REVIEW

Determination of bulky DNA adducts in biomonitoring of carcinogenic chemical exposures: features and comparison of current techniques

Shu-Xin Qu, Cheng-Long Bai and Neill H. Stacey

DNA adducts resulting from carcinogenic chemicals are becoming more frequently used as a powerful biomarker to determine the extent of exposure. The available techniques for determination of bulky DNA adducts can be classified into four major groups, these being immunological assay, fluorescence techniques, chromatography/mass spectrometry and the ^{32}P-postlabelling technique. In this review, the features of current techniques and the combined applications are summarized and compared. Among the available techniques, the ^{32}P-postlabelling assay seems to best meet the application criteria overall. In particular, ^{32}P-postlabelling is the most suitable technique for the detection of bulky DNA adducts induced by unknown environmental and occupational chemicals or mixtures.

Keywords: *Chemical carcinogenicity, DNA adduct, biomonitoring, cancer.*

Abbreviations: *2-AAF,2-acetylaminofluorene; AFB1, aflatoxin B1; B[a]P, benzo[a]pyrene; BPDE, benzo[a]pyrene-diol epoxide; DE, dihydrodiol epoxide; ELISA, enzyme-linked immunosorbent assay; FAB, fast atom bombardment; FTMS, fourier transform ion cyclotron mass spectrometry; GC, gas chromatography; HPLC, high performance liquid chromatography; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometry; PAHs, polycyclic aromatic hydrocarbons; RIA, radioimmunoassay; RP-HPLC, reverse-phase high performance liquid chromatography; SCE, sister chromatid exchanges; SFS, synchronous fluorescence spectroscopy; TLC, thin layer chromatography; UDS, unscheduled DNA synthesis; USERIA, ultra sensitive enzymatic radioimmunoassay; WBC, white blood cells.*

Introduction

Occupational and environmental exposures to carcinogenic chemicals are a great concern for human health. Carcinogenic risk assessment based on the estimation of environmental exposure is difficult because of the complexity of the underlying transformation process, involving several cellular and macromolecular reactions. They depend on absorption and distribution, penetration of cells by the carcinogen, its metabolic activation to the ultimate carcinogenic moiety, detoxification, interaction with DNA, removal of the bound carcinogen by a DNA repair process and elimination of cancerous cells. Many carcinogens react covalently with DNA to form DNA adducts (note: other adducts, such as haemoglobin adducts, will not be discussed here) thereby potentially initiating the multistage process leading to cell transformation and clinical malignancy (Hecht *et al.* 1993, Hemminki and Pershagen 1994, Legator and Au 1994, Carmichael *et al.* 1995, Fang *et al.* 1996). For instance, the general route of enzymatic bioactivation and DNA binding of polycyclic aromatic hydrocarbons (PAHs) has been elucidated in considerable detail for the well-known model compound benzo[*a*]pyrene (B[*a*]P). It has been established that B[*a*]P-diol epoxide (BPDE) is the ultimate carcinogen, i.e. the reactive species that binds to DNA and results in bulky adduct formation (Weinstein *et al.* 1976, Lutz 1978, Rojas *et al.* 1995). At present, it is generally accepted that the formation of carcinogen–DNA adducts constitutes the critical initial step in chemical carcinogenesis (Bishop 1987, Baan *et al.* 1994, Venkatachalam *et al.* 1995). As an important group of occupational and environmental pollutants, PAHs and their bulky DNA adducts have been well studied and will be emphasized in this review.

Since most carcinogens or their metabolites are large molecules and bind with DNA, detection of carcinogen-induced bulky DNA adducts has been increasingly used as a means of biomonitoring human exposure to carcinogenic chemicals, agents or mixtures, such as PAHs, aromatic amines, alkylating agents and cigarette smoke (Hemminki 1995, Rojas *et al.* 1995, Timbrell *et al.* 1996). Numerous reports have been published in the field of DNA adduct detection with experimental animals. However, the measurement of DNA adducts in a variety of human tissues or biological materials, such as white blood cells (WBC), liver and breast tissue and oral tissues, has provided considerable information for human cancer research (Jones *et al.* 1993, Carmichael *et al.* 1995, Rojas *et al.* 1995, Vaca *et al.* 1995, Zhang *et al.* 1995, Fang *et al.* 1996).

DNA damage and adduct formation

DNA is a dynamic molecule that undergoes conformational and structural changes during its physiological functioning. The integrity of its structure most be maintained and molecular mechanisms that correct damage induced by mutagenic agents are essential for the protection of this integrity. These mechanisms are often referred to as 'DNA repair mechanisms'. Mechanisms of self repair at the cellular level exist which protect DNA from damage, and repair damage once it has occurred (Bishop 1987, Beland and Poirier 1993, Scholl *et al.* 1995). DNA damage is manifested as either strand lesions (strand break) or direct alterations to the chemical structure (covalently bonded DNA adducts). DNA adducts interfere with normal DNA replication and can lead to mutation if they are not removed (Perera and Whyatt 1994).

Establishing the sequence of events that occur after DNA perturbation is difficult, either as a lesion or an adduct; suffice to say that the reaction of exogenous chemicals with DNA can

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1354–750X/97 $12·00

result in deleterious conditions for the host. As this DNA damage is often transient, it can only be detected when the effect of any mutagenic challenge is not fully compensated by DNA repair mechanisms in the host organism. Any loss of DNA integrity could therefore be used as a possible biomarker. The presence of DNA adducts is an indication of exposure to genotoxic pollutants, while the occurrence of elevated levels of secondary modifications, such as DNA strand breaks, can be used as an indicator of DNA perturbation arising because of exposure to genotoxic chemicals or mixtures (Perera and Whyatt 1994, Farmer 1995, Lloyd-Jones 1995).

Usually, DNA adducts are formed by the covalent bonding of reactive chemicals with macromolecules. Adduct formation often occurs after the metabolic activation of these chemicals to form reactive nucleophilic or electrophilic intermediates. DNA adducts are formed as a result of reactions with strong electrophilic regions in nucleic acids to form covalently bonded adducts. In DNA, these binding sites include the oxygen and nitrogen atoms in the purine and pyrimidine bases. Adduct-forming compounds are normally described as genotoxic since they can directly alter DNA structure; examples include some PAHs, heterocyclic polyaromatics, alkenyl benzenes, arylamines and amides, mycotoxins, alkylating agents and free radicals (Santella 1991, Shou *et al.* 1993, Farmer 1995, Scholl *et al.* 1995).

Detection of DNA adducts

Over the last decade various techniques for detecting bulky DNA adducts have been developed. These techniques can be generally classified into four major groups:

1. immunological,
2. fluorescence,
3. chromatographic/mass spectroscopic, and
4. ^{32}P-postlabelling techniques.

