only detectable in the lower exposed workers from the coke oven. A statistically significant effect of smoking was not observed.

Vu Duc and Lafontaine (1996) measured 1OHPy in human urine as a biomarker of exposure to PAC in various processes in an aluminium production plant, such as relining, starting up of a pot, working in a paste plant and at bake ovens. They found a trend for 1OHPy excretion similar to that of pyrene and $\Sigma 8$ PAHs.

Schoket *et al.* (1999) measured DNA adduct formation in blood and urinary 1OHPy excretion in aluminium production workers. DNA adduct levels in blood were significantly enhanced relative to

controls. 1OHPy levels were 13- to 70-fold higher than in the controls. No PAH exposure measurements were made. A significant linear relation between DNA adduct levels and 1OHPy concentrations was not found nor was a significant influence of smoking on DNA adduct formation observed.

Mannschreck *et al.* (1996) measured the exposure to PAHs of workers in a graphite electrode production plant and found a significant correlation between urinary 1OHPy excretion and the BaP and pyrene concentrations.

Using ^{32}P -post-labelling analysis coupled with HPLC, Tuominen *et al.* (2002) were able to resolve four DNA adducts from peripheral mononuclear blood cells of pot room workers and controls. For three of the DNA adducts there was no statistically significant difference between the exposed group and the controls. One adduct was, however, statistically increased in the exposed group. The levels of this adduct were shown to be related to the length of employment, genetic polymorphisms and use of respiratory protection.

Iron foundries. Phillips *et al.* (1988) measured DNA adducts in peripheral white blood cells of workers occupationally exposed to PAHs in a Finnish iron foundry and from control subjects not known to be occupationally exposed to PACs. Using exposures from previous industrial hygiene surveys, foundry workers were classified as belonging to high, medium or low exposure groups according to their exposure to airborne BaP (high, >0.2; medium, 0.05–0.2; low, <0.05 μg BaP/ m^3 air). The average DNA adduct level in the high and medium exposure groups was 1.8 adducts/ 10^8 nucleotides (range 0–10). DNA adduct levels of the low exposure group (average 0.06, range 0–0.6 adducts/ 10^8 nucleotides) were not different from those of the unexposed controls (average 0.2, range 0–1.9 adducts/ 10^8 nucleotides). A significant effect of smoking was not found. In the same foundry and using the same exposure classification, Reddy *et al.* (1991) investigated DNA adduct formation in white blood cells of 61 workers and 19 controls. There was a highly significant correlation between the estimated exposures and adduct levels as determined by analysis of variance. No effects due to age, sex or smoking habit of the subjects were observed. The results from this (larger) study confirmed the initial results reported by Phillips *et al.* (1988).

In separate studies at about a factor of 10 lower BaP exposure, Santella *et al.* (1993) used competitive ELISA to measure PAH–DNA adducts in white blood cells and urinary 1OHPy. Both the PAH–DNA and the 1OHPy levels showed an increasing trend with exposure. The inter-individual variability was high for both biomarkers.

The averaged results of three laboratories, measured on 53 foundry workers exposed to $<0.05\text{--}0.2\ \mu\text{g}/\text{m}^3$ BaP and six unexposed controls, were 16 (range 9–26) adducts/ 10^8 nucleotides and 2.4 (range 1.7–3.1) adducts/ 10^8 nucleotides, respectively. No significant effect of smoking was found (Savela *et al.*, 1989).

Hemminki *et al.* (1997) studied DNA adducts in leukocytes of workers exposed in a foundry over a period of 5 yr. The results indicated consistency in dose–response relationships for workplace BaP concentrations and DNA adducts. The post-labelling method was able to detect an increase in aromatic DNA adducts in leukocytes when exposure to BaP was $\sim 0.005\ \mu\text{g}/\text{m}^3$. It was concluded that at such low levels, smoking and consumption of charcoal broiled food may be important contributors to adduct levels.

Forni *et al.* (1996) studied cytogenetic end points in leukocytes of a cohort of coke oven workers in a steel plant, engaged in jobs involving different levels of exposure. No significant difference was found in CA for the exposed subjects, relative to the controls. The average exposure to BaP was $0.96\ \mu\text{g}/\text{m}^3$, versus <0.001 for the controls. The SCEs of the exposed groups were significantly increased only for smokers. The non-significance for CA is in line with the findings of Reuterwall *et al.* (1991) and Heussner *et al.* (1985).

Bitumen application workers. A considerable number of workers are involved in handling hot bitumen-derived products in, for example, road paving and roofing. In 1998 the US National Institute of Safety and Health (NIOSH) estimated the number of workers in the US asphalt and roofing industries to exceed 350000 (NIOSH, 1998). On the basis of the volumes of bitumen/asphalt produced the number of workers in Europe is at least half of the US number.

Road pavers. Pasquini *et al.* (1989) carried out biological monitoring to assess the mutagenic/carcinogenic hazards associated with exposure to bitumen fumes during paving operations, analysing some biological parameters in the urine of a group of exposed workers. The urine samples were studied for mutagenicity by the Ames test and for levels of thioethers and D-glucaric acid. Urinary mutagenicity data for exposed workers were statistically higher than those of a group of unexposed subjects. Thioethers were higher only in subjects exposed simultaneously to bitumen fumes and cigarettes. D-Glucaric acid excretion was not increased significantly due to bitumen fume exposure.

Fuchs *et al.* (1996) studied road pavers, roofers and painters exposed to bitumen materials and reported DNA adducts for the pavers of the order of a few adducts/ 10^9 nucleotides; these were very low, but no values were reported for the controls. Alkaline strand

breaks in pavers, roofers and painters were not significantly different from the controls.

Burgaz *et al.* (1988, 1991, 1992, 1998) surveyed asphalt workers, but did not report values for the levels of PAH exposure. In these studies the mean levels of urinary IOHPy in bitumen-exposed workers were significantly higher relative to those of the controls. However, the average level of urinary IOHPy was well below the $1.4\ \mu\text{mol}/\text{mol}$ creatinine proposed by Jongeneelen (2001) as the no effect level. Mean SCE values of exposed subjects (all and non-smokers) were significantly higher than those of controls. There was a significant difference between means of the exposed and control groups in the frequency of MN. Urinary thioethers measured in the exposed groups were higher than in controls, however, this increase was only significant in the smoking group, possibly due to differences in smoking habits. A correlation between urinary IOHPy and thioether levels was not found.

Hatjian *et al.* (1995, 1997) measured PAH exposures, SCE, urinary thioethers and urinary D-glucaric acid in pavers and roofers. No significant difference was found between exposed subjects and controls. An increase in urinary IOHPy over a 3 day work period was found for the pavers and roofers, but not for the controls.

In a study of 19 asphalt workers and 13 controls, Zhou (1997) reported airborne PAH exposures, PAHs in skin wipes, IOHPy pre- and post-shift and total DNA adducts in exfoliated urothelial cells. Although airborne PAH concentrations and PAH in skin wipes were 20 and 4 times higher, respectively, for the exposed workers compared with the controls, no significant difference in IOHPy or DNA adduct levels was found between controls and exposed workers. The post-shift IOHPy levels were below the proposed benchmark no effect level of $1.4\ \mu\text{mol}/\text{mol}$ creatinine.

