Yonsei Medical Journal
Vol. 37, No. 1, 1996

□ Review Article □

Molecular Biomarkers of Occupational Lung Cancer

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Occupational exposures to certain metals, hydrocarbons and ionizing radiation are associated with increased lung cancer in workers; because these exposures continue, lung cancer remains an important problem in industrialized nations. The gravity of the lung cancer, specifically the low cure rate associated with the disease, has forced researchers to focus efforts at developing biological indicators (biomarkers) of carcinogen exposure and early, reversible effects. This review examines critically the development of these biomarkers for occupational and environmental exposures to polycyclic aromatic hydrocarbons (PAH), a ubiquitous class of lung carcinogens. Biomarkers of several different stages of the carcinogenic process have been proposed. Industrial hygiene and occupational health emphasize exposure and disease prevention. For this reason, biomarkers useful in industrial hygiene practice are those which measure events prior to the initiation phase of carcinogenesis; markers of later events which have a greater positive predictive value may measure irreversible effects and are more appropriate for disease screening and epidemiology. One of the strengths of biological monitoring is that exposures and effects can be measured regardless of route. Data indicates that the dermal route may be a significant pathway for delivery of PAH to the lung. This finding has important ramifications because as airborne exposure limits decrease the relative impact of dermal absorption is increased.

Key Words: Occupational lung cancer, biomonitoring, biomarkers DNA adduct

INTRODUCTION

Lung cancer remains one of the most serious and troubling occupational diseases. The causes and pathogenesis of the disease make it is difficult to accurately estimate the proportion of lung cancer cases attributable to occupation and those caused by other environmental agents such as tobacco smoking. However, there is good epidemiological evidence to suggest that several occupational groups are at significantly increased risk for developing lung cancer. These include steelworkers who were found to have 33-fold greater risk of lung cancer than the controls (Parkinson, 1984), and coke oven workers who have a 5~10 fold increased lung cancer risk.

Table 1 gives a list of the agents identified or strongly suspected of being occupational lung carcinogens. For the purposes of this paper attention will be restricted to complex mixtures containing polycyclic aromatic hydrocarbons (PAH), except where noted. Table 2 gives a list of occupations with potential exposure to coal tar, while Table 3 lists complex mixtures containing PAH. Clearly, there are large number of workers who may

Received January 30, 1996

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Table 1. Occupational lung carcinogens

Arsenic	Asbestos
Beryllium	Bis(chloromethyl) ether
Cadmium	Chloromethyl ethyl ether
Chloroprene	chromates
Coke oven emissions	Lead
Mustard gas	Nickel
Soots and tars	Tobacco smoke
Uranium	Vinyl chloride

Table 2. Occupations with potential exposure to coal tar

Asphalt workers	Boat builders
Briquette makers	Coal tar workers
Coke oven workers	Creosoters
Electrode makers	Flue cleaners
Furnacemen	Pavers
Pitchworkers	Railroad track workers
Road workers	Roofers
Rubber workers	Shinglemakers
Tile pressers	Waterproofers

Table 3. Some complex mixtures containing polycyclic aromatic hydrocarbons

Aluminum reduction electrodes
Asphalt and crumb rubber asphalt
Coal tar pithch
Coke oven tar
Combustion products, in general, e.g., foundries
Marijuana smoke
Roofing tar
Tobacco smoke
Used gasoline engie oil

be exposed to these agents. The focus of this paper will be to discuss the advances that have been made in developing biomarkers of exposure and effects for lung carcinogens and to put these markers in a theoretical framework relevant to all of us who work in occupational health, namely, the prevention of occupational disease.

Lung cancer is one of the most important disease in the world as there were 885,000 cases with 770,000 deaths worldwide in 1985 (Parkin *et al.* 1993). The lung is the site of 11.9% of all tumors worldwide, making lung cancer the most common neoplastic disease in the world. The prognosis for diagnosed cases remains grim, with only 13% of the patients alive five years following diagnosis. In the United States, lung cancer accounts for 28% of all cancers. There were 170,000 cases of lung cancer in the United States in 1993 and 149,000 deaths (Boring *et al.* 1993) and there is virtually no difference in the 5 year survival rate in the United States and the rest of the world. Thus, an educated public and the availability of modern medical services which should provide for early diagnosis and treatment have had little impact on the course of this disease.

It has been estimated that 76% of all US lung cancer cases are due to tobacco smoking (Samet and Lerchen, 1984). It is amazing how the curves for tobacco sales and lung cancer rates overlap given a 20~25 year latency period (Fig. 1)(Boring *et al.* 1993). Tobacco smoking has a further impact, particularly important in lung cancer, in our ability to differentiate cases arising from occupational exposure and from those due to cigarette smoking. This problem stems from the fact that while there is a fairly long list of occupational lung carcinogens (Table 1), the great majority of workers at risk are exposed to soots, tars and related combustion products and other complex mixtures which have similar chemical composition to tobacco smoke. Therefore, the specific chemical agents which cause lung cancer from smoking and those caused by most occupational exposures will be the same or very closely related. Therefore, the "trail" left by both of these exposures in terms of the biomarkers are very similar. The potential for interaction (potentiation, additive and synergistic effects) between occupational and environmental exposures has made it difficult to determine the proportion of total lung cancers directly attributable to occupation. Various estimates of the population attributable

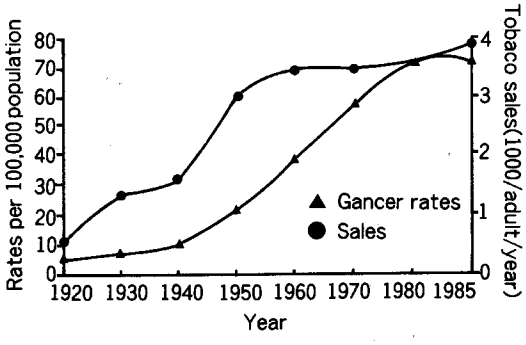


Fig. 1. *Relationship between cigarette sales and lung cancer in U.S. males.*

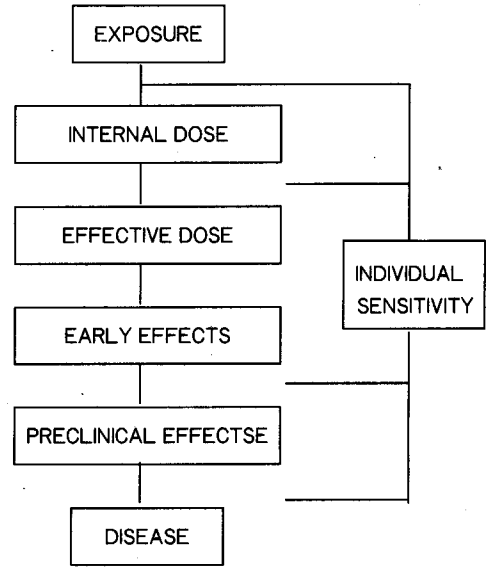


Fig. 2. *Model continuum between environmental exposure and disease, with various levels of biomarkers indicated.*