Most of the techniques may be readily used in toxicological studies (Hatch and Thomas 1993, Legator and Au 1994). In a number of published review articles, each of the individual techniques has been discussed (Watson 1987, Santella 1991, Beach and Gupta 1992, Hemminki 1993, 1995, Farmer 1995, Keith and Dirheimer 1995). However, there has not been a comparison and evaluation of these techniques concurrently. In this review, the ^{32}P-postlabelling technique will be highlighted.

Regarding the sensitivity of the methods, currently available techniques are able to quantitate carcinogen–DNA adducts at extremely low concentrations, such as the femto- or atto-mole level, which means the detection of one adduct in about 10^{8-10} nucleotides. Such trace amounts of DNA adducts normally reflect a very early stage of potential carcinogenesis or cancer development. Thus, detection of DNA adducts provides initial warning information about human exposure to carcinogenic chemicals, hence assisting the ultimate goal of biomonitoring studies of providing accurate risk assessment including, eventually, identification of individuals at elevated risk for cancer development (Beach and Gupta 1992, Hemminki 1995). In comparison to data obtained from environmental monitoring, application of this biomarker takes into account individual differences in absorption, metabolism and repair (Watson 1987, Legator and Au 1994, Keith and Dirheimer 1995).

To define the relationship between DNA adduct formation and tumorigenesis, many experiments have been conducted to measure DNA adducts in target tissues from experimental animals during continuous exposure to carcinogens. For some chemicals, such as aflatoxin, aromatic amines, and aromatic hydrocarbons, tumour induction appears to be associated with the major DNA adduct detected, whereas with *N*-nitrosamines the response is normally correlated with minor forms of DNA damage (Groopman *et al.* 1992a, Poirier 1993, Scholl *et al.* 1995). During continuous carcinogen administration, steady-state adduct concentrations are generally obtained in the target tissues, and there is often a linear correlation between the carcinogen concentrations and the steady-state DNA adduct levels. Steady-state DNA adduct levels are also often linearly related to the tumorigenic response and cell proliferation. Because DNA adducts detected in humans are chemically identical to those found in experimental animals, DNA adduct data in animals have certainly contributed to our understanding of human cancer risk caused by carcinogenic chemicals (Beland and Poirier 1993, Qu and Stacey 1996). So far, detection of DNA adducts has been applied to human peripheral blood cells and other human tissues obtained from surgery or biopsy of individuals who have been exposed to carcinogens (Beland and Poirier 1993, Jacobson-Dram *et al.* 1993, Kang *et al.* 1995, Vaca *et al.* 1995, Fang *et al.* 1996). However, detection of DNA adducts in humans is limited by the availability of samples. Therefore, more accessible tissues such as white blood cells, placenta and surgical specimens have been more frequently used. Reported inconsistencies between white blood cells and target tissue DNA adducts (van Schooten *et al.* 1992) indicate the need for care in the interpretation of such data.

Currently available techniques

Immunological assays

Development of immunoassays and immunoaffinity chromatographic methods for determining carcinogen–DNA adducts rests upon eliciting and characterizing polyclonal and monoclonal antisera against these haptens. The use of such antisera has wide applications in investigating chronic administration in animal models and in monitoring human tissues for evidence of carcinogen exposure (Corley *et al.* 1995, Hsu *et al.* 1995, Tilby *et al.* 1995, De Pooter *et al.* 1996). Polyclonal and monoclonal antibodies have been developed against carcinogen–DNA adducts and oxidized or UV damaged bases. Santella (1991) has published an article with a table of the available antisera. The most common procedure for coupling monoadducts to protein is periodate oxidation of the ribose sugar, but carbodiimide procedures have also been used for monophosphate. Antisera specificity varies with the type of antigen used. Antisera generated against monoadducts normally recognize the monoadduct itself as well as the adduct in intact DNA. In contrast, antisera generated against modified

DNA tend to recognize the adducts only in intact DNA and not the isolated adducts. The latter types of antisera recognize some surrounding DNA structure as well as the adduct itself. Unfortunately, they also sometimes have cross-reactivity with non-modified DNA which confounds interpretation of data (Poirier 1993, Lee and Strickland 1993, Booth *et al.* 1994, Tilby *et al.* 1995, De Pooter *et al.* 1996).

Several types of quantitative immunological assays have been used for adduct measurement. It must be mentioned here that the enzyme-linked immunosorbent assay (ELISA) is used most frequently in the immunological assays, because they (1) do not require radiolabelled substances, (2) have high sensitivity and (3) can easily be applied to a large number of samples (Kriek *et al.* 1993, Mumford *et al.* 1993, Hsu *et al.* 1995, De Pooter *et al.* 1996). Based on similar immunological mechanisms and by introducing radioactive isotopes, DNA adducts can also be easily identified by radioimmunoassay (RIA) or ultra sensitive enzymatic radioimmunoassay (USERIA). With well-developed antisera, DNA adducts in femtomole amounts (10^{-15}) can be readily detected. Both RIA and USERIA have been used in human samples in occupational and environmental studies (Assennato *et al.* 1993, Jacobson-Kram *et al.* 1993, Mumford *et al.* 1993, Schoket *et al.* 1995, Zhang *et al.* 1995). Not only DNA damage can be quantified directly by immunological assays, but antisera have also been used to isolate DNA adducts of particular chemical class by immunoaffinity chromatography before application of more chemically-specific endpoints. These methodological approaches have made seminal contributions to the newly emerging field of molecular epidemiology and chemotheraputic monitoring (Poirier 1993, Tilby *et al.* 1995).

Using ELISA on intact DNA, a maximum of 50 μg is normally assayed per well. Both ELISA and RIA developed with carcinogen–DNA adduct antisera are exceedingly sensitive, measuring one adduct in 10^8 nucleotides. Assays for monoadducts can have even higher theoretical sensitivity if the adduct is first isolated by chromatographic methods. Isolation of adducts prior to immunological analysis, while time consuming, also eliminates potential problems with antisera showing cross-reactivity with normal nucleotides. Polyclonal antisera are technically much easier to produce, but more limited in quantity. For monoclonal antisera, once a good one has been developed, much more is available either as hybridoma supernatant or ascites fluid (Venkatachalam and Wani 1994). However, Venkatachalam *et al.* (1995) claimed that in their study the polyclonal antiserum (BP1) seemed to have higher affinity, avidity and sensitivity than the monoclonal antibody (5D2) for the assessment of bulky *anti*-BPDE–DNA adducts. Under normal conditions, the sensitivity between the effective polyclonal and monoclonal antisera are not significantly different (Assennato *et al.* 1993, Lee and Strickland 1993, Hsu *et al.* 1995).