No difference was found between pre- and post-shift urinary IOHPy in a study by Järholm *et al.* (1999), who monitored Swedish non-smoking asphalt workers and controls for PAHs in the breathing zone, urinary IOHPy and SCE and MN in peripheral lymphocytes. For a PAH exposure in the ranges found in other studies (Brandt *et al.*, 1993; Watts *et al.*, 1998) the road pavers had no significant increase in SCE and MN.

In a long term (4 yr) monitoring study of a group of asphalt pavers, Major *et al.* (2001) measured CA, SCE and hypoxanthine phosphoribosyltransferase (HPRT) variation frequencies and compared these with the values for unexposed controls. During the study period a number of hygiene measures were instituted, such as improvements in personal protection, switching from crude oil to detergents to clean equipment and introducing proper ventilation in the cabins of the roller drivers. This resulted in a drop in

CA in the study period from 3–3.6% to values between 1 and 1.2%, the level of the controls. A CA level <1 has been reported by the European Study Group on Cytogenetic Biomarkers and Health (Hagmar *et al.*, 1994, 1998) as having no increased cancer risk. The SCE frequencies increased significantly relative to the controls in the first year of the study, but then decreased to the control level. HPRT values in the exposed groups did not differ in any of the study years. The authors concluded that the application of tar-free asphalt by itself does not increase the genotoxic risk when adequate personal protection is provided.

Szanişzlo and Ungvary (2001) monitored urinary PAH metabolites in asphalt pavers in the city of Budapest and measured atmospheric PAHs in the working environment. A group of non-PAH exposed health care workers was chosen as controls. In the same study, policemen working in the city were also monitored. Although total PAHs and pyrene were similar for policemen and asphalt pavers, the $\Sigma(5-6)$ ring PAHs was much lower for the asphalt pavers (7 ng/m³) than for the policemen (26 ng/m³). Urinary 1OHPy excretion was similar for the non-smokers in all groups and similar to the level of the controls. Levels for the smokers were about 5 times higher than those of the non-smokers. Hence, with this external PAH exposure level ($\Sigma 7$ PAHs 5–54 ng/m³) smoking was the dominant factor affecting 1OHPy excretion. Other PAH metabolites (3-hydroxybenzo[*a*]anthracene and 3-hydroxybenzo[*a*]pyrene) were not detected in the urine of any of the groups.

Roofers. Hatjian *et al.* (1995, 1997) monitored roofers, exposed only to bitumen fumes, for PAH exposure, urinary thioether excretion, urinary 1OHPy excretion and SCE. For a $\Sigma 8$ PAH exposure level of 0.2 µg/m³ no significant difference relative to the controls was found for any of the biomarkers.

Herbert *et al.* (1990) reported on a study of roofers involved, during the first half of the shift, in removal of an old coal tar roof and, during the second half, in application of a new bitumen-based roof. Personal air samples and skin wipes were taken and DNA adducts in white blood cells were determined by ³²P-post-labelling. The levels of BaP relative to the total PAHs measured, in both personal air samples (BaP 0.8 µg/m³, $\Sigma 7$ 3–6 ring PAHs 8.3 µg/m³) and skin wipes, indicated that the main PAH exposure came from coal tar dust that the workers were exposed to during the removal of the old roof. The median level of DNA adducts was 1.3 per 10⁸ nucleotides (83% above detection limit). The controls were 0.13 adducts/10⁸ nucleotides (17% above detection limit). For the roofers the DNA adduct levels correlated with the post-shift levels of PAHs in the skin wipes.

In a separate study, the contribution of coal tar exposure was confirmed by Toraason *et al.* (2001),

who measured DNA strand breaks and 1OHPy in roofers with coal tar pitch dust and/or bitumen fume exposure. The assessments of 1OHPy indicated that exposure to bitumen fumes might be a factor in elevated urinary 1OHPy, but that PAC exposures during removal of roofs containing coal tar was the primary contributor. 1OHPy levels in roofers with only bitumen fume exposure were <1 µmol/mol creatinine, whilst those in roofers co-exposed to coal tar were 6 times higher. Elevated strand breaks were only found in roofers with exposure to coal tar.

In a factory manufacturing clay pigeons, Lafontaine (2000) monitored five workers and two external observers by personal air and urinary 1OHPy sampling for a four shift working week. No respiratory protection was worn during the manufacture of the targets, which were moulded from coal tar pitch at 190°C. Atmospheric concentrations of pyrene and BaP ranged from 0.66 to 5.05 and 0.037 to 0.270 µg/m³, respectively. The correlation between atmospheric pyrene and urinary 1OHPy concentrations (increase over the shift) was poor ($r = 0.37$). It improved greatly ($r = 0.74$) if the amount of pyrene inhaled over the shift and the corresponding amount of urinary 1OHPy excreted were considered. This observation is in line with the findings of Grimmer *et al.* (1994) for coke plant workers.

Diesel exhaust. Diesel exhaust has been classified as a probable human carcinogen (Group 2A) by the International Agency for Research on Cancer (IARC, 1989). Epidemiological data suggest an increased risk of lung cancer for workers exposed to diesel exhaust (Lipsett and Campleman, 1999) at a level comparable with that for environmental tobacco smoke (Comstock, 1998). Using lung implantation of condensed exhaust fractions in rats, Grimmer *et al.* (1987) found that in a group of 35 rats the fraction of PAHs with four or more aromatic rings caused six out of seven total cancers, whereas the nitro-aromatic fraction contributed only one out of seven cancers.

Shu-Xin *et al.* (1997) studied coal miners over a period of weeks of extra high exposure to diesel exhaust that led to an increase in DNA adducts in white blood cells, relative to an already relatively high adduct level during normal work (non-exposed, 5.1 adducts/10⁸ nucleotides, versus 10.4 adducts/10⁸ nucleotides for normal exposure level and 13.9 adducts/10⁸ nucleotides after extra high exposure).

In non-smoking bus garage workers exposed to an estimated ~0.7 mg/m³ total particulate matter and ~15 ng/m³ BaP, increased DNA adduct levels were found (Hemminki *et al.*, 1994).

Nielsen *et al.* (1996a) studied DNA adduct formation, 1OHPy excretion and hydroxyethylvaline (HOEtVal) adducts in haemoglobin in a group of bus garage workers exposed to diesel exhaust. The exposed workers had significantly higher levels of all

three biomarkers compared with the controls. The authors suggested that skin exposure to lubricating oil was a major confounder for biomarker formation. However, in our view the high level of HOEtVal in haemoglobin relative to the controls (33 versus 22 pmol/mol) suggested an important role for exhaust gas exposure. This was corroborated by the finding that the HOEtVal adduct levels correlated with the urinary 1OHPy levels.

DNA adduct formation and urinary 1OHPy excretion were measured in a group of garage mechanics exposed to 0.05–0.18 $\mu\text{g}/\text{m}^3$ BaP (controls were exposed to 0.0006–0.0009 $\mu\text{g}/\text{m}^3$). No difference in levels of DNA adducts was found between mechanics and controls. Significant differences were observed between smoking and non-smoking workers for both DNA adducts and 1OHPy excretion, but not between smoking and non-smoking controls. Approximately two to three orders of magnitude difference in ambient PAH levels gave rise to only a 2-fold increase in 1OHPy concentration (Schoket *et al.*, 1999). In these types of studies it seems probable that skin co-exposures to diesel fuel and lubricating oil could have affected the results, a conclusion also made by Nielsen *et al.* (1996a).