risk for lung cancaer have been made and range from 1~2% to 15% in males and 5% in females to 12% in English males (Samet and Lerchen, 1984). However, even if the lowest of these estimates are accurate, it is clear that the health effects of certain occupations is profound; some groups of exposed workers have been shown to be at from 10~50 times greater risk than were their controls (Parkinson, 1984; Gibbs and Horowitz, 1977).

There are several important reasons why the development of biomonitoring methods is highly desirable for occupational lung cancer and carcinogens. The main function of industrial hygiene and all occupational health professions is to prevent occupational disease. Lung cancer is a deadly disease, with such a low cure rate and a high rate of reoccurrence that efforts should be focussed toward primary prevention, i.e., removal of exposure. Lung cancer advances in discrete premalignant stages including hyperplasia, metaplasia, dysplasia and carcinoma in situ. If biomarkers could be found that are indicative of earlier stages it is possible that preventive measures (e.g., vitamin or nutrient therapy) could halt the progress of the disease. On the other hand there is some doubt that secondary prevention of this type would be useful because even with early detection as currently defined, the mortality rate from lung cancer is extremely high. In addition, there is strong evidence for the theory of "field cancerization" with lung cancer. This theory is based on the random probability of critical mutations in populations of cells in the lung exposed to carcinogens. Because with exposure magnitude or duration, most cells will sustain mutagenic hits and there is increasing probability that there will be multiple, independent tumors. To support this theory it has been shown that the lungs of persons with primary tumors have many areas of aneuploidy and other atypical molecular and cytogenetic aneuploidy distant from the primary tumor. In addition it has been reported that about 50% of long term survivors of lung cancer develop new primary tumors (Gazdar and Carbone, 1994). These data reinforce the notion that primary prevention should be the major tool against lung cancer.

The most promising aspect for biomarkers of occupational carcinogens is their potential use in primary prevention as sentinels of significant exposure and early effects of carcinogens. Biomarkers can be used to guide and monitor the efficacy of exposure intervention strategies. Fig. 2 displays a model for a linear relationship between exposure and disease with several intervening stages. If biomarkers

are available which represent these stages, they can be exploited to characterize exposure in an increasingly selective way relative to the disease. It is clear from the graph that when a marker is measured that is closer to the occurrence of disease, the better will be the ability to predict the disease. On the other hand, implicit in Figure 2 is the possibility that certain markers may be too closely related to the disease outcome to be useful in the prevention of occupational disease. To review the figure briefly: a fraction of the total external dose is absorbed. Measuring the internal rather than the external dose incorporates exposure from all routes and the use of personnel protective equipment. A fraction of the internal dose either is or becomes metabolized to a form capable of interacting with the critical cellular targets. This is the effective dose. A fraction of this dose actually does interact with those targets to cause early effects. These effects can range from those which are reversible and those which have a very low probability of developing into a neoplasm to those which are more closely related to the disease process.

The marker should measure an event in the disease process, making it biologically relevant to the disease. Measurement of the marker should also theoretically accommodate individual differences in exposure and susceptibility. The marker should be readily detectable and show a dose-response. A most important criteria for a marker in occupational health is that the marker measures either a reversible event or one that has a low probability of resulting in disease so that if interventions are utilized, both the level of the marker and the risk of disease will be decreased.

BIOMOLECULAR MARKERS OF INTERNAL DOSE

The two most frequently used measures of internal dose for PAH carcinogen exposure are urinary levels mutagenicity and 1-hydroxypyrene, the initial metabolite of pyrene a common component of most complex mixtures (Lesage *et al.* 1987). Urinary mutagenicity using a modified Ames Salmonella typhimurium assay was proposed for use in the mid-1970's as a general human monitor. This is a simple assay which can be done using a single urine sample from each individual. While this assay has been losing favor to more molecular based assays, it is useful to examine its potentials and shortcomings in some detail. It is important to appreciate that particular biomarkers reflect facets of biology and have strengths and weaknesses based on what measurements are made. For example, because the *Salmonella* assay measures total mutagenic activity in the urine, the assay integrates for exposures by all routes as long as the putative mutagens are excreted by this route. On the other hand, the assay as usually performed in monitoring studies incorporates enzymatic procedures to activate the unmetabolized parent compound (S-9 mix) and break conjugations to release the active forms of the detoxified agent, β-glucuronidase and sulfatase. Understanding this, it can be readily appreciated that persons who might be more apt to excrete detoxified forms would be reported at increased risk when in fact they are likely to be at lower risk for the same amount of exposure. This potential problem can be remedied by running the assays separately both with and without the individual enzymes.

Another measure of internal dose which has found increasing favor in occupational monitoring studies is measurement of urinary excretion of 1-hydroxypyrene, the initial metabolite of pyrene, a non-carcinogenic compound commonly found in complex mixtures of PAH. Since pyrene is a similar carcinogenic PAH in terms of vapor pressures and lipophilicity, this measurement is anticipated to serve as a surrogate marker for exposure to all PAH in a mixture. Clearly, it is not a measure of risk because no estimate is made of absorption of carcinogenic components in the mixture, and hydroxylations tend to be detoxification reactions for carcinogenic PAH. Therefore 1-hydroxypyrene measurement is also a non-specific marker which is not directly related to the carcinogenic process. There have been several studies indicating that 1-hydroxypyrene measurement can be very use-

ful in exposure assessment. Jongeneelen *et al.* (1988) examined the excretion of 1-hydroxypyrene in 40 workers exposed to coal tar derived road tars. They found that there was a consistent and significant difference between the pre- and post-shift levels of this metabolite at 4 different paving sites. They also found that there was a significant correlation between the amount of pyrene recovered from the hands of the workers and the post-shift 1-hydroxypyrene levels, suggesting that dermal absorption was a major route of exposure. In order to resolve the relative contributions of the inhalation and dermal routes to total uptake of PAH, dermal and breathing zone pyrene measurements were taken on a group of 12 coke oven workers (VanRooij *et al.* 1993). There were exposure related increases in post-shift 1-hydroxypyrene levels which were highly correlated with dermal and inhalation exposure and less so with smoking and alcohol use. Using a multiple regression model, the authors estimated that 75% of the total pyrene dose entered the body through the skin, documenting the importance of this route.