Fluorescence techniques

Fluorescence techniques have been used for many years as a major analytical tool in medical science because of their great sensitivity (Kennaway 1955, Phillips 1983). Conventional fluorescence spectroscopy usually seeks an excitation or emission spectrum. Making fluorescence measurements with both monochromators fixed is possible, but this approach is seriously limited in its specificity. Normally, both excitation and emission wavelengths are scanned with a fixed wavelength under spectroscopy. The fluorescence properties of a number of carcinogens including B[*a*]P and aflatoxin B1 (AFB1) have been used to develop a method for sensitive detection. In recent years, some newly developed methods have been reported in a number of laboratories. Several fluorescence techniques are possible and have been suggested for human biomonitoring of DNA adducts (Hemminki *et al.* 1990, Rojas *et al.* 1994, 1995, Sandrelli *et al.* 1995). Furthermore, urine samples from a population in Africa exposed to aflatoxin-contaminated food have been studied by Sep-Pak extraction followed by a high performance liquid chromatography analysis with a fluorescence detector (Groopman *et al.* 1992a, b).

In a newly developed method, synchronous fluorescence spectroscopy (SFS), both the excitation and emission monochromators are driven simultaneously. This technique has the advantage of greatly simplifying the spectrum (Weston and Bowman 1991, Weston 1993). Measurement of B[*a*]P exposure has utilized release of B[*a*]P tetrols from the DNA adducts by acid treatment followed by quantitation with SFS (Corley and Hurtubise 1993). For B[*a*]P tetrols, a single peak appears around 380 nm in the fluorescence spectra. However, different proportions of positive peaks at 380 nm have been reported in different human samples. Because of the broad peak observed in most human samples, it probably results from multiple adducts (Vahakangas *et al.* 1985, Hemminki *et al.* 1990, Weston and Bowman 1991, Rojas *et al.* 1994, 1995). In a study by Vahakangas *et al.* (1985), SFS with a fixed wavelength difference of 34 nm between excitation and emission was used to quantitate BPDE–DNA adducts. Fluorescence emission maxima occurred at 382 nm for BPDE–DNA and 379 nm for B[*a*]P-tetrols and triols. Apart from these data, SFS measurements of organic extracts of acid-treated human placental DNA also suggested the presence of multiple fluorescent components. To evaluate the stability of BPDE–DNA adducts, studies using SFS were carried out in rats treated with B[*a*]P in a number of laboratories under different experimental conditions. Samples stored for various times from 16 to 72 h and temperatures from 4 to 20 °C showed no significant variation of fluorescent adducts for samples from lung and liver. Using fluorescence assays, the BPDE-adducts in other tissues, such as mouse skin, human placenta and lung, have also been measured (Manchester *et al.* 1990, Alexandrov *et al.* 1992, Bjelogrlic *et al.* 1994, Rojas *et al.* 1995).

Overall, the major limitations of the use of fluorescence spectroscopy for the detection of carcinogen–DNA adducts in tissues are prior knowledge of the chemistry of the adducts concerned and a requirement that the adduct be fluorescent. Therefore, fluorescence assays have been restricted to the detection of some particular adducts, such as BPDE-adducts for PAHs, AFB1 adducts and O^6-methyldeoxy-guanosine adducts (Zhang *et al.* 1991, Groopman *et al.* 1992a, Bjelogrlic *et al.* 1994). A key to further improvement in the sensitivity for fluorescence detection was the introduction of

immunofluorescence analysis with polyclonal and monoclonal antibodies developed to recognize BPDE–DNA adducts and to recognize other PAH–DNA adducts (Sandrelli *et al.* 1995), but not aflatoxin-modified DNA or aromatic amine-modified deoxyguanosine (Weston and Bowman 1991). A lower-temperature fluorescence technique may also be a possible direction for DNA adduct detection (Zhao *et al.* 1992, Corley and Hurtubise 1993, Corley *et al.* 1995).

Chromatographic and mass spectrometric analyses

Analysis of carcinogen–DNA adducts from biological matrices is a challenging task that requires a method to have high sensitivity and specificity. Chromatography techniques provide one possible component for analysing carcinogen–DNA adducts and other adduct complexes. Thin-layer chromatography (TLC), a classic chromatographic technique, has been widely used in this area and will be discussed in the section of ^{32}P-postlabelling of this review. Gas chromatography (GC), another relatively modern technique, has also been developed for adduct analysis, but mostly in combination with mass spectrometry (MS) to reach high resolution and sensitivity.

Application of high performance liquid chromatography (HPLC) is one of the directions for chromatographic techniques in DNA adduct measurement (Carmichael *et al.* 1995, Rojas *et al.* 1995). Radioactively labelled carcinogens are normally involved in the HPLC methods (Stanton *et al.* 1985, Shou *et al.* 1993, Nair *et al.* 1995, Fang *et al.* 1996) with the radioactivity providing the actual detection and quantitation aspects. For example, when measuring arylamine–DNA adducts *in vitro* and *in vivo*, tritium-labelled carcinogens have been used with HPLC (Beland *et al.* 1983). Adducts were isolated by HPLC and then characterized by ^{1}H nuclear magnetic resonance and electron impact in-beam desorption MS. For 1-nitropyrene-induced DNA adducts, ^{3}H-nitropyrene was administered i.p. to rats. DNA was isolated from a number of tissues. In their HPLC procedure, a UV detector was applied to monitor the eluent at 280 nm. Fractions were collected every 0.5 min and counted for radioactivity by scintillation counting. Reverse-phase PHLC (RP-HPLC) was also used to detect PAH-induced DNA adducts. With this method, radioactive fractions obtained from Sep-Pak columns were analysed on a C18 Bondapak column which was eluted with a linear gradient of 20 to 60% methanol in water. Fractions were collected every minute and subjected to scintillation counting (Shou *et al.* 1993) or measurement with an on-line radioactivity detector (Carmichael *et al.* 1995, Fang *et al.* 1996). Optimum separation and compound class separation of the metabolites of B[*a*]P-induced DNA adducts were also obtained with RP-HPLC (Rozbeh and Hurtubise 1994). One of the recent studies used HPLC to separate the normal nucleotide in the DNA samples and was also used to help ascertain the level of RNA contamination in the DNA preparation and the extent of enzymatic hydrolysis of DNA. Data obtained using HPLC have significantly facilitated DNA adduct detection (Nair *et al.* 1995) with ^{35}S- and ^{14}C- as well as ^{3}H-labelled carcinogens being used (Jackson *et al.* 1985, Lau and Baird 1991, 1994).