Seidel *et al.* (2002) monitored underground salt miners exposed to diesel exhaust by air sampling for PAHs and nitroarenes and by measuring the urinary excretion of 1OHPy, hydroxylated phenanthrene metabolites and some aromatic amines as markers for exposure to nitro compounds. An increased urinary level of phenanthrene metabolites was found in diesel exhaust exposed workers that was further enhanced by smoking. The excreted amounts of aromatic amines found as metabolites of the nitroarenes were about 5- to 10-fold higher than expected from the levels determined by personal air sampling at the workplace of the individuals. The authors suggested that urinary 3-aminobenzanthrone be used as a specific biomarker of exposure to diesel exhaust.

Non-occupational exposures to PAH

Environmental. Nielsen *et al.* (1996b) monitored urban and suburban non-smoking bus drivers and rural controls (smokers and non-smokers) for DNA adducts. A significant influence of urban air pollution on the level of DNA adducts (40 adducts/ 10^8 nucleotides for urban and 18 adducts/ 10^8 nucleotides for suburban bus drivers versus 2.4 adducts/ 10^8 nucleotides for local controls) was found. No significant influence on adduct levels was associated with potential confounders, including smoking and diet.

Binkova *et al.* (1995) monitored non-smoking and non-occupationally exposed working women in an area in the Czech Republic heavily polluted by coal combustion. The authors found a significant correlation between PAH exposures ($\Sigma 8\text{PAHs}$ between low

<0.01 and high >0.02 $\mu\text{g}/\text{m}^3$, BaP between low <0.002 and high >0.004 $\mu\text{g}/\text{m}^3$) measured in the 24 h period prior to blood sampling and the DNA adducts in white blood cells (4–6.5 DNA adducts/ 10^8 nucleotides).

Lewtas *et al.* (1997) studied the effect of personal exposure to air pollution on DNA adducts in a group of women working outdoors in the Czech Republic. 'Low' levels of PAH exposures ($\Sigma 8\text{PAHs}$ average 0.01 $\mu\text{g}/\text{m}^3$) showed a significant correlation with DNA adducts in white blood cells.

Hemminki *et al.* (1990a,b) found that persons living in the vicinity of coke ovens in Poland (BaP exposure 0.015–0.057 $\mu\text{g}/\text{m}^3$) had 2–3 times higher DNA adduct levels than persons living in the countryside. Furthermore, the results showed that the levels of aromatic adducts in white blood cell DNA did not linearly relate to ambient air levels of PAHs. It was concluded that other sources, such as food, might have been important contributors. In studies in the same geographical area and on similar groups, Perera *et al.* (1992) measured a number of biomarkers of exposure. Their results showed that exposure to environmental pollution (0.006 < BaP < 0.057 $\mu\text{g}/\text{m}^3$) was associated with significant increases in DNA adducts, in SCE, including HFC, and in CA. Furthermore, the DNA adducts were significantly correlated with CA.

Poirier (1999) studied US army soldiers (61) normally stationed in Germany but deployed in Kuwait in the aftermath of the Gulf war. PAH exposures in Kuwait were low (BaP < 0.00023 $\mu\text{g}/\text{m}^3$) compared with BaP concentrations (0.0015–0.007 $\mu\text{g}/\text{m}^3$) in Germany. A significant difference in blood DNA adducts was found (1.7 adducts/ 10^8 nucleotides in Kuwait versus 2.9 adducts/ 10^8 nucleotides in Germany, before and after, respectively). The authors suggested that in addition to the environmental contribution, the difference in diet (e.g. consumption of charbroiled meat) might have played a role.

Darcey *et al.* (1992) reported no significant differences in DNA adduct levels (average 2.6 adducts/ 10^8 nucleotides) of nine US firefighters before and after 6 weeks of fighting oil fires in Kuwait in 1991. Their diet was almost exclusively imported.

Eder (1999) reviewed a number of the foregoing studies, with a particular focus on intra-individual variations, and found that environmental exposure can have a larger effect on PAH adduct levels than occupational exposure, food or smoking. The author indicated that the most important changes in PAH-DNA adduct levels were caused by environmental exposure due to heating fuels.

Effects of smoking and diet. Burgaz *et al.* (1988, 1992, 1998) surveyed groups of asphalt workers and controls, but reported no data for actual PAC exposures. Determinations of mean levels of urinary

1OHPy showed no significant difference between smokers and non-smokers in both groups. In both groups SCE frequencies of smokers were significantly higher than those of non-smokers. Smoking had no significant effect on MN. However, a significant effect of smoking on urinary thioether excretion was reported and a linear correlation between number of cigarettes smoked and thioether excretion was found. In contrast, at relatively low external PAH exposure levels ($\Sigma 8\text{PAHs} < 0.06 \mu\text{g}/\text{m}^3$), Szaniszló and Ungváry (2001) reported smoking to be the dominant factor influencing 1OHPy excretion. The exposures of the smokers were about 5 times higher than those of the non-smokers.

Savela and Hemminki (1991) compared a group of 11 otherwise unexposed male and female smokers with a similar group of non-smokers (10) and determined levels of white blood cell DNA adducts in T lymphocytes and in granulocytes. In T lymphocytes, with half-lives of several years, much greater (and significant) differences (31 versus 13 adducts/ 10^8 nucleotides) were found between smokers and non-smokers than in granulocytes (9.6 versus 7.6 adducts/ 10^8 nucleotides), with short half-lives (7–24 h). van Schooten *et al.* (1990) studied 56 coke oven workers and 44 non-exposed controls for PAH–DNA adducts using ELISA. In both groups smokers had significantly higher levels of adducts than non-smokers.

Phillips *et al.* (1990) analysed non-tumourous bronchial tissue of 37 cigarette smokers, eight former smokers and eight non-smokers for the presence of aromatic DNA adducts by ^{32}P -post-labelling assay. Adduct levels detected in DNA from non-smokers, former smokers and current smokers were 3.45 ± 1.62 , 3.93 ± 1.92 and 5.53 ± 2.13 adducts/ 10^8 nucleotides, respectively. The differences in adduct levels between current smokers and former or non-smokers were statistically significant. Among the smokers, significant correlations were found between adduct levels and both daily cigarette consumption and total cigarette consumption. DNA from peripheral blood leukocytes of 31 heavy smokers (>20 cigarettes/day) and 20 non-smokers was also analysed by ^{32}P -post-labelling. Adduct levels in the samples from smokers were not significantly different from those of non-smokers. The authors concluded that measuring DNA adducts in peripheral white blood cells may not be a good procedure for monitoring inhalation exposure to carcinogens. In a study comparing 4-aminobiphenyl haemoglobin and aromatic adducts (detected by ^{32}P -post-labelling) in lymphocytes of smokers it was found that the levels of both of these types of adducts saturated with high tobacco usage, ~ 30 cigarettes/day (Dallinga *et al.*, 1998). Eder (1999) reported that DNA adduct levels were not significantly influenced by smoking cessation, whereas SCEs significantly decreased after cessation.

Several authors (Hemminki *et al.*, 1997; Poirier, 1999) have suggested that at relatively low exposure levels the influence of diet might be an important contributor to biomarker formation. Consumption of charbroiled meat has a marked influence on urinary 1OHPy excretion; increases in the range 10- to 80-fold have been observed (Kang *et al.*, 1995). Other studies indicate that recent consumption of charbroiled food contributes to the PAH–DNA adduct load in peripheral white blood cells, albeit with large inter-individual variations (Rothman *et al.*, 1990, 1993a,b; Poirier, 1999).

