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Chemical and Biochemical Dosimetry of Exposure to Genotoxic Chemicals

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Epidemiologic studies designed to evaluate the health significance of environmental chemicals are compromised by the lack of quantitative exposure data for individuals in exposed populations. Monitoring data on levels of compounds in environmental media often represent the only information available, and average population exposure is therefore the only quantitative parameter that can be calculated. Biological monitoring, i.e., measurements on cells, tissues or body fluids of exposed persons, has the objective of defining the so-called "internal dose" or "effective dose" on an individual basis. Such measurements can be used to ensure that current or past exposure does not entail unacceptable health risks, or can detect potentially excessive exposure before the appearance of adverse health effects. Results obtained through this approach can be interpreted on an individual basis and also used to estimate for that individual the amount of chemical absorbed during a specific time interval or the amount bound to critical sites. They may also be useful for characterization of community exposure by analyzing results obtained in groups of individuals within the general population. In this respect, biological monitoring data complement environmental measurements but have certain advantages in estimating health risks. Most importantly, the data obtained are more directly related to adverse effects and thus provide a better estimate of risk than ambient monitoring. Biological monitoring also takes into account absorption by all routes, integrates exposure from all sources, and therefore can be used as a basis for estimate of total risk from multiple chemicals.

Strategies for Exposure Monitoring

Two categorical types of measurements can be made in biological monitoring: measurements of levels of chemicals, their metabolites and/or derivatives in body fluids or excreta and measurements of biological responses such as mutation, sister chromatid exchange, chromosome aberrations, etc., in cells and tissues of exposed individuals.

Quantification of chemicals in body fluids (e.g., blood, breast milk, saliva, semen) or in excreta has been carried out by direct chemical analysis, immunologic analysis, or can be inferred from results of bioassays for mutagenicity. Most of the existing chemical methods and available data relate to occupational exposure, since such measurements have been used in occupational hygiene programs for many years. Immunoassays and bioassays are more recent developments that have as yet found only limited application. With the recognition that most genotoxic chemicals require metabolic activation to electrophilic forms in order to express their effects, an additional strategy for chemical dosimetry has developed based upon the detection and quantification of covalently bound derivatives formed between activated chemicals and cellular macromolecules such as nucleic acids and proteins. Immunologic and chemical analytical methods have been developed for this purpose which are sufficiently sensitive to detect the consequences of ambient exposure in the workplace and general environment.

Prevention of excessive exposure to chemicals in industry has been approached traditionally by setting standards for the concentration of compounds in ambient air. Air monitoring has therefore constituted the principal means of assessing exposure, an approach that obviously takes into account only exposure via the pulmonary route and cannot estimate total exposure. Over the past two decades, analytical methods have been developed for many compounds representing a variety of chemical classes to which people are exposed, principally in the workplace. Lauwerys (*1*) has summarized these methods from the perspective of their usefulness in biological monitoring programs and described a total of 69 chemicals for which analytical methodology was considered to be sufficiently well developed for application in worker surveillance programs. Lauwerys (*1*), as well as Baselt (*2*) and Linch (*3*), summarized the analytical methodology as well as additional pertinent information including representative values for each of the chemicals in body fluids.

In addition to their use in monitoring programs in the workplace, a few of these methods have also been applied to population studies involving large numbers of subjects. For example, chlorinated hydrocarbons have been extensively studied with respect to their storage

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and accumulation in adipose tissue and other body compartments. Hayes (*4*) summarized the literature concerning levels of DDT and its derivatives in adipose tissue and other body compartments in large numbers of subjects studied over a period of two decades. In a related area, analysis of human milk has been employed to determine exposure to chlorinated pesticides (*5*) and to polychlorinated biphenyls (*6,7*).

With reference specifically to environmental carcinogens and mutagens, the monitoring field is in a more primitive state of development. The main focus of research has been on the development of analytical methods for detection of carcinogens that can occur as contaminants of food, and consequently most of the existing methods were intended for the purpose of food analysis. Methods have been published for some *N*-nitroso compounds, polycyclic aromatic hydrocarbons, aromatic amines, vinyl chloride, and mycotoxins. The suitability of these methods for analysis of media other than food has not usually been evaluated, and in many instances they are impractical for analysis of large numbers of samples.

A larger number of analytical methods are available for aflatoxins than for any other class of carcinogen. In this case, some methods originally developed for analysis of oilseeds and grains have been adapted for analysis of edible tissues and milk of animals in order to minimize human exposure through residues of the parent compounds or metabolites, and have also been applied in analysis of tissue of people suspected of having been exposed to aflatoxins.

In studies of the putative etiologic role of aflatoxins in cases of Reye's syndrome, an acute highly fatal disease of children, aflatoxin B_1 (AFB_1) was detected in samples of liver, stool, brain, and kidney at levels in the order of 100 ng/g through application of a thin-layer chromatographic (TLC) method with fluorescence detection (*8,9*). These results were extended and substantiated in more recent studies in which HPLC separation with fluorescence detection was used (*10–12*). Aflatoxins have also been detected in human tumors of the liver (*13,14*) and lung (*15*) with TLC methodology.