Mass spectometry is a technique with high specificity and recent developments have increased sensitivity to the point where analysis of human DNA samples by this method may now yield pronounced results. The common goal in DNA adduct analysis by MS is to measure the particular carcinogen adducted to DNA. These carcinogen–DNA adducts can be detected as parent chemical compounds released from DNA or as the adducted bases, nucleosides, or nucleotides. This is the only technique that measures the molecular weight of the substance. However, adducts must first be released from the DNA and derivatized to give increased volatility and thermal stability (Farmer 1995). Highly halogenated derivatizing agents increase detectability to the femtogram range. For example, derivatization with pentafluorobenzyl bromide can facilitate the determination of polar compounds by GC with electron capture detection or detection by negative ion chemical ionization MS. The goal of measuring a single carcinogen molecule or DNA adduct from biological matrices is sufficiently challenging; however, as MS techniques continue to advance, a survey method may be developed that could attain the goal of complete multi-analysis of different types of DNA adducts in biological matrices. Following recent developments, the GC–MS technique has been applied to the detection of alkylated adducts in a variety of animals including humans (Weston 1993, Farmer 1995, Friesen *et al.* 1996). In one study, a similar derivatization procedure was used for detection of alkyl purine, 3-Me-ade, 7-Me-Gua and 8-hydroxyguanine in human urine (Farmer *et al.* 1991). Levels of adducts observed were similar to SFS at 1 adduct in 107–108 nucleotides (Weston 1993, Barry *et al.* 1996). Other developments, such as combination with a laser technique, fast atom bombardment (FAB) and stable-isotope-labelled internal standards, may have certain advantages as discussed below.

Matrix-assisted laser desorption/ionization (MALDI) and Fourier transform ion cyclotron mass spectrometry. (FTMS) have been applied for the structural characterization of four PAH dihydrodiol epoxide (DE) adducts, including the 5,6-dimethylchrysene DE adduct of 2-deoxyadenosine, the 5-methyl- and 5,6-dimethylchrysene DE adducts of 2-deoxyguanosyl 3-phosphate. Measurement of positive and negative ion mass spectra, accurate mass determinations, and collisionally induced dissociation experiments were carried out using 10–40 ng (20–70 pmol) of samples (Weston 1993). Compared with FAB or FAB-MS in the analysis of underivatized PAH–DE adducts, MALDI-FTMS signals are long-lived, the direct MALDI-FTMS showed more structurally informative fragments spectra, and accurate mass and collisionally induced dissociation experiments required lower sample quantities (Stemmler *et al.* 1994). In another approach, sample stacking has been used to improve the detection limits of capillary zone electrophoresis coupled to continuous flow FAB-MS for the analysis of DNA adducts. It was found that, with stacking, the concentration detection limit of deoxynucleotide adducts could be improved by as much as three orders of magnitude, thereby bringing it into the 10–8 M range. In addition, detectable mass limitation by MS for a model acetylaminofluorene deoxyguanosine 5-monophosphate

adduct was found to be in the low picomole range for full scanning and the low femtomole range for multiple reaction monitoring of a selected fragmentation. The techniques have been applied to the analysis of adducts in an *in vitro* reaction of *N*-acetoxy-*N*-acetyl-2-aminofluorene with DNA and ethylene oxide-related *N*7-guanine DNA adducts (Saha *et al.* 1995, Wolf and Vouros 1995).

Though a wide range of GC–MS methods has been developed, it has so far been limited in its application for the detection of carcinogen–DNA adducts in humans, partly because of the great expense and the relatively large amount of sample required for analysis. However, this technique has been more successfully applied in the measurement of carcinogen–protein adducts (Farmer *et al.* 1991, Farmer 1995).

^{32}P-postlabelling technique

The technique of ^{32}P-postlabelling was first established in the early 1980s by Randerath *et al.* (1981) and Gupta *et al.* (1982) and was followed by some modifications (Gupta 1985, Gupta and Earley 1988, Randerath and Randerath 1994, Reddy and Randerath 1994, Oakley *et al.* 1996). In general, ^{32}P-postlabelling involves first isolating DNA from the test specimen; this DNA is then enzymatically digested with RNase A, T1 and Proteinase K to yield its component nucleotides, which are then subjected to 5-phosphorylation with ^{32}P-adenosine triphosphate, and the labelled adduct nucleotides can then be separated on a TLC plate (Gupta 1985, Oakley *et al.* 1996). This is commonly considered as a prominent technique for monitoring DNA adduct levels in experimental animals and humans. It is suitable for the detection of a broad range of hydrophobic xenobiotic contaminants that can form adducts with DNA (Perera and Whyatt 1994, Lloyd-Jones 1995, Keith and Dirheimer 1995, Qu and Stacey, in press).

DNA adducts measured by ^{32}P-postlabelling with TLC have been compared with other conventional genotoxic parameters. It was found that B[*a*]P induced time- and dose-dependent increases in DNA adducts showed good correlation with changes in unscheduled DNA synthesis (UDS) in hamster tracheal organ culture (Wolterbeek *et al.* 1993). Treatment of both rats and mice with the same doses of B[*a*]P induced dose response curves for DNA adducts and sister chromatid exchange (SCE) which were similar in nature. However, the major DNA adduct was about 10-fold more prevalent in the peripheral blood cells of the mouse than those of the rat (Kligerman *et al.* 1989). The induction of SCE also showed good correlation with increases of DNA adducts *in vitro* (Kelsey *et al.* 1994).

To boost the sensitivity of this assay, enhancement techniques have been developed in a number of laboratories (Reddy and Randerath 1987, Vaca *et al.* 1992, Hatcher and Swaminathan 1995a, Oakley *et al.* 1996). The major enhancement methods are (1) nuclease P1 digestion of the deoxynucleotide-3-phosphates to remove normal nucleotides, (2) *n*-butanol extraction of the carcinogen–DNA adducts, which involves the preferential partitioning of aromatic and/or hydrophobic adducts into this organic solvent over an aqueous phase. Each enhancement method is based on the differential behaviour of non-adducted nucleotides from carcinogen–DNA adducts and the removal of the majority of the hydrolysed, non-adducted 3-phosphodeoxynucleotides prior to ^{32}P-postlabelling (Gorelick and Reeder 1993). Because relative enrichment of carcinogen–DNA adducts over several orders of magnitude can be attained sometimes, there is the added benefit that the amount of radioactive material which must be handled can be reduced. Concentration steps to enrich the adducted nucleotides can enhance detection limits to one adduct per 1010 nucleotides (Randerath and Randerath 1994, Oakley *et al.* 1996).