It is important that a biomarker respond to changes in exposure, decreasing when exposures are decreased and *vice* versa. Air, skin and 1-hydroxypyrene levels were determined in a group of workers exposed to creosote for two consecutive weeks (VanRooij *et al.* 1993). Workers wore coveralls and gloves one week, but not the other. There were significant reductions in the dermal exposures and in the levels of 1-hydroxypyrene when the coveralls were worn. These data reinforce the notion that dermal exposure plays a significant role in total exposure and that urinary 1-hydroxypyrene levels can be used to assess changes in exposures. Similarly, simple hygienic interventions were emplaced in a group of coke oven workers (VanRooij *et al.* 1994). These consisted of frequent hand and face washing and more regular changes of work-clothes. Again, there was a significant decrease in the excretion of 1-hydroxypyrene in the workers when they reduced the amount of pyrene and PAH on their skins.

Several workers have determined the effect of tobacco smoking on excretion of 1-hydroxypyrene. VanRooij *et al.* (1994) saw that exposed smokers excreted higher levels of 1-hydroxypyrene than did non-smokers and that there was a linear dose-response between number of cigarettes smoked and levels of the biomarker. However, when there is exposure to PAH atmospheres in coal tar pitch, coke ovens or in creosote plants, smoking appears to have a lesser effect than does occupational PAH exposure (VanRooij *et al.* 1993; 1994). So, although there is an effect of smoking on urinary excretion of 1-hydroxypyrene, unlike urinary mutagenicity, this does not seem to be a main effect. Diet is another potential confounder because fairly large amounts of pyrene and other PAH may be absorbed from the gastrointestinal tract from food that is fried or broiled. Buckley and Lioy (1992) saw that there was no real effect of increasing dietary PAH. VanRooij *et al.* (1994) saw that while dietary effects were significant in a large group of non-exposed smokers and nonsmokers, diet explained only a very small proportion of the variance. Nonetheless, these potential confounders should be examined in any study involving measurement of 1-hydroxypyrene.

More serious potential problems using 1-hydroxypyrene as a monitoring method include the apparent facts that there are variable amounts of pyrene in different exposures and, more importantly, that there appear to be real differences between individuals in the

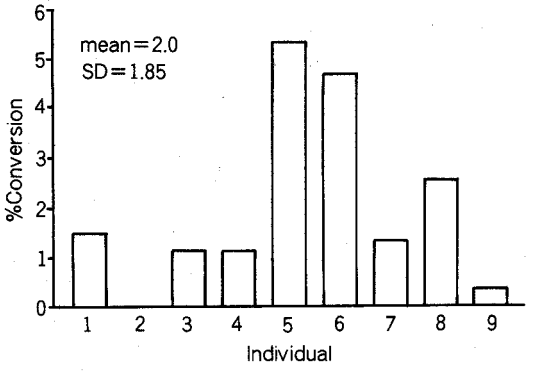


Fig. 3. *Variability in the in vitro conversion of pyrene to 1-hydroxypyrene.*

metabolism of pyrene to 1-hydroxypyrene. Figure 3 displays work presented by Grimmer (1990) who saw that there was at least a 10 fold variability between the abilities of microsomal preparations from 9 individuals to convert pyrene to 1-hydroxypyrene.

This data suggests the potential for misclassification exists. More work must be done to determine the impact of this in vitro variability in vivo.

New, more sensitive methods of performing 1-hydroxypyrene determinations have lately been published (Strickland *et al.* 1994; Hansen *et al.* 1993). These will decrease the exposure necessary to obtain a positive result, but will require field validation.

Again, since 1-hydroxypyrene is a metabolite of a non-carcinogenic PAH, it is not related to risk of genetic disease except inasmuch as presence of this marker indicates exposure to this class of compounds. To examine risk and the individual variability in susceptibility, measurements must be made of the amount of ingested carcinogen converted to an active form.

Other markers of internal dose are now being investigated. Most promising is the measurement of benzo(a)pyrene (BAP) tetrol by synchronous fluorescence (Weston *et al.* 1993). This metabolite of BAP represents the diol epoxide fraction of the total dose which did not bind to other macromolecules, but was oxidized and hydrolyzed twice by P-450 enzymes and epoxide hydrase. As such, this metabolite represent the fraction of the activated compound which is detoxified. No human studies using this marker have been reported to date, however.

BIOMARKERS OF CARCINOGEN EFFECTIVE DOSE

The effective dose of carcinogen is that fraction of the total absorbed dose which is in a form capable of reacting with DNA to produce a mutagenic alteration. This dose can be measured directly in DNA, or, surrogates biomolecular markers which trap the reactive species can be measured as surrogates.

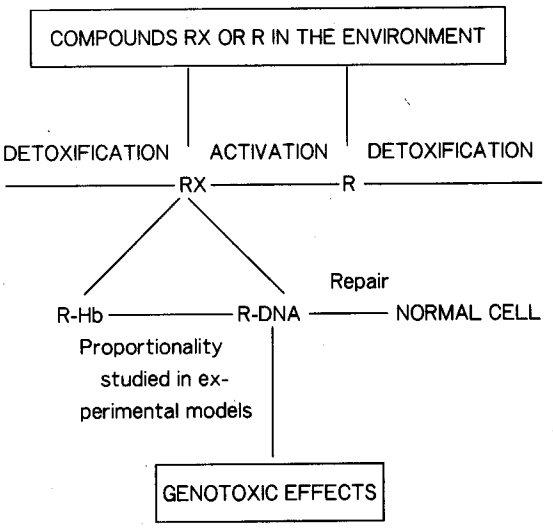


Fig. 4. *Scheme for the activation, detoxification and repair of carcinogen-induced damage. The potential for biomarker application and interaction is indicated.*