Human variability and effects of polymorphisms

Carcinogenesis may be modulated by host polymorphism in genes for PAC metabolism and DNA repair enzymes. This has become an important area of research. Studies relating phenotype/genotype to cancer have examined measurable early end points such as DNA adduct formation and/or cytogenetic damage. There is now substantial evidence emerging from numerous laboratories showing considerable inter-individual human variation in levels of adducts formed due to environmental and occupational exposures to PACs. It is evident that groups of predisposing polymorphic genes exist, for example, those involved in PAC metabolism and DNA repair (reviewed in Bartsch *et al.*, 1998). These factors may increase the degree of adduct formation and thus the cancer risk in certain exposed subjects, even when only low level exposure has occurred. Biomonitoring and molecular epidemiology could thus play an important role in identifying susceptible individuals, particularly those suffering a combination of high risk factors, namely a high level of exposure to PACs, inherited cancer predisposing genes and a deficiency of protective factors such as occur in the diet. Once identified, cancer predisposing genes can be used as intermediate risk markers for cancer.

A number of studies have investigated the relationship between occupational (Hemminki *et al.*, 1997; Kalina *et al.*, 1998; Pan *et al.*, 1998; Rojas *et al.*, 1998, 2000; Pavanello *et al.*, 1999; Zhang *et al.*, 2000) and environmental (Butkiewicz *et al.*, 1998, 2000) exposure to PACs and genetic polymorphisms. Coke oven workers are a group that suffers high exposures to PACs, as detailed in several studies on this occupation (Kalina *et al.*, 1998; Zhang *et al.*, 2000). Kalina *et al.* (1998) studied 64 coke oven workers and monitored exposure to eight PAHs. The exposure levels varied widely from 0.6 to $547 \mu\text{g}/\text{m}^3$ for total PAH exposure, compared with 0.07– $1.51 \mu\text{g}/\text{m}^3$ for the 34 controls studied. Measuring the cytogenetic markers CA, SCE, cells with high frequency of SCE, the heterogeneity index SCE and genetic polymorphisms of glutathione *S*-transferase (GST) M1 and *N*-acetyltransferase 2 (NAT2), it was found that all the cytogenetic markers were signifi-

Table 2. Biomarker levels and PAH exposures for the occupational groups [mean (min–max)]

Rank order for PAH exposure		Σ 8PAHs ($\mu\text{g}/\text{m}^3$)	BaP ($\mu\text{g}/\text{m}^3$)	IOHP ($\mu\text{mol}/\text{mol}$ creatinine)	DNA (^{32}P -post-labelling) (adducts/ 10^8 nucleotides)	CA (%)	SCE (SCE/cell)	MN (%)
1	Coke oven	15.4 (0.1–547)	3.3 (0.002–90)	3.7 (0.2–70)	8.1 (0.5–32)	1.6–2.3	6 (3.8–10)	4.1 (2.7–5)
1	Aluminium mfg.	12 (1–153)	4.4 (0.02–24)	7 (0.06–18)	2.6 (1.4–6.7)			0–4.7
2	Foundries		0.2 (0.05–1)	2.7 (0.3–10)	4.5 (0.1–26)	0.5	8	1.7
3	Bitumen applications	0.15 (0.01–0.5)	0.01 (0.0001–0.01)	1.1 (0.1–2)	0.09 (0.01–0.3)	1–1.2	4.6 (2.8–6.6)	1.6–2.9
4	Diesel exhaust	0.04	0.008–0.018	0.31 (0.07–0.4)	1.7 (0.65–4)			2.3
4	Environmental	0.002–0.03	0.01 (0.0001–0.06)	0.11–0.6	8.6 (0.3–19)	3.5 (2.8–4.3)	8.9 (6–15)	–

cantly increased in the exposed versus control groups. However, in this study no effect of GSTM1 and NAT2 genotypes were observed individually or in combination with the cytogenetic markers. The frequency of CA and SCE/cell were found to be related to exposure to carcinogenic PAHs. Using ^{32}P -post-labelling for PAH–DNA adducts in white blood cells of coke oven workers, Zhang *et al.* (2000) found that subjects homozygous for the rare allele of CYP1A1 showed significantly higher DNA adduct levels than other CYP1A1 genotypes. This area has been the subject of an extensive review by the IARC, which related the observed adducts and other biomarkers to phenotype (IARC, 1999). This appears to be a very promising area of research with significant potential for a role in cancer prevention. However, in view of the overall variation in results observed by different laboratories, it is evident that more research is necessary for this potential to be fulfilled.

ANALYSIS OF THE DATA

The data reported in the articles reviewed are tabulated in the Appendix (Table A1), which lists the average values and the minima and maxima reported in those studies. In the discussion that follows we provide an overview of these data. Although we are aware of the difficulty of comparing results from different laboratories, we have nevertheless attempted to establish whether trends can be observed between the measured biomarker levels and the corresponding external PAC exposures (recorded in Table A1). As a marker for external PAC exposure we have selected the sum of eight carcinogenic PAHs (Σ 8PAHs), since these values are reported in many studies. Where a value for Σ 8PAHs was not available, we have estimated it from BaP exposure or other reported data, such as the sum of the reported PAHs or BSM.

Factors for the conversion of BaP concentrations into Σ 8PAHs have been derived from publications where both BaP and Σ 8PAHs were reported. Values for BaP can be translated into values for Σ 8PAHs because the boiling point of BaP (496°C) is approxi-

mately in the middle of the relatively narrow boiling point range for all eight PAHs (425–534°C). This makes the ratio Σ 8PAHs to BaP mainly dependent on the PAH profile for the specific industrial process, which is reflected in different factors for the different processes. We estimated the following factors for the conversion of BaP concentrations into Σ 8PAH concentrations: for coke ovens, 10; for bitumen fume exposures, 12.5; for environmental exposures, 5. We then compared the results of this analysis of all studies with the conclusions of the authors of the original papers. Most of these papers only covered one type of occupational group, with its specific quantitative and qualitative exposure characteristics. In Table 2 an overview is given of the biomarker levels and external PAH exposures for each occupational group. The groups have been ranked according to their external PAH exposures.

The highest external PAH exposures were observed for workers in coke ovens and the aluminium industry, followed in order by those in foundries, in bitumen and asphalt applications, in those exposed to engine (diesel) exhaust and in those classified as being only environmentally exposed. Between the highest and the next exposure category there was about an order of magnitude difference in PAH exposure levels.

In this review we attempt to correlate each biomarker separately with the corresponding Σ 8PAH exposures. Although the PAH exposures and urinary IOHPy measurements were low in the environmentally exposed groups relative to all the occupationally exposed groups, the DNA adduct levels were remarkably similar in the environmentally exposed group and the coke oven workers.

However, a comment on the PAH exposures and biomarker levels of the different groups is first necessary. Although in the environmentally exposed group the PAH exposures and urinary IOHPy excretions were very low, relative to the occupationally exposed groups, the DNA adduct levels reported were at a comparable level to those reported for coke oven workers. These workers have three orders of magni-

tude higher Σ 8PAH exposures than the environmentally exposed group. The CA and SCE values of the environmentally exposed group were even higher than those of the coke oven workers. There are two possible explanations, which are not mutually exclusive. First, it is possible that exposures to other genotoxic compounds produced larger effects than those from PAHs and these other genotoxic agents were more abundant among environmentally exposed subjects. It is also possible that the exposure regimen played some role because environmentally exposed persons are exposed 24 h/day, for their lifetime, whereas occupationally exposed persons are exposed 8–10 h/working day, during their working life. Because of this difficulty in characterizing the actual exposures of the environmental group, the environmental data have not been included in the following analyses.