One example of use of these extraction techniques is found in a study of cigarette smokers using ^{32}P-postlabelling where both butanol extraction and nuclease P1 enhancement consistently showed discrete radioactive spots and diagonal zones in all smokers but not in the non- or ex-smokers. The two different enhancement methods generated similar profiles with the exception for some special adducts, which were only observed with butanol extraction (Degawa *et al.* 1994). In a study by Jones *et al.* (1993), DNA was extracted from samples obtained from 20 tobacco smokers, four ex-smokers, and nine non-smokers and analysed for the presence of aromatic DNA adducts using ^{32}P-postlabelling with two different enhancements. With butanol extraction enhancement, a much wider range and substantially higher levels of DNA adducts were identified than with nuclease P1 enhancement. It is generally accepted that butanol extraction is a more effective enhancement for DNA detection of adducts induced by aromatic amines, such as the dG-C8 derivatives of 4-aminobiphenyl and 4-acetyl benzidine (Chacko and Gupta 1988, Beach and Gupta 1992, Neilsen *et al.* 1996, Oakley *et al.* 1996). However, for detection of PAHs-induced DNA adducts, nuclease P1 enhancement has been widely used (Grzybowska *et al.* 1993, Hou *et al.* 1995, Qu and Stacey 1996, Szyfter *et al.* 1996). For detecting other carcinogen–DNA adducts, either butanol extraction or nuclease P1 enhancement methods have been used depending upon conditions of individual experiments (Reddy 1993, Hemminki *et al.* 1994, Kelsey *et al.* 1994, Hatcher and Swaminathan 1995a, b, Hemminki 1995, Stone *et al.* 1995, Oakley *et al.* 1996). In addition, Reddy (1991) also investigated the utility of another 3-dephosphorylating enzyme, nuclease S1, and found it to be as effective as nuclease P1. When assaying for B[*a*]P, benzoquinone and 2-acetylaminofluorene (2-AAF), the results showed that the nuclease S1 can complement the nuclease P1 assay, with improved recoveries for some adducts.

Most applications of ^{32}P-postlabelling have been directed at the detection of adducts involving *in vitro* and *in vivo* studies with experimental animals (Kligerman *et al.* 1989, Randerath *et al.* 1992, Wolterbeek *et al.* 1993, Degawa *et al.* 1994, Hemminki 1995, Oakley *et al.* 1996, Qu and Stacey 1996). Dose–response experiments have produced data which show a clear relationship between carcinogen challenge and adduct accumulation. Both the range of species including humans studied and the xenobiotics involved are ever increasing (Beach and Gupta 1992, Nesnow *et al.* 1993, Schell *et al.* 1995, Qu and Stacey 1996). The detection of adducts in human DNA has been associated with exposure to genotoxic environmental contaminants. A major goal of the application has been the

elucidation of the aetiology of some human cancers which are believed to be induced by carcinogens. In recent years, extensive ^{32}P-postlabelling studies have been done in cultured human tissues or tissues directly obtained from humans (Routledge *et al.* 1992, Grzybowska *et al.* 1993, Jones *et al.* 1993, Degawa *et al.* 1994, Perera *et al.* 1995, Li *et al.* 1996). In a number of recent publications, the ^{32}P-postlabelling technique, rather than other techniques, has been used in experimental animals for DNA adduct toxicokinetic studies (Ross *et al.* 1990, Walker *et al.* 1992, Nesnow *et al.* 1993, Qu and Stacey 1996).

Since ^{32}P-postlabelling assays were first developed, chromatographic techniques have become an important part of the assay. In most of the cases, TLC is employed to separate DNA adducts from digested DNA samples because it is economic and convenient. TLC results can also provide clear pictures of the DNA adduct patterns (Culp and Beland 1994, Schell *et al.* 1995, Qu and Stacey 1996, in press). However, TLC analyses are limited in terms of separation when complex patterns of compounds are found. In particular, development of TLC sheets in solvents can take from hours to days with considerable risk of contamination in the laboratory. For these reasons, Spencer *et al.* (1993) studied different solvents for the development of TLC plates, especially the D3 and D4 phases of the chromatography. They found 0.2 M ammonium hydroxide worked well for the recovery, separation, and resolution of a wide array of adducts derived from highly lipophilic aromatic hydrocarbons and aromatic amines. This solvent required much less time (<25%) as compared with the other solvents and more importantly allowed the separation of adducts which otherwise co-migrated and were not visible when using the other three D4 solvents. Background radioactivity per cm^2 of the thin layer was also reduced two- to three-fold with the ammonia solvent versus the urea-based solvents. Thus, the use of this dilute ammonia system may provide powerful resolution in a more time-efficient manner. Autoradiography of TLC plates is still routinely used to locate the radioactive regions and then these areas are excised for analysis by liquid scintillation spectrometry, and in some cases coupled with scanning densitometry, to quantitate radioactivity on the chromatography. However, interpretation visually or by densitometry of autoradiographic film images is subject to significant restriction because of limited dynamic range for film. At low levels of radioactivity the faint spots on an autoradiogram may be only 20–30% of expected autoradiographic densities based on radioactivity, whereas at higher levels of radioactivity, film becomes insensitive to the increasing levels of radioactivity. Thus, determination of background radioactivity levels in various regions of TLC plates for subtraction from individual spot values can be difficult, particularly on complex chromatograms where there are multiple overlapping spots (Reichert *et al.* 1992, Hatcher and Swaminathan 1995a, Keith and Dirheimer 1995).

In a study reported by Zeisig and Moeller (1995), HPLC was used to separate DNA adducts formed from 38 PAHs and biphenyls *in vitro* and followed with ^{32}P-postlabelling. The ^{32}P-HPLC method proved to be useful for separation, detection and characterization of DNA adducts from most of the substances. The results showed that the ^{32}P-HPLC method was suitable for characterizing DNA adducts from many substances. From 35 of the tested substances, 365 adducts were detected and characterized on the basis of retention times. Furthermore, HPLC was also applied in the separation of the purine nucleoside 3′-phosphate adducts formed by reaction with the pure enantiomers of *anti*-dihydrodiol epoxide of B[*a*]P. For the deoxyguanosine 3-phosphate adducts good separations were achieved using a 2 to 40% acetonitrile gradient over 60 min in 0.05 M potassium phosphate buffer, pH 7.2 (Peltonen *et al.* 1992).

Ion-pair RP-HPLC coupled with ^{32}P-postlabelling has been used to detect 7-methyl-dGp from human lungs, but it has also identified other nucleotides and unknown adducts. This combination attains chemical specificity, retains sufficient sensitivity and should be useful in human biomonitoring studies (Kato *et al.* 1993). In another study, ion-exchange chromatography and RP-HPLC were combined followed by ^{32}P-postlabelling to characterize the DNA adducts formed in the forestomach of rats. Combination of two independent chromatographic systems considerably enhances the fidelity of identification of DNA adducts with the ^{32}P-postlabelling assay (Stiborova *et al.* 1994, Hatcher and Swaminathan 1995a).

Distinct from traditional HPLC, where eluent is collected and measured with a scintillation counter, researchers have developed a new method of analysing ^{32}P-postlabelled DNA adducts on RP-HPLC with on -line detection of ^{32}P-radioactivity (Moeller and Zeisig 1993, Fang and Vaca 1995, Zeisig and Moeller 1995, Fang *et al.* 1996). This method permits direct injection of ^{32}P-postlabelling mixture into the analytical system without prior purification with low background radioactivity. The method can be used in conjunction with TLC analyses of ^{32}P-postlabelled DNA adducts to improve the analytical capacity. Normally, typical times for analysis of a single sample by HPLC and TLC were about 30–40 min and 4–24 h, respectively (Peltonen *et al.* 1992, Moeller and Zeisig 1993, Weston 1993, Zeisig and Moeller 1995). The method has been applied to 2-nitrofluorene, a carcinogenic pollutant, and 2-AAF, a model carcinogen which is also a metabolite of nitrofluorene (Moeller and Zeisig 1993) and a number of PAHs and their derivatives (Zeisig and Moeller 1995). It is worth noting here that the introduction of standard DNA adducts in the ^{32}P-postlabelling with HPLC may further boost the application of this technique (Li *et al.* 1995, Nath *et al.* 1996).