Carcinogen-hemoglobin adducts

Fig. 4 shows the rationale for selection of effective dose biomarkers. Some carcinogens in the environment can be absorbed in an reactive form (RX in the figure), such as is the case with bis(chloromethyl)ether, dimethyl sulfate, ethylene imine, or ethylene oxide. More frequently, however, the carcinogen is absorbed in a procarcinogenic form ("R") which is then metabolically activated to the reactive moiety by the metabolic enzymes of the host. PAH in the environment are good examples of the latter class. Once absorbed a procarcinogen is metabolized to either a detoxi-fied or active form. Which path is taken depends on the interaction between the specific substrate(s) and the metabolism of the host. This initial metabolism appears to be a major factor in individual specificity (Talaska et al. 1992). Once activated (RX) the carcinogen may react with any of several biomolecules, proteins, sugars, RNA or DNA. Since DNA is the genetic material, reactions with the other biomolecules actually consti-

tutes a detoxification pathway. There are several biological molecules, e.g., glutathione, glucuronic acid, which can conjugate activated carcinogens and facilitate their excretion and result in detoxification. These react with electrophilic carcinogens because they have nucleophilic character. Other nucleophilic sites are found on certain proteins. One protein which has been proposed and used for monitoring purposes is hemoglobin (Hb). While the reaction of a carcinogen is not important biologically, the same reactive form of the carcinogen reacts with either DNA or Hb. As indicated in the figure, there likely exists a proportionality between the Hb and DNA binding for any given compound. Once the proportionality is determined, it can be used to predict the DNA-mediated effects. The best examples of the use of Hb adducts in monitoring for genotoxic exposure are found in the literature of tobacco smoking-related human urinary bladder cancer and 4-aminobiphenyl-hemoglobin adducts (Bartsch *et al.* 1990; Bryant *et al.* 1988; 1987).

Thioethers

The active form of the carcinogen may be detoxified by binding to nucleophiles on biomolecules other than DNA. Important among these nucleophilic "sinks" is glutathione a tri-peptide containing a cysteine residue and a nucleophilic sulfur. Once carcinogen binding occurs, glutathione is hydrolyzed to the corresponding mercapturic acid and excreted in the urine. It has been proposed that measurement of the excreted mercapturic acids as generic thioethers can be used to monitor exposure and effective dose. There has been one occupational study using this marker (Pasquni *et al.* 1989). Pasquini *et al.* (1989) monitored the excretion of thioethers and mutagens in the urines of road pavers and their controls. There was a consistent, but very modest increase in the excretion of thioethers in the exposed group and in smokers, however, the differences were not statistically significant.

Carcinogen-DNA adducts

Carcinogen-DNA adducts are the products of reactions between electrophilic carcinogens and nucleophilic sites on DNA. These are the initial genetic lesions induced by many, but by no means all, chemical carcinogens (Talaska *et al.* 1992; Kaderlik *et al.* 1992; Hemminki, 1993). DNA adducts can cause the DNA polymerase

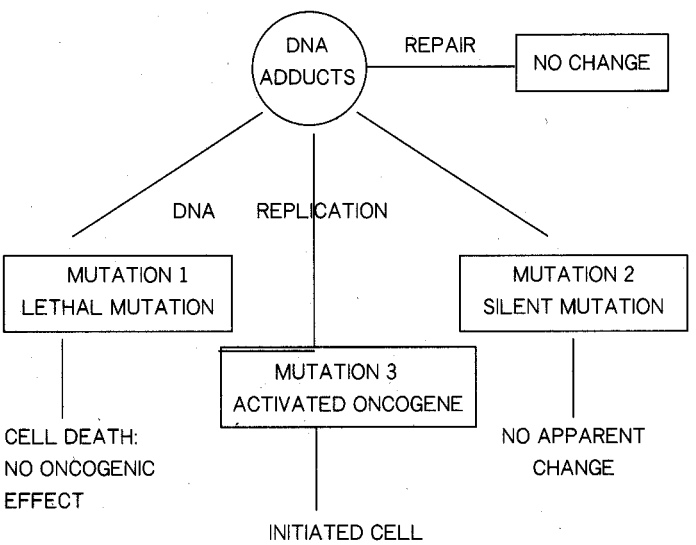


Fig. 5. *Biological effects which could happen subsequent to the presence of carcinogen-DNA adducts in a cell.*

to make errors during replication and induce mutations. Adducts have been shown to be a better predictor of mutation, chromosome aberrations and cancer than is crude applied dose in animal studies. The advantages of using carcinogen-DNA adduct analysis for carcinogen monitoring include integration of exposure and individual risk factors, direct linkage to genetic effects, and, for industrial hygiene purposes, the fact that there is a low probability that any single adduct will result in the development of a tumor. Figure 5 shows that there are many possible outcomes following the formation of carcinogen-DNA adducts. Therefore, if carcinogen-DNA adducts are detected and then the exposure is terminated there is a low probability that a neoplasm will develop. For these reasons we feel that measurement of carcinogen-DNA adducts has the potential to be an excellent tool to determine significant carcinogen exposure. Research has been done showing that measurement of adducts responds in a dose-dependent fashion to exposure, interventions and genetic effect modifiers. A recent prospective study has shown that the presence of aflatoxin-DNA in the urine of exposed persons is associated to an increased liver cancer rate in those persons (Ross *et al.* 1992).

The most successful method to monitor carcinogen-DNA adducts has been ^{32}P-postlabelling. This technique involves the labelling of adducted nucleotides with [^{32}P]ATP followed by chromatography to resolve the various adducts. The technique has been shown to be most suitable for bulky aromatic carcinogens like PAH, but has been used with a variety of carcinogens. There have been several recent reviews of this method (Talaska *et al.* 1992; Kaderlik *et al.* 1992). The major advantages of ^{32}P-postlabelling are sensitivity and ability to detect carcinogen-DNA adducts from a wide variety of carcinogens. The major disadvantages of the technique are the variable recovery of particular adducts and the lack of information on adduct identification. Although this technique is still considered experimental, it has been used in many studies of exposed human populations.