The increments in the biomarker levels for the exposed subjects over the corresponding control values (e.g. 1OHPy_{Inc}) have been correlated with Σ 8PAH exposures. We have checked our simple linear regression analyses by two separate statistical approaches: a weighted regression analysis and an analysis whereby the exposed were divided into low and high exposure groups. Statistical analyses were conducted using the GLM procedure in the Statistical Analysis System (SAS), version 8.02. Group mean biomarker levels were considered by analysis of covariance on Σ 8PAH and the study. Observations were weighted according to group size. A second approach was used whereby study groups were allocated to exposure levels of 0, >0–<2 and ≥ 2 mg/m³. Group mean biomarker levels were considered by analysis of variance by exposure level, allowing for the study from which the data was taken. 1OHPy and DNA adduct data were log transformed prior to analysis. Observations were weighted according to group size. For the weighted regression analysis it was not possible to include standard deviations, because these were only reported for some studies.

Urinary 1-hydroxypyrene excretion (1OHPy)

For 1OHPy excretion at the end of shift there is an increasing trend with increasing PAH exposure in the range 0–150 $\mu\text{g}/\text{m}^3$ ($1\text{OHPy}_{\text{Inc}} = 0.12 \times \Sigma 8\text{PAH} + 0.65$, $R^2 = 0.95$, $\text{df} = 11$) (Fig. 3). Above 20 $\mu\text{g}/\text{m}^3$ only 2 of 12 data points are found at high exposures. When the two highest exposure values are omitted and only the range 0–20 $\mu\text{g}/\text{m}^3$ is taken, the slope increases 2-fold relative to that of the full range of exposures and the fit is less good. No differences were found with the weighted regression in which a statistically significant increase in 1OHPy with Σ 8PAH at the 1% level was found.

In the seven publications on CTPV exposures (Σ 8PAH exposure 0.5–150 $\mu\text{g}/\text{m}^3$) that have been reviewed, a significant correlation between external

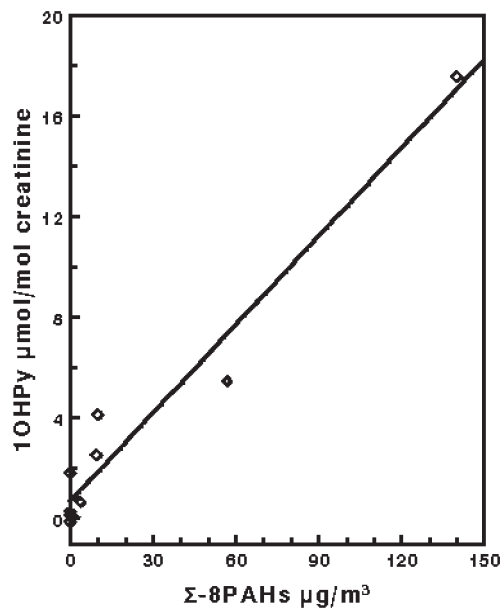


Fig. 3. Relationship between urinary 1OHPy excretion and airborne PAH exposures (0–150 $\mu\text{g}/\text{m}^3$) in personal exposure studies in occupational surveys involving exposure to coal tar fumes, bitumen fumes and diesel exhaust. Increments above the corresponding control values are shown, plus the respective linear fit (see Analysis of the Data).

PAH exposure and urinary 1OHPy excretion has been reported in four cases. In the three others a trend was observed. Also, in a roofing survey, where exposure to coal tar (0.01–8.5 $\mu\text{g}/\text{m}^3$) occurred, a significant correlation was found. Furthermore, in a study in a plant manufacturing clay pigeons, where coal tar was used as the binder, high PAH exposures were recorded and a trend was found between pyrene exposure and urinary 1OHPy excretion.

In five cases where exposure to bitumen fumes only occurred in paving and roofing operations (Σ 8PAHs 0.01–9.6 $\mu\text{g}/\text{m}^3$), no relation was found between the external PAH exposure and urinary 1OHPy excretion. In a study on garage workers exposed to diesel exhaust a trend between PAH exposure and urinary 1OHPy excretion was reported.

Chromosome aberrations (CA)

The only other biomarker that showed an increasing trend with increasing external PAH exposures was CA ($\text{CA}_{\text{Inc}} = 0.08 \times \Sigma 8\text{PAHs} - 0.53$, $R^2 = 0.919$, $\text{df} = 4$) (Fig. 4). However, these correlations are based on only five observations. This was confirmed by the weighted regression, in which a statistically significant increase in CA with Σ 8PAH at the 1% level was found. After dividing the subjects into low and high exposure groups, no significant difference was observed between exposure levels. A statistically significant relationship was not seen by analysis of variance, possibly because of the small number of

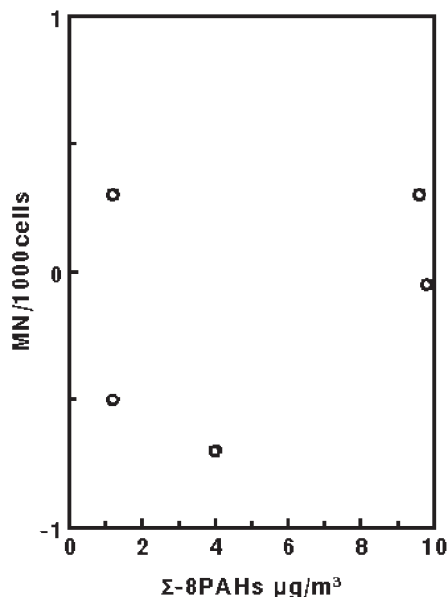


Fig. 7. Relationship between MN and PAH exposure for data collected during occupational surveys involving exposure to coal tar fumes, bitumen fumes and diesel exhaust. Increments above the corresponding control values are shown (see Analysis of the Data).

Out of a total of 15 studies reviewed, seven of these reported a non-significant difference between the exposed groups and controls or no relation between PAH exposure and adduct levels. Of the remaining studies, three reported a trend and four reported a significant correlation between PAH exposure and adduct levels. These were all studies relating to CTPV exposures. One study of coal miners showed a semi-quantitative relation between diesel exhaust exposure and DNA adduct level, but no actual PAC exposures were measured. From the reported correlations or trends between DNA adduct levels and PAH exposure levels, it is evident that there is no relation between the magnitude of the PAH exposure and whether or not a correlation was found by the authors.