In the early 1990s, a technique known as storage phosphor imaging has been adapted for quantitation of radioactivity on TLC sheets (Johnson *et al.* 1990). It was found that storage phosphor imaging was about 10 times more sensitive than screen-enhanced autoradiography at –80°C for the detection of ^{32}P, exhibits a greater linear range of response, has a resolution that compares favourably to film and has lower background than does liquid scintillation spectrometry (Reichert *et al.* 1992). In a very recent study, Qu and Stacey (1996) have used a bio-imaging analyser with quantification of DNA adducts by computer. This new imaging analyser can effectively scan a special radioactive transmission plate within a few minutes without any direct exposure of the operators to radioactivity at this stage. A record of the distribution and intensity of radioactive spots (DNA adducts) allows for computer assisted

assessment of adduct profiles and can facilitate quantitation of individual adducts and radioactive zones comprising multiple overlapping adducts in complex chromatograms. Additionally, the permanent record created by the imaging technology permits easy retrospective analysis of samples, whereas with conventional autoradiography and liquid scintillation spectrometry reanalysis of a replicate sample is required.

One interesting feature from DNA adduct detection is the application of the ^{32}P-postlabelling technique in environmental monitoring, which has provided very promising data in regard to marine life exposure to carcinogenic chemicals (Masfaraud *et al.* 1992, Nishimoto *et al.* 1992, van Schooten *et al.* 1995). In the liver of eel (*Anguilla anguilla*), PAH–DNA adducts were detected in the fish collected from six freshwater sites in the Amsterdam area with different levels of PAH contamination in their sediments. Significant correlations were found between the aromatic DNA adduct levels and the levels of PAH in sediments ($p < 0.01$) (van Schooten *et al.* 1995). Using the white sucker fish (*Catostomus commersoni*) from the basin of the St Lawrence River (Canada), the genotoxic potential of industrial pollution was evaluated by measuring the levels of DNA adducts in the hepatic tissues by the ^{32}P-postlabelling technique. At the highly contaminated sites, total adduct levels in all fish ranged from 25–178 adducts/10^9 nucleotides. The mean levels of adducts were significantly higher than those from the St Francois River, a less contaminated site. The effluent from some heavy industries and pulp and paper mills may be significantly associated with the overall genotoxic damage to fish (El-Adlouni *et al.* 1995). More recently, other carcinogen-induced DNA adducts have also been detected with ^{32}P-postlabelling in different species, such as trout, English sole and earthworm with either nuclease P1 or butanol enrichment (Masfaraud *et al.* 1992, Stein *et al.* 1993, Potter *et al.* 1994, van Schooten *et al.* 1995, Walsh *et al.* 1995).

Other available techniques

Immunohistochemical method

Exposure of animals to carcinogenic PAH may cause tumour development, which in some cases is highly specific both with respect to tissue and cell type (van Schooten *et al.* 1991, Curigliano *et al.* 1996). Methods for dosimetry of adducts in DNA isolated from homogenized organs or tissues, such as *in vitro* immunoassay or ^{32}P-postlabelling, do not provide information about differences in adduct induction and repair between different cell types within that organ or tissue. Immunohistochemical detection of DNA adducts at the single cell level offers the possibility to analyse the distribution of DNA damage in different types of cells using considerably less cells than the ^{32}P-postlabelling and ELISA methods (Johnsson *et al.* 1995, Motykiewicz *et al.* 1995). Immunofluorescence microscopy has been applied with adduct-specific antibodies to investigate BP-adduct induction in various cell types in the hamster trachea epithelium. With this method, morphological changes such as squamous metaplasia and hyperplasia can be observed in the epithelial cells (Baan *et al.* 1994). Immunohistochemical methodology with microfluorometry has also been applied to the determination of hepatic DNA adducts in rats fed 2-acetylaminofluorene (Huitfeldt *et al.* 1994).

By using the immunohistochemical technique to quantify 4-aminobiphenyl (4-ABP)–DNA adducts and p53 nuclear overexpression in T1 bladder cancer of smokers and non-smokers, Curigliano *et al.* (1996) suggested that this technique has sufficient sensitivity for detection of these adducts in human bladder samples. The advantages of this method include small sample size, the possibility of retrospective analysis of stored paraffin blocks, the ability to analyse binding in specific cell types, and relatively low cost. On comparison of the DNA adduct levels measured by immunohistochemical method to those measured with GC/MS in R52 cells (a mouse NIH3T3 cell line expressing high levels of cytochrome P450 1A2) and mouse liver tissue exposed to 4-ABP, a good correlation ($r = 0.98$, $p < 0.0001$) was found. Based on adduct levels determined by GC/MS in both R52 cells and liver tissues, the immunohistochemical method has a limit of sensitivity of approximately 1 adduct/10^{7-8} nucleotides (Al-Atrash *et al.* 1995).

Combination of immunological, fluorescent and chromatographic analyses

Because humans are chronically exposed to diverse kinds of chemicals and eventual DNA structural modifications are supposed to be a complex mixture of adducts at very low levels, using an assay with extremely high sensitivity and specificity is essential. In the early 1990s Weston and Bowman (1991) reported on how to use immunoaffinity chromatography and SFS, together with HPLC and the generation of complete fluorescence excitation–emission matrices to identify unequivocally BPDE–DNA adducts in samples of human lungs. This technique has been further developed in other tissues obtained from humans and rodents (Nair *et al.* 1995). In brief, BPDE nucleotide adducts were isolated with immunoaffinity chromatography columns bearing antibodies raised against the *anti*-7,8-diol-9,10-epoxide-deoxyguanosine adduct of B[*a*]P. These adducts were hydrolysed to tetrahydrotetrols and the hydrolysis products subjected to HPLC. Major products isolated by HPLC were then determined by SFS. Using this method, levels of BPDE–DNA adducts in the range of 1–40 in 10^8 nucleotides were measured in 6 out of 25 samples, with a lower detection limit of one adduct in 10^8 nucleotides. Kang *et al.* (1993) have established another quantitation method called PREPI (prefractionation by HPLC, ^{32}P-postlabelling, and immunoprecipitation), for detecting O^6-methylguanine, O^4-methylthymine, and O^4-ethylthymine, with a combination of prefractionation by HPLC, ^{32}P-postlabelling, and immunoprecipitation. The detection limit was about 1 fmole for all three adducts, enabling the analysis of about 1×10^{-8} levels as a molar ratio to normal counterparts using 100 μg of DNA. Immunoaffinity purification combined with ^{32}P-postlabelling for the detection of O^6-methylguanine in DNA was also applied to other human tissues, such as liver and

peripheral blood leucocytes (Kang *et al.* 1995). The minimum detection limit of the combined method is expected to be about 1 O^6-methyldeoxyguanosine in 10^8 normal 2-deoxyguanosines (i.e. up to 30 lesions per human cell) when 100 μg of DNA is used (Cooper *et al.* 1992). Thus, joint application of fluorescence spectroscopy and HPLC is an interesting development for DNA adduct detection (Alexandrov *et al.* 1992, Manchester *et al.* 1992, Tilby *et al.* 1995).