The enzyme linked immunosorbant assay (ELISA) is another technique for carcinogen-DNA adduct monitoring that has proven to be useful in human monitoring studies. This assay is based on the development of a polyclonal antibody raised against a specific carcinogen-DNA adduct. Because the antibody is polyclonal it will recognize the adduct against which it was raised plus adducts which have similar idiotypes (Roberts *et al.* 1988). Thus, closely related PAH adducts can often be detected using an antibody raised against the N2-adduct of BAP (Zhang *et al.* 1990; Santella *et al.* 1993). Several of the studies discussed below have made use of this assay. To be useful in human monitoring a method must undergo validation at several levels with increasing stringency of response (Schulte and Talaska, 1994). Early studies of carcinogen-DNA adduct levels in groups exposed to tobacco smoke demonstrated a dose-response with increasing exposure for lung tissue. Figure 6 plots the data from Philips *et al.* (1988). As can be seen there appears to be considerable variability in response. This variability could be due to assay or individual variability. Figure 7 shows data from our lab indicating that there is significant variability when the same human urinary bladder DNA samples were reanalyzed. Similar results in human lymphocytes have been published (Szyfter and Hemminki, 1992). Fig. 8 gives data from our lab showing that there were

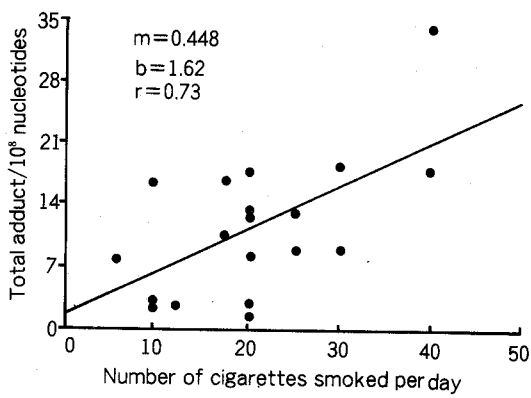


Fig. 6. *Carcinogen-DNA adducts in the lungs of smokers. Data are from Phillips et al. 1988.*

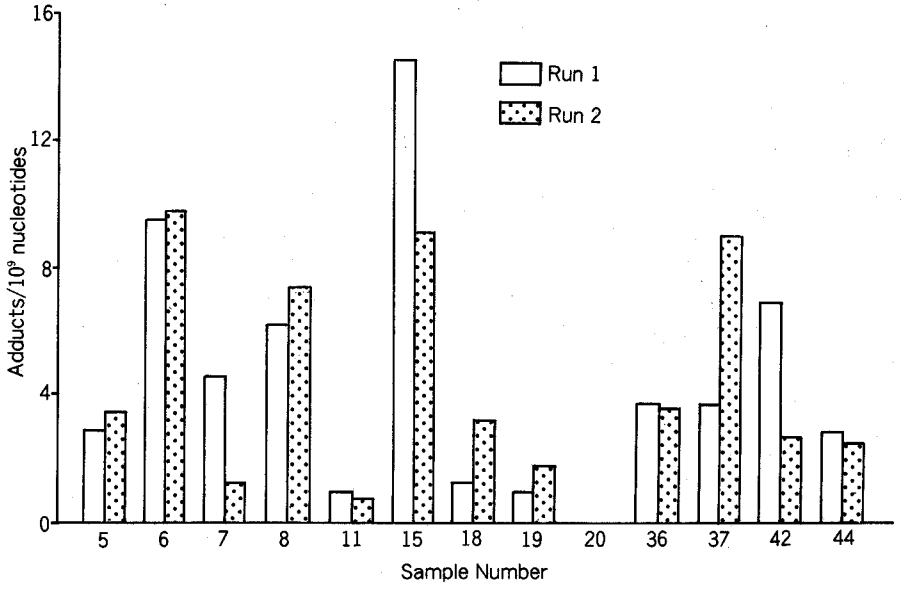


Fig. 7. *Repeat variability in adduct levels in human urinary bladder biopsy samples.*

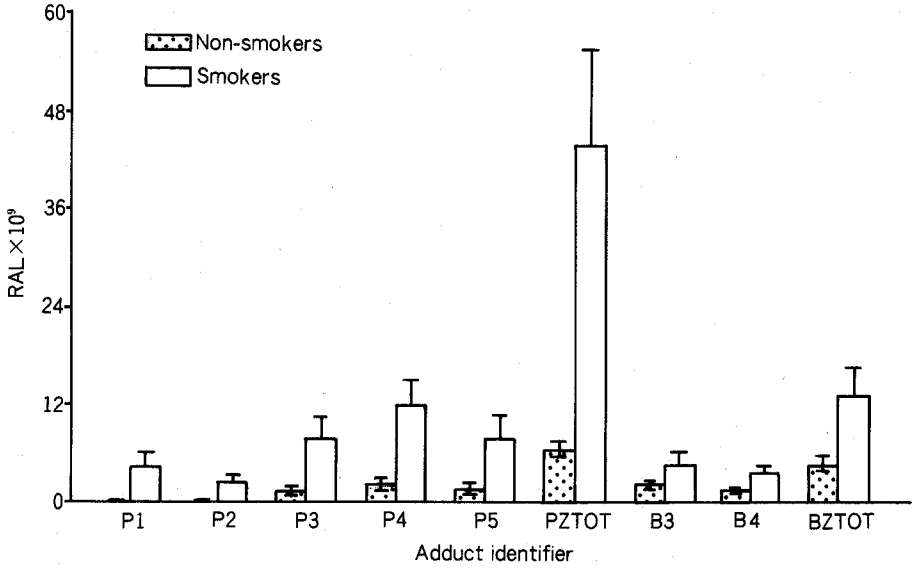
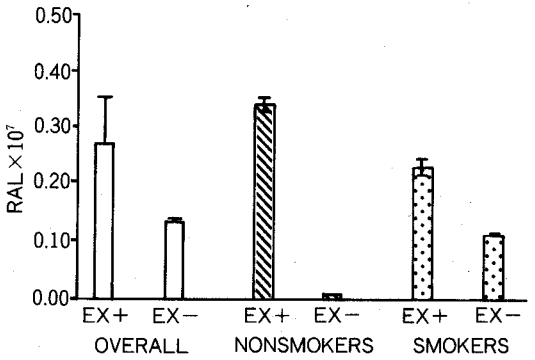


Fig. 8. *Significantly elevated carcinogen-DNA adducts in the lungs of tobacco smokers versus non-smokers. Adducts denoted as P1 through PZTOT are those which are seen when the samples were analyzed using the nuclease P1 variant of the ^{32}P-postlabelling method. Adducts indicated as B3 through BZTOT are those significantly elevated when hydrolyzed DNA's were extracteed with n-butanol prior to ^{32}P-postlabelling.*

statistically significant differences in the mean levels of carcinogen-DNA adducts in the lungs of smokers and non-smokers (Ketters *et al.* 1992). These data are interesting because they show a 4-9 fold increase in adduct levels in the smokers; consistent with the increased cancer risk seen in these persons (Boring *et al.* 1993).