In the foregoing discussion we suggested that in environmental exposures genotoxic compounds other than PAHs could be responsible for the observed high biomarker levels. However, the above mentioned trend suggests that the levels of these genotoxic compounds correlate with PAH exposure levels. A decreasing degree of DNA adduct formation with increasing PAH concentration has been reported in several studies (Lewtas *et al.*, 1997). This absence of clear-cut dose-response relationships for complex PAHs has also been noted by Hemminki *et al.* (1997) in studies in which apparently high exposures to PAHs caused little increase in adduct levels. This might be explained by the finding that aromatic adducts in lymphocytes of smokers saturated at

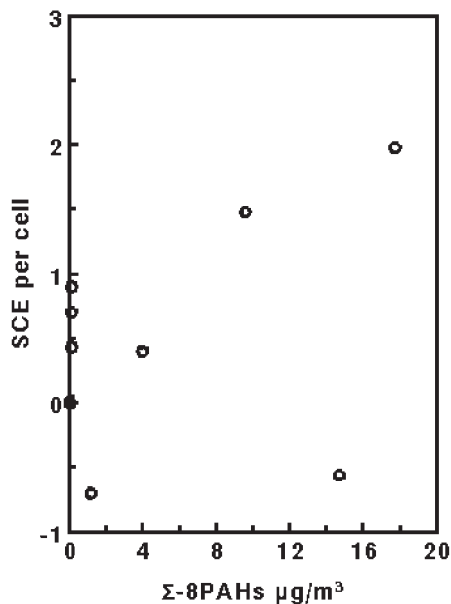


Fig. 8. Relationship between SCE and PAH exposure for data collected during occupational surveys involving exposure to coal tar fumes, bitumen fumes and diesel exhaust. Increments above the corresponding control values are shown (see Analysis of the Data).

around 15 cigarettes/day (Dallinga *et al.*, 1998). The suggested saturation levels are similar to those that could be caused by a high dietary intake of PAHs (Hemminki *et al.*, 1997).

Micronuclei

For MN, no relation with PAH exposure ($0 < \Sigma 8\text{PAHs} < 10 \mu\text{g}/\text{m}^3$) was found (Fig. 7). The MN_{Inc} values are practically constant and almost 1, i.e. the values for the exposed are no higher than the corresponding control values (MN_{Inc} , range -0.7 to 0.46 , average -0.03 , $n = 6$). After dividing the subjects into low and high exposure groups, no significant difference was observed between exposure levels. In the articles reviewed where MN was studied, no significant differences between exposed subjects and controls were found.

Sister chromatid exchange

The SCE values reported (range 0.1 – 2) (Fig. 8) show an almost imperceptible increasing trend ($\text{SCE}_{\text{Inc}} = 0.05 \times \Sigma 8\text{PAHs} + 0.23$, $R^2 = 0.15$, $\text{df} = 9$). After dividing the subjects into low and high exposure groups, no significant difference was observed between exposure levels. Of the articles reviewed, one study (Kalina *et al.*, 1998) reported a non-linear dose-response relation between PAH exposure and SCE values. All other studies (four for CTPV exposure and four for bitumen fume exposure) show either no significant difference between exposed

subjects and controls (six) or no dose–response relationship.

INHALATION VERSUS DERMAL EXPOSURE

A number of authors have suggested that, in addition to the inhalation route, the dermal route of exposure may also play an important role (Herbert *et al.*, 1990; van Rooij *et al.*, 1992, 1993; Kuljukka *et al.*, 1996; Nielsen *et al.*, 1996a,b; Gundel *et al.*, 2000; Lafontaine *et al.*, 2000, 2002; Major *et al.*, 2001). For example, Lafontaine *et al.* (2002) and van Rooij *et al.* (1993) have estimated the contribution of the dermal route of exposure to be between 25 and 75%. From results of animal exposure studies there are also indications that rather high doses via the inhalation route are necessary to evoke a genotoxic effect. In controlled skin painting experiments with laboratory animals genotoxic effects seem much easier to demonstrate. In animal studies only very high doses of bitumen fume condensates, which had been found to be carcinogenic in rodent skin painting studies (Niemeier *et al.*, 1988), produced DNA adducts in the lungs when administered by tracheal instillation (Qian *et al.*, 1998). In a study by Genevois-Charneau *et al.* (2001) only one adduct was observed in the lung of rats exposed nose-only to a high concentration of bitumen fumes ($\Sigma 8\text{PAHs}$ $12.3 \mu\text{g}/\text{m}^3$). Inhalation exposure to coal tar fumes ($1.9 \mu\text{g}/\text{m}^3$) did not give any adduct formation. Dermal application of bitumen or coal tar fume condensate, however, gave rise to adducts in skin, lungs and lymphocytes (Genevois *et al.*, 1996). In another nose-only inhalation study of bitumen fumes ($\Sigma 8\text{PAHs}$ $19 \mu\text{g}/\text{m}^3$), using transgenic mice, exposure for 6 h/day for 5 days did not give rise to DNA adducts or mutagenicity (Micillino *et al.*, 2002). This study differed from the previous ones in that the rest time after termination of the exposure was 24 h in the first study and 30 days in the second, giving ample time for repair of adducts. The long rest time was necessary for the fixation of possible mutagenicity. However, in contrast to these controlled laboratory studies, it is extremely difficult to compare the effective magnitude of the absorbed dose via the two routes for occupational exposures to PAHs.

DISCUSSION

In this review we have attempted to relate the occurrence of biomarkers measured during occupational exposure to PACs in various processes and environments with the external, mostly airborne, PAC exposure. In 45 of the studies reviewed quantitative data were reported for some or all of the biomarkers. These data are summarized in Table A1. As a marker for external exposure, either a single PAH (BaP) or the sum of a number of specific PAHs

was normally reported. Because in many of the studies reviewed the sum of eight carcinogenic PAHs ($\Sigma 8\text{PAHs}$) has been taken as a marker, we have also adopted this marker for our review and analyses. Sometimes it was necessary for us to estimate the $\Sigma 8\text{PAH}$ exposure from other data reported, e.g. BaP measurements. Although external exposures contain not only $\Sigma 8\text{PAHs}$ but also heterocyclic PACs, $\Sigma 8\text{PAHs}$ can be considered a good marker, because the concentrations of heterocyclic PACs are generally proportional to those of the corresponding PAHs.

In our analysis of relationships between external exposure to $\Sigma 8\text{PAHs}$ and measured biomarker levels we have compared the absolute levels of the biomarker, together with its ratio and the increment, relative to the corresponding level measured in the control subjects. Overall, this analysis showed that the incremental values above the controls gave the best correlations with external exposure. There are examples of studies where ratios have been used to determine whether there are biological effects associated with occupational exposures, e.g. for styrene (Bonassi *et al.*, 1996). However, in the present analyses plots of ratio of exposed mean to control mean suggest that ratios are not independent of the control mean for this data and therefore it was not appropriate to analyse the data on the basis of ratios.

Our analysis shows that the use of such markers for comparison of exposure to atmospheres from completely different processes, such as coal tar pitch volatiles versus bitumen fumes, can lead to an erroneous judgement of the 'real' load of potentially carcinogenic PACs. This observation is also true for IOHPy as a biomarker of exposure. It is, therefore, somewhat surprising that a correlation is often found between urinary IOHPy excretion and external PAH exposure, not only within studies, but also for the whole range of different processes, work environments and exposure levels that have been reviewed. An explanation could be that the ratio of pyrene to the $\Sigma 8\text{PAHs}$ in fumes from processes involving CTPV, bitumen fumes or diesel exhaust only ranges between 0.24 and 0.6 and that even the average ratio found in urban air in the UK is only 0.7. This can be interpreted as meaning that $\Sigma 8\text{PAHs}$ is a reasonable marker for pyrene exposure.