In conventional HPLC analysis, methanol/aqueous buffer solution gradients are employed; the fluorescence yields of BPDE–dG adducts increase strongly with increasing methanol concentration, while the yields of BPDE–dA adducts are less sensitive to the solvent composition, particularly in degassed solution. This work demonstrated that these fluorescence characteristics can be used to quantitate the relatively low yields of BPDE–dA adducts by fluorescence detection when BPDE-modified DNA is subjected to enzymatic degradation to the mononucleoside level, followed by HPLC analysis of the digests (Chen *et al.* 1993, Rojas *et al.* 1995).

Comparison and evaluation

Each technique possesses its own advantages and disadvantages in relation to the different principles they are based on. Therefore, a comparison of the different available techniques will provide indications for selecting a suitable assay to detect the DNA adducts under study.

In general, ^{32}P-postlabelling and ELISA techniques have been widely adopted in human studies for detecting of DNA adducts, in which ELISA has been shown to detect adducts from a variety of PAHs, and ^{32}P-postlabelling has a broad specificity for aromatic DNA adducts. In workers from the primary aluminium industry, Schoket *et al.* (1993) found these two techniques have resulted in the same major conclusions, where significant differences of DNA adducts were demonstrated between the unexposed and exposed groups, and the two exposed groups were similar to each other. The overall detection limit was approximately eight-fold lower with the ^{32}P-postlabelling assay than ELISA, and the maximum values were about three-fold higher by the antibody assay, in spite of the much broader adduct specificity of the ^{32}P-postlabelling technique. In another study on detection of platinum–DNA adducts by ^{32}P-postlabelling with TLC or HPLC, good correlations with other existing methods, such as atomic absorption spectrometry, immunohistochemistry and ELISA, were found in DNA samples treated *in vitro* with cisplatin or carboplatin. Nevertheless, the authors have specifically indicated that after enzymatic digestion of DNA, the positively charged platinum adducts were not able to be purified from unplatinated products with normal conditions and strong cation exchange chromatography had to be used. Subsequently the samples were deplatinated with cyanide, because platinated dinucleotides are very poor substrates for polynucleotide kinase (Blommaert and Saris 1995). This result suggests that when negative results for adducts are obtained, some modification of the detection conditions may be required.

Using a rank correlation test, statistical evaluation of the positive samples by ELISA and of their corresponding ^{32}P-postlabelled pairs suggested a weak but significant correlation between the two methods (r rank =–0.219, $p < 0.05$). The diversity may primarily come from different labelling efficiencies of aromatic DNA adducts by ^{32}P-postlabelling and differential recognition of PAH–DNA adducts by ELISA (Schoket *et al.* 1993). Therefore, although there is a degree of uncertainty surrounding absolute quantitation by either method, each is internally consistent, and statistically significant differences in adduct levels between exposure groups and controls were clearly demonstrated both by ^{32}P-postlabelling and immunoassay (Mumford *et al.* 1993, Blommaert and Saris 1995, Hemminki 1995, Nair *et al.* 1995). It is worth noting here that, in ELISA, antibodies normally raised in rabbits against BPDE–DNA cross-react with other PAH–DNA adducts (Watson 1987).

Using coded WBC DNA samples, BPDE–DNA adduct levels were measured by fluorometry of the B[*a*]P-tetrols and then compared with results by ^{32}P-postlabelling (nuclease P1 enrichment) and ELISA measurements. A good correlation and proportionality were found between the levels of BPDE–DNA adducts measured by fluorometry and ^{32}P-postlabelling (r = 0.95, $p < 0.01$). The correlation between fluorometry and ELISA was much lower and not significant (r = 0.61, p = 0.1). It is possible that ELISA grossly overestimated BPDE–DNA adduct levels measured by the other two methods. However, the results show that the highly sensitive and specific fluorometric assay is still suitable for measuring BPDE–DNA adducts in WBC from humans exposed to B[*a*]P (Rojas *et al.* 1994, 1995). In a paper by Zhang *et al.* (1991), a quantitative indirect immunofluorescence technique was developed utilizing a monoclonal antibody (6A10) recognizing the imidazole ring-opened form of the major N-7 guanine adduct of AFB1. Quantitation of AFB1–DNA adducts was carried out by densitometric analysis of photographic slides, competitive ELISA with antibody 6A10 and fluorescence spectroscopy. The results showed a significant correlation of the quantitative immunofluorescence intensity with levels of AFB1 adducts detected by ELISA (r = 0.61) and spectrofluorescence (r = 0.78). This technique was recently used for the detection of DNA damage in single cells (Tilby *et al.* 1995, Zhang *et al.* 1995). In another study, Misra *et al.* (1994) compared two ultrasensitive methods for measuring two adducts, 1, N^6-etheno-2-deoxyadenosine (edA) and 3, N^4-etheno2-deoxycytidine (edC), in cellular DNA. It was found that the combination of the immunoaffinity and ^{32}P-TLC procedure proved to be more sensitive and more reproducible than the combination of HPLC and ^{32}P-TLC method. On comparison of an HPLC and a fluorometric assay for dosimetry of BPDE–DNA adducts in smokers' lung tissues, a crude linear correlation between the amounts of the adducts measured by HPLC and those of bulky DNA adducts determined by ^{32}P-postlabelling was observed for the same samples (r = 0.78, $P < 0.02$) (Alexandrov *et al.* 1992).

It is worth pointing out here that in the detection of DNA adducts, particularly in humans, there is still significant variability (van Schooten *et al.* 1992, Schoket *et al.* 1993, Hou *et al.* 1995, Szyfter *et al.* 1996, Totsuka *et al.* 1996). In our

laboratory, considerable variability in DNA adduct levels was found for blood samples from coal miners when the same DNA sample was separated on TLC plates purchased from different companies. Even when the same sample was analysed using TLC plates from different batches from the same supplier there was often considerable variability in the results for DNA adducts. It seems that other laboratories may experience similar difficulties but this is not readily apparent from the literature. In a very recent publication Reichert and Stein (1996) have discussed some similar experiences with regard to TLC plates. Therefore, to maintain the same quality of TLC plates in different experiments, the application of self-made TLC plates has been suggested (Vock *et al.* 1995, 1996). In our laboratory, in order to minimize the variability, the same TLC plate (cut in half) was used for the two samples (before and after exposure) for each worker. This provided the considerable advantage of removing plate to plate variability in the ^{32}P-postlabelling study for samples from the same worker, thereby markedly increasing our confidence when comparing the results for before and after exposure.