In the studies mentioned above, section of tissue were obtained from donors during surgery. Non-invasive techniques must also be developed before a monitoring method can be useful in population studies. Human lymphocytes are attractive for this purpose because they are exposed to agents which enter the blood and can be obtained easily. Some very interesting occupational studies have been performed using blood lymphocytes. Increases in adduct levels were seen in Finnish foundry workers (Hemminki *et al.* 1990). Categorical analysis of the data indicated that 3 of 4 persons with high exposure ($>2\,\mu g/m^3$) were positive for adducts as were 8 of 10 with medium exposure, 4 of 18 with low exposure and 1 of 9 unexposed controls. Quantitatively, the adduct levels were 9 times higher in the highest exposed group relative to the controls and any exposure led to a 4 fold increase in adduct levels. Somewhat surprisingly, there was no co-chromatography of any of the adducts seen in these samples with an authentic BAP-DNA adduct standard. These authors also found that person working in specific operations (e.g., sand preparation and transport), which involved the highest levels of exposure to PAH also had the highest levels of carcinogen-DNA adducts. Polish coke oven workers were also seen to have greatly increased levels of carcinogen-DNA adducts (Hemminki *et al.* 1990; 1990; Philips *et al.* 1988). In these studies workers employed in the coke oven batteries had significantly higher levels of adducts than did persons who did no battery work. Roofers are exposed to significant levels of coal tar pitch and the PAH associated with this complex mixture (Herbert *et al.* 1990). Figure 9 shows that the carcinogen-DNA adduct levels in the lymphocytes of roofers was slightly greater than twice that of non-exposed controls. When stratified by



Herber, et al, Scand. J. Work Environ. Health, 16 : 135.

Fig. 9. *Lymphocyte DNA adduct levels in roofers by exposure and smoking status. Data are from Herbert et al. 1990.*

smoking status, the effect of exposure can still be seen, particularly in the non-smoking group. This data indicates that adduct levels may be more selective in their response to occupational exposure than is urinary mutagenicity, for example. This has been seen by several workers (Hemminki *et al.* 1990; Philips *et al.* 1988; Herbert *et al.* 1990). The relative importance of dermal exposure to total exposure may play a role in this responsiveness. Schoket *et al.* (1991) measured the carcinogen-DNA adduct levels lymphocytes of two groups of aluminum reduction plant workers. They found that the levels of adducts were about 3 times higher in the lymphocytes of each of the plants versus the controls. An effect of smoking was also reported but this was said to be of less import than occupational exposure. These data suggest that blood lymphocytes are specifically responsive to occupational exposures and could thus be used in human monitoring methods. However, the relationship between lymphocyte adduct levels and cancer risk is not known. In a chronic dog exposure study we saw that levels of adducts in the blood lymphocytes were on the order of 1/10 to 1/50th that in the target organ, and that there was no correlation between lymphocytes levels and those in the target organ. Reddy and Randerath (1990) saw that the adduct levels in lymphocytes for animals exposed to

BAP orally were correlated with the levels in the lung and liver. However, for two other carcinogens, dibenz(c, g)carbazole and safrole, no correlation was seen. Lewitas *et al.* (1993) saw that adduct levels in lymphocytes were consistently lower than in the target organs.

One approach to less invasive monitoring of the lung in person with carcinogen exposure is by using bronchoalveolar lavage. DeFlora *et al.* (1993) used synchronous fluorescence to determine the levels of BAP adducts in BAL of cigarette smokers. They found that adducts were not detectable in samples from non-or ex-smokers whereas positive responses were seen in 85% of current smokers. Unfortunately, Lewitas *et al.* (1993) reported that the levels of carcinogen-DNA adducts in bronchoalveolar lavage were as low or lower than those in blood lymphocytes.

Carcinogen-DNA adduct monitoring methods using cells exfoliated from the lung and urinary bladder using sputum and urine were developed. The majority of these efforts have been directed in developing the exfoliated urothelial cell assay. In an animal model, the levels of carcinogen-DNA adducts in the exfoliated urothelial cells were approximately comparable to those in the target organ (Talaska *et al.* 1990). The levels of carcinogen-DNA adducts were higher in smokers relative to non-smokers and that there were statistically significant correlations between the amount smoked, the levels of hemoglobin adducts and the urinary mutagenicity for each individual (Talaska *et al.* 1990). The regression lines for Hb and DNA adducts in this study were parallel, suggesting that either could be used.

In order to be able to predict individual risk it is necessary that a marker respond to differences in important metabolic capabilities between individuals. Glutathione-Stransferase mu (GST-μ) is reported to important in the detoxification of lung carcinogens (Sipes and Gandolfi, 1991). In a study involving biopsy samples of human lung, there was an inverse correlation between the levels of carcinogen-DNA adducts and GSTm activity in the individual lung samples (Ketters *et al.* 1992). It was also reported that individuals who are

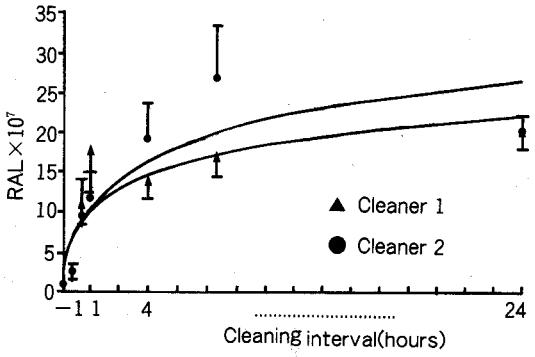


Fig. 10. *DNA adduct levels using nuclease P1 in the lungs of mice treated topically with used gasoline engine oils.*

slow acetylators are more likely to have specific carcinogen-DNA adducts in exfoliated urothelial cells (Vineis *et al.* 1994). These data suggest that analysis of carcinogen-DNA adducts may be response to these important individual differences in metabolic status.