In our analysis of studies on biomarkers of internally effective dose, DNA adducts were reported in 20 of the studies, of which 15 could be used for our dose–response estimations. In many controlled animal studies with PAHs positive dose–response relationships between exposure concentrations and adduct levels have been found. In our analysis of all the data by weighted regression a significant statistical correlation was only found because of two data points from studies in which high $\Sigma 8\text{PAH}$ exposures occurred and high DNA adduct levels were found. When these two data points were not included in the

analysis there was no correlation. After dividing the subjects into low and high exposure groups, no significant difference was observed between exposure levels. In half of the individual studies reported no significant relation between external dose and adduct formation was observed. In all the occupational studies where a relation was observed between DNA adduct formation and PAH exposure, these related only to exposure to CTPV, and hence to relatively high exposure levels of PACs. This poor correlation may be due to PACs from other sources, such as from food or from environmental exposures. Sources other than occupational exposure may start to dominate DNA adduct formation at low occupational PAH exposures (Hemminki *et al.*, 1997; Eder, 1999; Poirier, 1999). It is also possible that the duration of exposure rather than the magnitude of the recent dose is the determinant of the level of DNA adducts (Vineis and Perera, 2000). It is unlikely that variations in the adduct analysis procedures could have given rise to the high values found in the environmentally exposed groups. From our analyses of the reported data it appears, therefore, that DNA adduct measurements cannot be used as a reliable quantitative biomarker of occupational exposure to PACs. However, because DNA adducts are associated with mutation and cancer, measurement of an increase in DNA adduct levels relative to the corresponding control levels is, in principle, an indication of increased risk. It is not possible at this time to quantify that increment in risk. Although DNA adduct formation is related to tumorigenesis in animal models, different adducts have different mutagenic and carcinogenic responses (Poirier and Beland, 1992). Many of the human DNA adducts have been determined using DNA from white blood cells and these may not reflect levels in the target tissues (Hemminki *et al.*, 2001). Some adduct determinations have been made in short-lived cells such as granulocytes, rather than longer lived cells such as T lymphocytes, which may better reflect steady-state PAH levels.

The percentage of CA (range 0.5–2.3%) was the only biomarker of effect found to show a positive correlation with external exposure to PAH (range 0.1–18 $\mu\text{g}/\text{m}^3$ $\Sigma 8\text{PAH}$). It should be noted, however, that this dose–response relation was based on only five observations. Of the studies that reported CA, only one showed a (non-linear) dose–response relation. In the other four studies no significant difference between exposed subjects and controls was found. Hence, more studies are necessary to firmly establish the value of CA as a biomarker of exposure to PACs. CA is also the only cytogenetic marker that has been reported to have predictive value for cancer risk.

Overall for SCEs and MN, no relation with PAH exposures was found. In only one study was a non-

linear dose–response relationship found for SCE. This indicates that these biomarkers are less useful for monitoring the effect of PAC exposures.

In a number of biomarker studies a decreasing ‘formation efficiency’ (see Lewtas *et al.*, 1997, p. 13) of the biomarker was found with increasing $\Sigma 8\text{PAH}$. From our analysis this was particularly evident for DNA adduct formation and probably also for CA (see Analysis of the Data, Chromosome aberrations). Because of the difficult nature and variability of the adduct measurements, we are uncertain as to the reliability of the measurements to firmly establish the existence of such a trend. For the other biomarkers our analysis of the data suggests that at very low exposures to PAHs the poor precision of the PAH exposure data and of the biomarker data can give meaningless values for the biomarker measurements and ‘formation efficiency’. Moreover, at very low PAH exposure levels, other sources, such as diet, become major contributors to biomarker formation, as has been suggested by several authors.

For people living in areas with high air pollution, high biomarker levels are often observed relative to their PAH exposures. In such areas, studies of occupational exposure in work situations involving moderate PAH exposures can give rise to a non-significant production of biomarkers from occupational exposure, relative to controls. Smoking can also have a significant effect on the measured biomarker levels. Selection of the control subjects should, therefore, take smoking and environmental factors into consideration.

CONCLUSIONS

This review has shown that if the sum of a limited number of PAHs or a single PAH is chosen as a marker to monitor external exposure to PACs, then it is important to recognize that such a marker may represent a different set of potentially carcinogenic PACs when exposures in different processes and jobs are compared. For those biomarkers for which we have found a good correlation between biomarker levels and external PAH exposures, the increments in biomarker levels above the corresponding control values gave the best correlations.

Our review of data showed that urinary 1OHPY excretion correlates well with external PAH exposure and that this metabolite appears to be a suitable marker for internal exposure to PAHs. Measurements of 24 h urine appear to give the best representation of the cumulative exposure during a work shift.

In contrast, measurements of DNA adducts, which are mostly determined in white blood cells rather than in target organs, do not show good correlations with exposure to PAHs in a variety of workplace and exposure situations. This is perhaps surprising in view of the numerous laboratory studies that have

shown correlations between exposures to PAHs and tissue levels of adducts in exposed animals. It is not clear which factors are responsible for this poor correlation for human exposures. Possible explanations are that other sources of PACs dominate DNA adduct formation at low occupational PAC exposures, that the duration of exposure determines the levels of DNA adducts or, simply, that adducts are not measured in the target organs. At this stage DNA adducts measured in lymphocytes are, therefore, not recommended quantitative biomarkers of exposure to PACs. MN and SCE in peripheral white blood cells are also viewed as unsatisfactory as biomarkers for PAC exposure.

The limited amount of data available indicates that levels of CA show a correlation with exposure to PAHs. Because of their accepted association with cancer, CA may, therefore, be suitable indicators of increased cancer risk from exposure to PACs. Additional data for PAH exposures are necessary to firmly establish this.

All of the above end points are modulated by host-dependent factors. Human polymorphisms therefore represent factors that influence individual response and risk. The identification of individuals at highest risk therefore needs to be taken into account. Individual smoking habits and lifestyle in an area with high environmental exposures can also influence biomarker levels.

For exposures to complex mixtures such as PACs, not only biomarkers, but also external exposure measurements, are necessary to get a complete picture of the exposure scenario and also to aid the identification of possible anomalies and confounders.

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APPENDICES

Table A1 lists the data reported by the papers reviewed.

Glossary

Aneuploidy is the gain or loss of individual chromosomes from the normal diploid set of 46.

Chromosome aberration. An abnormality of chromosome number or structure

Genotoxic. Able to cause harmful heritable changes in DNA

Genotype. The genetic constitution of an organism as revealed by genetic or molecular analysis, i.e. the complete set of genes, both dominant and recessive, possessed by a particular cell or organism.

Hazard. A qualitative term expressing the potential that a chemical can harm health under the conditions of exposure.

Lymphocytes. Lymphocytes are a type of white blood cell present in blood, lymph nodes, spleen, thymus gland, gut wall and bone marrow. Important to the immune system, they produce circulating antibodies and T lymphocytes, which are primarily responsible for cell-mediated immunity and can differentiate into helper, killer or suppressor cells.

Phenotype. The observable structural and functional characteristics of an organism determined by its genotype and modulated by its environment.

Polymorphism. Genetic polymorphism is the variation in the sequence of DNA among individuals that can lead to the expression of different metabolizing enzymes resulting in differences in the metabolism of foreign compounds, such as PACs, between individuals.

Red blood cells (erythrocytes). Erythrocytes are the most numerous type in the blood and are responsible for the transport of oxygen and carbon dioxide. The mature cells have no nuclei.

Risk estimation. The quantification of dose–effect and dose–response relationships for a substance and linking exposure to the probability and nature of an effect.