In addition, interlaboratory differences when using ^{32}P-postlabelling and other assays remain an issue for DNA adduct detection. A comparison of antisera and immunoassays for BPDE–DNA found quite disparate results amongst laboratories and resulted in standardization of antisera, competitors and ELISA assay conditions (Santella *et al.* 1988, Hsu *et al.* 1995). The data suggested that when a biological sample is assayed against a BPDE–DNA standard modified in the same range as the biological samples (4.5 fmol g^{-1}), quantitative recovery of adducts is achieved by ELISA. The interlaboratory differences in immunoassay procedures can have significant consequences for data comparison and suggest that using the same antisera across laboratories and modified DNA standards would be preferable. An interlaboratory trial of ^{32}P-postlabelling resulted in good qualitative and reasonable quantitative agreement (Phillips and Castegnaro 1993). A difference in resolution of spots and zones was also noted amongst participant laboratories. The standardization of the detection techniques for DNA adducts remains an important, but difficult, task in the field and will require considerable attention (Shields *et al.* 1993, Scholl *et al.* 1995).

The potential usefulness of DNA adduct detection seems to be constrained by a number of factors. Since it is apparent that adducts may be formed from substances of both synthetic and natural origins, the detection of adducts may not therefore reflect the true result of anthropogenic stress alone. This complicates the use of DNA adducts as dosimeters of exposure to occupational or environmental genotoxic agents as the time of exposure may not always be clear-cut (Hatch and Thomas 1993, Baan *et al.* 1994, Lloyd-Jones 1995, Rojas *et al.* 1995, Vaca *et al.* 1995). For human exposure, pollution levels encountered may not be sufficiently high to elicit statistically significant increases in adduct levels (Kriek *et al.* 1993, Hou *et al.* 1995). Alternatively, increased exposure to genotoxic agents may result in the induction of DNA repair processes which mask the level of DNA adduct formation through an increased rate of DNA repair (Beach and Gupta 1992, De Pooter *et al.* 1996). For biomarker evaluation to be valid, extrapolating observed effects from one population to another must be possible, and more significantly from test species under artificial conditions to actual human populations (Gorelick and Reeder 1993, Neilsen *et al.* 1996). Because experimental animals are normally used prior to human studies, the species response is a significant component of not only exposure levels, but also the metabolism of genotoxic chemicals. Some test species may exhibit a greater capacity for genotoxic activation, while others will show less metabolic activity especially for some particular substances (Kligerman *et al.* 1989, Legator and Au 1994). Furthermore, as biomarkers will be used in epidemiological studies generating relatively large numbers of samples, the resource implications will need to be as low as possible (Schoket *et al.* 1995, Neilsen *et al.* 1996).

On comparison of the two most commonly used techniques, ^{32}P-postlabelling and ELISA, some interesting data have been reported. In an aluminium production plant, Kriek *et al.* (1993) analysed WBC DNA from coke-oven workers and from aluminium production workers and demonstrated the presence of PAH–DNA adducts. About 47% of the coke-oven workers had detectable levels of PAH–DNA adducts in their WBC compared with 27% of controls, as measured by both ELISA and ^{32}P-postlabelling. In both groups, smokers had significantly higher levels of PAH–DNA adducts than did non-smokers. In the aluminium workers, no PAH–DNA adducts were detected by ELISA, although the B[*a*]P concentrations in the work atmosphere were comparable to those of coke-oven workers. The more sensitive ^{32}P-postlabelling assay showed the presence of PAH–DNA adducts in 91% of the aluminium workers. Regarding the differences in DNA adduct levels obtained from the different techniques, it is possible that the different techniques recognize different types of DNA adducts. It is also possible that methodological differences affecting the specificity of adduct assays in biological samples could account for these apparently disparate results. These findings are important because they confirmed the formation of DNA adducts from BPDE and other PAHs in humans and because they identify specific problems that will need to be considered in future investigations of DNA damage in humans (Manchester *et al.* 1990, Ovrebo *et al.* 1992, Schoket *et al.* 1993, 1995).

In addition, with the ^{32}P-postlabelling technique, the enhancement procedures appear to act selectively, where nuclease P1 and butanol extraction procedures differently enhanced detection of some known adducts. However, to efficiently analyse biological samples for unknown adducts major enhancement may be required at the early stage of each study (Gupta and Earley 1988, Oakley *et al.* 1996). One study has demonstrated that when some 'negative' samples, which were analysed by ^{32}P-postlabelling with nuclease P1 enhancement, were re-analysed by the same technique but with another enhancement procedure (butanol extraction), putative BPDE-adducts and/or other PAH–DNA adducts could be detected (Manchester *et al.* 1990). In the detection of DNA adducts generated from the peroxidase-mediated activation of *N*-hydroxy-4-acetylaminobiphenyl *in vitro*, two individual adducts were identified. Amongst these adducts, adduct 1 was detected by butanol extraction but it was insensitive to nuclease P1. However, adduct 2 was only detectable when

using nuclease P1 enhancement (Hatcher and Swaminathan 1995a). Once a suitable enhancement procedure has been selected, the ^{32}P-postlabelling technique seems to be the most promising assay for detection of DNA adducts induced by chemical carcinogens (Martin *et al.* 1995, Neilsen *et al.* 1996).

Although the presence of DNA adducts will indicate recent past and present exposure to genotoxic chemicals, the point should also be made that any failure to detect DNA adducts cannot be taken as an indication of the absence of genotoxic compounds from the living or working environment. Ultimately, this may make detection techniques directed at a specific single adduct a more appropriate technique for occupational and environmental monitoring. This would help to overcome the semi-quantitative nature of the DNA adduct detection techniques and allow for a more definitive determination of specific DNA adducts (Beach and Gupta 1992, Hemminki 1993, Baan *et al.* 1994, Keith and Dirheimer 1995).

Conclusions

A variety of techniques is available for detecting DNA adducts induced by carcinogenic chemicals or mixtures. It is considered that for an assay to be applicable in a human exposure setting, it must (1) be sensitive enough to detect low levels of adducts; (2) require only microgram quantities of DNA; (3) give results quantitatively related to exposure; (4) be applicable to unknown adducts that may be formed from complex mixtures such as cigarette smoke or diesel engine emissions; (5) be able to resolve, quantitate and identify adducts; and (6) have a cost advantage. Among the available techniques, the ^{32}P-postlabelling assay seems to best meet these criteria overall. In particular, ^{32}P-postlabelling is the most suitable technique for the detection of DNA adducts induced by unknown environmental and occupational chemicals or mixtures.

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Received 27 August 1996, revised form accepted 9 October 1996