Another criteria for the use of an assay as a biomarker carcinogen exposure or effect is the marker's response to exposure intervention. There have been several studies showing that carcinogen-DNA adduct analysis does respond to smoking cessation and when worker go on extended vacations (Hemminki *et al.* 1990; Philips *et al.* 1988). Carcinogen-DNA adduct analysis were used to determine the effectiveness interventions which reduce the absorption of carcinogens from the skin. Fig. 10 shows the plot of the DNA adduct levels in the lungs of animals treated topically with used gasoline engine oils (UGEO), a complex mixture known to contain PAH and be carcinogenic. It was found that when the skin was cleaned quickly after the exposure that the level of lung adducts remained relatively low. However, if cleaning was delayed for 4 or 8 hours, then there were no significant differences between the resulting lung adduct levels and those if cleaning was not done. It is interesting that the lung is still a major target for PAH-DNA adducts when complex mixtures are applied topically. It has been estimated that the skin has metabolic activity of about

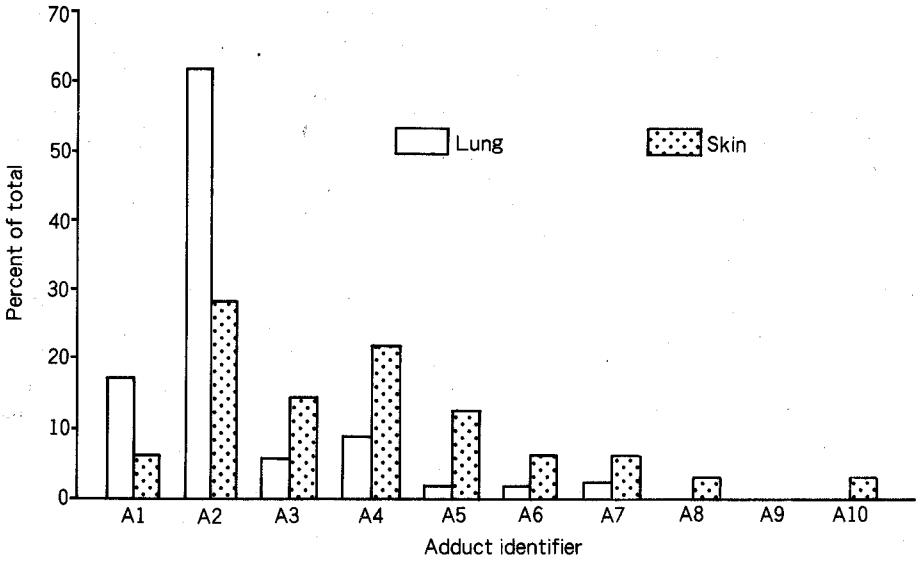


Fig. 11. *Relative proportions various DNA adduct from used gasoline engine oil treatment in the lungs and skins of mice treated topically.*

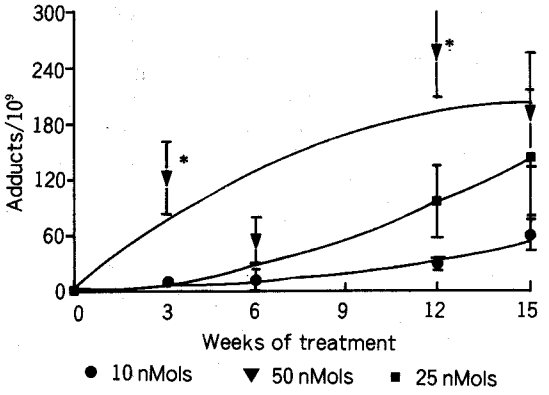


Fig. 12. *Accumulation of BAP-DNA adducts in skin during chronic topical exposure.*

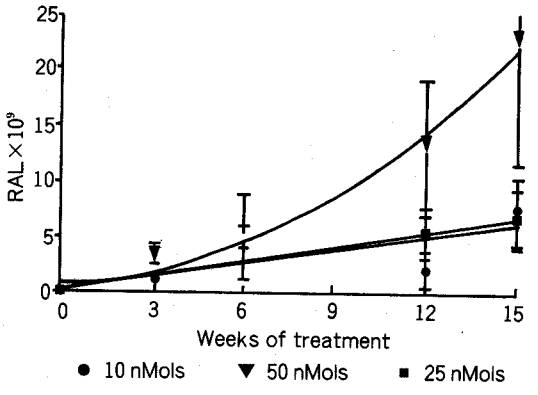


Fig. 13. *Accumulation of BAP-DNA adducts in the lung during chronic topical exposure.*

1/100th and 1/10th the liver and lung, respectively (Sipes and Gandolfi, 1991). Fig. 11 gives the relative proportion of individual adducts in lung and skin following topical application of UGEO. Although both tissues have some evidence of almost each adduct, adduct 2 (which we suspect is from BAP) is proportionately much higher in the lung than in the skin. In another study mice were treated twice weekly with 10, 25 or 50 nMols of BAP topically. Fig.12 shows the adduct levels in the skin for each dose and each dose has a distinct adduct accumulation curve. Notably, the 50 nMol dose appears to reach steady state in about 12 weeks. Fig. 13 gives the adduct accumulation curves in the lung. Note

that at the same time the adduct levels in the skin reach steady state, those in the lung increase rapidly. This indicates that metabolism in the skin becomes saturated and allows more parent compound to enter the blood to be transported to the lung where it is activated rapidly owing to the higher metabolic capability of that organ.

The analysis of carcinogen-DNA adduct levels is responsive to occupational and environmental lung carcinogens via all routes of exposure and to interventions and individual differences in metabolism. These markers can be chemically specific if standards are available (Talaska *et al.* 1991). The areas of further research that are needed include analysis of day-to-day variability in reported marker levels and a standard protocol to deal with daily and interlaboratory variability (Schulte and Talaska, 1994; Savela *et al.* 1989).

BIOMARKERS OF EARLY CARCINOGEN EFFECT

The heritable damage caused by carcinogen-DNA adducts is fixed during DNA replication and results in a change in the genetic material. Most often this damage occurs at sites which are not related to the carcinogenic process, silent or lethal mutations, for example. While these mutations are not directly related to carcinogenesis their occurrence can be exploited for monitoring purposes. There are generally two classes of markers in this regard: mutation assays which detect point mutations and others which cause loss of gene activity; and, cytogenetic markers which detect gene mutations.

Cytogenetic markers of early effect

Chromosome breakage results from DNA adduction due to the damage itself or because of misrepair. Most of the damage that occurs from the breakage of chromosomes involves significant genetic material and is, for this reason, lethal. More important for carcinogenesis are the genetic fragments which occur at breaks on different chromosomes which reanneal with the wrong chromosome before replication. Since there is no net loss of genetic material these translocations can be viable and, if they occur at critical sites can initiate carcinogenesis. It has been shown that the myc oncogene is activated in this manner (Battey *et al.* 1983). Translocations are relatively rare events, and, until recently, were very difficult and laborious to detect. The advent of chromosome "painting" techniques may make detection of these lesions easier, although they remain relatively rare events. It is more common to detect the more frequent lethal damage that occurs after exposure. The most commonly used and widely accepted assay of this type is the micronucleus assay.