Risk. The likelihood of suffering a harmful effect resulting from exposure to a risk factor. Risk is usually expressed as the probability of occurrence of an adverse effect.

Sister chromatid exchange. A reciprocal exchange of DNA between the two DNA molecules of a replicating chromosome.

White blood cells (leukocytes). White blood cells are involved in protecting the body from infection and consist of lymphocytes (20–40%) and monocytes (2–10%), with relatively clear cytoplasm, and three types of granulocytes, whose cytoplasm is filled with granules. In contrast to red blood cells, white blood cells have nuclei.

Details of common assays for monitoring PAH exposure

³²P-post-labelling. ³²P-post-labelling procedures for detecting PAC–DNA adducts are all based on a method originally developed by Randerath *et al.* (1981). Following isolation of DNA it is first enzymically digested to deoxyribonucleoside 3′-monophosphates. Adducts are then enriched by a variety of methods, including butanol extraction (Gupta, 1985), nuclease P1 digestion of normal nucleotides (Reddy and Randerath, 1986) or immunaffinity chromatography. Adducts are then radiolabelled by phosphorylation using high specific activity [³²P]ATP and T4 polynucleotide kinase. Labelled adducts are usually separated as 3′,5′-bisphosphates by multidimensional ion exchange thin layer chromatography. Typical sensitivity of detection for PAC adducts is ~1 adduct/10⁹ normal nucleotides using microgram quantities of DNA. This correlates to ~1 adduct/cell.

Immunoassays. Both polyclonal and monoclonal antibodies have been generated against a variety of PAC carcinogen adducts for their immunoassay, although some show considerable cross-reactivity. The most common procedures include enzyme-linked immunoabsorbent assay (ELISA), competitive radioimmunoassay (RIA) and ultra-sensitive enzymatic radioimmunoassay (USERIA). Immunochemical methods for detecting PAC–DNA adducts are generally less sensitive than ³²P-post-labelling, but are simple and relatively inexpensive. An important use of antibodies is in immunoaffinity chromatography methods that are very effective for enrichment of adducts prior to analysis by physicochemical methods, such as post-labelling or mass spectrometry. Details of the use of immunoassays for detecting carcinogen–DNA adducts have been reviewed by Poirier (1991).

Table A1. Continued

Cat.	N	Exposure		Σ8PAHs (µg/m ³)		BaP (µg/m ³)		1-Hydroxypyrene [urinary 1OHPy excretion (µmol/mol creatinine)]		DNA adducts ³² P-post-labelling (adducts/10 ⁸ nucleotides)		Chromosome aberrations (% CA)		Sister chromatid exchange (SCE/cell)		Micronuclei (MN/100 cells)		Reference	
		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean		Min
exp high	76	4.5	0.135	200	0.006	42				4.1	2.47	5.7							Lewtas <i>et al.</i> (1997)
envir	51	0.01	0.002	0.03	0.0001	0.005				4.7									
exp	23	15			1.7					7.1				6.2					Popp <i>et al.</i> (1997)
c										4.5				6.7					
exp	68									1.7									Ovrebo <i>et al.</i> (1992)
c	13									1.5									
exp	56	11*	1	186	0.98	0.1	7.80			5.1	ELISA								van Schooten <i>et al.</i> (1990)
c	44			*sum 4-6 ring						2.7									
exp	63	34			1.9					1.6	0.5	4.4							Zhang <i>et al.</i> (2000)
Aluminium mfg																			
exp	94	9.8	1.0	153	0.97	0.02	24	4.3	0.09	17.7	2.6	1.4	6.7			0.7	0	4.7	Carstensen <i>et al.</i> (1999a,b)
c	54	nd			nd			0.13	0.06	0.8	2.5	1.3	6.9			0.8	0	3	
exp	6	9.3			0.93			3											Tjoe Ny <i>et al.</i> (1993)
exp	10	140			14			18											
c	10				-			0.43											
exp	2	14.6			1.58			2.9											Yu Duc and Lafontaine (1996)
exp	67	4.7			0.01	1.2		10	38										Mannschreck <i>et al.</i> (1996)

Table A1. Continued

Cat.	N	Exposure	Σ 8PAHs ($\mu\text{g}/\text{m}^3$)			BaP ($\mu\text{g}/\text{m}^3$)			1-Hydroxypyrene [urinary 1OHPy excretion ($\mu\text{mol}/\text{mol}$ creatinine)]	DNA adducts ^{32}P -post-labelling (adducts/ 10^8 nucleotides)	Chromosome aberrations (% CA)	Sister chromatid exchange (SCE/cell)	Micronuclei (MN/100 cells)	Reference			
			Mean	Min	Max	Mean	Min	Max							Mean	Min	Max
1990																	
exp		0.05			0.005				2								
c									1								
1993																	
exp																	
c									1								
									1								
Pavers																	
Coal tar																	
exp	28				2.5	0.9	3.2							Jongeneelen <i>et al.</i> (1988)			
c	90				0.27	0.02	1.3										
Bitumen																	
exp	39				0.61									Burgaz <i>et al.</i> (1991, 1992)			
c	28				0.28												
exp	28				0.78	0.32	2.2				5.1	4	6.6	2.3	1.6	2.9	Burgaz <i>et al.</i> (1998)
c	28				0.52	bdl	1.7				4.7	3.6	6.1	1.8	1.3	2.6	
exp	3				0.6												Jongeneelen <i>et al.</i> (1988)
c	90				0.27	0.02	1.3										
exp	14	0.027	0.001	0.081	0.0027	0.0001	0.0081	0.5	0.1	1.4	36	2	122	in exfoliated			Zhou (1997)
c	11				0.2	0.1	0.2	0.2	0.1	0.2	22	2.2	74	urothelial cells			

Table A1. Continued

Cat.	N	Exposure			1-Hydroxypyrene [urinary 1OHPy excretion (μmol/mol creatinine)]			DNA adducts ³² P-post-labelling (adducts/10 ⁸ nucleotides)			Chromosome aberrations (% CA)			Sister chromatid exchange (SCE/cell)			Micronuclei (MN/100 cells)			Reference
		Σ8PAHs (μg/m ³)			BaP (μg/m ³)			Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	
		Mean	Min	Max	Mean	Min	Max													
Rural																				
winter	22			0.006						3.1	0.52	7.5	3.5	3	4	8.1	6	10		
summer	22									2.9	0.6	11.1	2.8	2	5	8.7	7	11		
Urban																				
winter	24						0.34	0.11	0.57											Szaniszo and Ungvary (2001)
urban bus drivers	49		**	0.0039	0.001	0.02				40										Nielsen <i>et al.</i> (1996a)
suburban bus drivers	41									18										
rural	60		**		8E-05	0.0005				2.4										
urban	56	>0.02	0.01	0.02						6.5										Binkova <i>et al.</i> (1995)
		<0.01			0.002	0.004				4.4										
					<0.002					4.0										
envir	51	0.01	0.002	0.03						4.7										Lewtas <i>et al.</i> (1997)
near coke ovens	19				0.015	0.057				10	5.6	19								Hemminki <i>et al.</i> (1990b)
rural	14									5.6	2.7	12								
Urban Germany	61				0.015	0.007				2.8	3									Poirier (1999)
Kuwait	61				<0.00023					1.7										

N, no. of workers; exp, exposed; c, controls. Estimated Σ8PAH exposures in italics. Deviating units or methods highlighted in bold.

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