Mutational markers of early effects

Several assays have been developed to measure the mutation rate using surrogate gene markers in humans. These represent extremely exciting areas of research and application to human monitoring. Two of these assays will be discussed here, the hypoxanthine-guanine phosphoribosoyl transferase (HGPRT) assay and the glycophorin A assay.

The HGPRT assay has been the most widely developed of the mutations assays and mutation rates have been determined for many exposures including ionizing radiation and tobacco smoking (Albertini *et al.* 1993). The assay detects mutant cells, deficient in HGPRT activity, by the fact that these cells will remain viable when grown in media containing 6-thioguanine. Wild-type cells incorporate toxic nucleotide analog into their DNA and are killed. The mutation determination is made in T-lymphocytes; these cells can incorporate damage over their lifespan (about 4 years). Several studies indicated that cigarette smokers and persons exposed to ionizing radiation have higher mutant frequency rates than non-smokers. Perera and co-workers have recently reported on results of a study done in foundry workers exposed to PAH (Perera *et al.* 1993). Determinations were made of PAH-DNA adducts and HGPRT mutations in lymphocytes and glycophorin A mutations on red blood cells. The authors reported in-

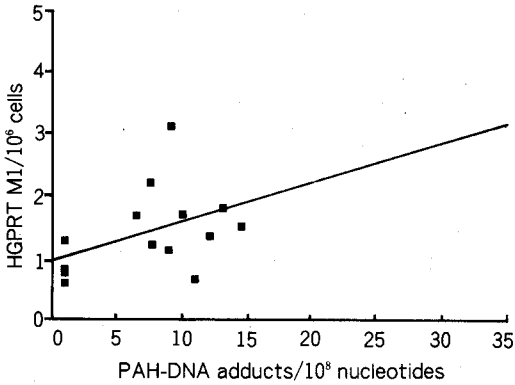


Fig. 14. *Correlation between PAH adducts and HGPRT mutaions in lymphocytes of foundry workers.*

creases in both PAH-DNA adduct levels and HGPRT mutations which were consistent with increasing exposure. Glycophorin A mutations were not increased. Interestingly, there was a better correlation between HGPRT mutations and PAH-DNA adducts than there was with airborne levels of PAH. This finding corroborates the role that DNA adducts as a better dosimeters of effective dose (Fig. 14).

The glycophorin A assay is based on the loss of a cell surface marker that occurs with several different types of mutation. The assay can only be done on 50% of the population, i. e., with those heterozygotic for glycophorins M and N. On the other hand the assay has the potential to detect a wider array of mutations than does the HGPRT assay, and also detect chromosome-chromosome interactions because the genes are located on an autosome (Chromosome 4) (Compton *et al.* 1991; Comptom-Quintana *et al.* 1993). The assay has been less well validated than the HGPRT assay, and has higher variability which precluded positive findings in an exposed foundry population.

PRECLINICAL MARKERS OF EFFECT

Markers of oncogene activation

Chemical carcinogens have been shown to mutate and activate oncogenes (Weinberg *et al.* 1989; Bailleul *et al.* 1989; Balmain *et al.* 1988; Bizub *et al.* 1986; Taylor *et al.* 1992). While the relationship between the activated oncogenes and cancer is not known with surety, activated oncogenes often appear in tumors and it appears that oncogene activation has a high positive predictive value for disease (Bennet *et al.* 1993). Unfortunately, the detection of activated oncogenes occurs at such a late stage in the exposure-response process so that disease might not be preventable in a significant number of cases. On the other hand, such information might prove extremely valuable in a medical surveillance program if it could be coupled with effective therapy.

Two oncogenes, in particular, appear to be associated with lung tumors. Activated ras oncogenes are found in about 30~50% of all lung tumors, particularly in adenocarcinomas. The mutations found on these activated genes at codons 12 and 61 are consistent with the sites mutated by PAH. In addition, overexpression of the ras gene product (*p21*) has been seen in about 50% of squamous cell carcinoma of the lung (Brandt-Rauf, 1991). In this regard, it has been proposed that measuring the levels of *p21* in serum could be used as a screening device to identify those workers with a high probability of developing disease. In a study of foundry workers who were shown to have increased PAH-DNA adduct levels in lymphocytes, increased levels of *p21* were seen in one worker. However, it has not been reported whether this person has developed a neoplasm (Brandt-Rauf *et al.* 1990). There are current investigations examining the role of other oncogenes in lung cancer, but these are at very early stages of development (Brandt-Rauf *et al.* 1994).

The *p53* oncogene has also been shown to be related to lung cancer. Studies by Harris and co-workers have shown that *p53* mutations are fairly early events in lung carcinogenesis, often occurring in pre-invasive lung lesions, as in dysplasia. This group has found that missense mutations in the *p53* gene often result in a protein that is only slowly degraded intracellularly and which can be detected by immunohistochemistry (Bennet

et al. 1993). Using these techniques this group screened tissues evaluated pathologically at various stages of malignancy from a series of 34 patients. They found immunohistochemical evidence for the mutated gene product in 0% of normal tissues, 6.7% of squamous metaplasias, 29.5% of mild dysplasias, 59.7% of severe dysplasias, 67.5% of microinvasive carcinomas, and 79.5% of invasive tumors. These data strongly indicate that mutations in p53 are intimately involved in the progression phase of carcinogenesis, after the cells are initiated.

These results will spur research for circulating markers of p53 oncoprotein mutations as it is clear that such invasive techniques as employed above will not be useful in population or occupational screening. However, this situation is reminiscent of that of the status of carcinogen-DNA adduct analysis 5 years ago before non-invasive techniques were developed and validated.

CONCLUSIONS

Biomolecular markers have been found which are indicative of the entire spectrum of the carcinogenic process, from exposure to disease. These biomarkers can be used to monitor primary and secondary prevention strategies as well as therapies. Which particular tool will be used will depend upon the outlook of the occupational health professional. From the industrial hygiene point of view, i.e., primary prevention, markers which indicate significant exposure and reversible effects are most attractive. Medical surveillance of already exposed populations is more interested in the markers with high positive predictive capabilities, like the activated oncoproteins. Clearly, the next 5 to 10 years will be devoted to research and validation of non-invasive markers of surrogate and target organ marker levels.

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