Studies in human populations consuming aflatoxin-contaminated peanut butter revealed the presence of aflatoxin M_1 in urine, as detected by TLC analysis (*16*), and aflatoxins were also found in the urine of Sudanese children suffering from kwashiorkor examined by HPLC analysis (*17*). Chemical screening methods for detection of aflatoxin and metabolites in human urine (*18*) and serum (*19*) have been reported.

The rapidly developing field of immunoassay has up to now principally been applied to the detection of covalent adducts of carcinogens with DNA, as will be discussed subsequently. However, methods based on radioimmunoassays have been reported for two carcinogens. Johnson et al. (*20*) developed a radioimmunoassay procedure for 4-acetamidobiphenyl, a metabolite of the carcinogen 4-aminobiphenyl, in urine. Rabbit polyclonal antibodies with high affinity (2.8×10^8 L/mole) were produced which were capable of detecting the metabolite at levels of about 1 ng (4.8 pmole) in human urine.

Sizaret et al. (*14*) similarly developed rabbit polyclonal antibodies which detected not only aflatoxin B_1 but also cross-reacted with various aflatoxin metabolites including M_1, the principal urinary excretory product discussed above. The radioimmunoassay developed using these antibodies was capable of following urinary metabolites of aflatoxin administered to rats at doses of 600 pmole or less. The authors thus propose that the assay would be suitable for use in human population studies.

Covalent Adducts as Dosimeters

The rationale underlying the strategy of chemical dosimetry by determining levels of derivatives covalently bound to cellular macromolecules is based on current understanding of the mode of action of genotoxic carcinogens and mutagens. Chemicals that are active as carcinogens and mutagens have electrophilic properties or are metabolically converted into electrophiles. These reactive derivatives undergo attack by nucleophilic centers in nucleic acids and proteins, resulting in the formation of covalent adducts. Particular attention has been paid to DNA adducts, since these are thought to represent initiating events leading to mutation and/or malignant transformation. Covalent adducts formed in RNA and proteins have no putative mechanistic role in carcinogenesis, but may relate quantitatively to total exposure and activation, and could therefore contribute to dosimetry of both exposure and activating capability.

Covalent nucleic acid adducts have differing levels of stability, some being removed spontaneously through chemical depurination, whereas others are removed enzymatically, in the process of DNA repair. A small proportion remain in DNA over long periods of time. In the few experimental models in which appropriate measurements have been made, adducts removed spontaneously or enzymatically from DNA are excreted in urine in amounts reflective of total binding levels. In contrast, protein adducts are stable over the life span of the protein, and therefore accumulate with multiple exposures.

These properties collectively form the basis for several complementary approaches to development of chemical dosimeters, providing different kinds of information. Measurements of DNA adducts *in situ* in the DNA of cells should give the most direct evidence of genotoxic exposure; excretion of DNA adducts (or products of them) in urine should give an indication of total, recent exposure; whereas protein adducts should provide an index of total exposure integrated over the lifespan of the target proteins.

Protein Adducts

Ehrenberg and Osterman-Golkar (*21*) reviewed the rationale and technical requirements for the use of pro-

tein alkylation for detecting mutagenic agents. Important among these requirements is that exposure must result in the formation of stable covalent derivatives of amino acids for which assay methods of adequate sensitivity and specificity can be devised. Further, the target protein should be found in easily accessible fluids (e.g., blood) and should be present in concentrations adequate to provide sufficient material for analysis. Among the amino acids likely to be alkylated following exposure are cysteine, histidine, the N-terminal amino acid of protein, and lysine. Although any protein could in principle be used for monitoring of alkylated derivatives, hemoglobin was suggested by Osterman-Golkar et al. (*22*) as a suitable dose-monitoring protein, and nearly all of the relative literature concerns studies of hemoglobin alkylation.

Osterman-Golkar et al. (*22*) established the stability of alkylated residues in hemoglobin modified by ethylene oxide or dimethylnitrosamine, and the equivalence of the half-life of alkylation levels produced by a single dose to the life span of hemoglobin in the mouse. Alkylation of hemoglobin in mice treated with vinyl chloride was described by the same investigators (*23*). Segerback et al. (*24*) further characterized the experimental model in mice treated acutely and chronically with the direct-acting alkylating agent methyl methanesulfonate in which they demonstrated the validity of the steady-state level of alkyl residues in hemoglobin as a measure of chronic repeated exposure.

Subsequently, Calleman et al. (*25*) carried out a study of hemoglobin alkylation in people occupationally exposed to ethylene oxide. Blood samples were obtained from persons exposed to doses of ethylene oxide established through continuous air-monitoring. Hemoglobin was analyzed for the presence of *N*-3-(2-hydroxyethyl)histidine by mass spectrometry and by ion-exchange amino acid analysis. The authors concluded that the hemoglobin alkylation values accurately reflected exposure, and were in good agreement with earlier data derived for ethylene oxide in the mouse.

Pereira and Chang (*26*) surveyed the ability of carcinogens and mutagens representing a broad spectrum of chemical classes to bind covalently to hemoglobin in rats. Animals were dosed with ^{14}C-labeled test compounds at levels of 0.1 to 10 μmole/kg body weight and blood was collected 24 hr later. Covalent binding was determined by analysis of purified hemoglobin for covalently bound radioactivity. All carcinogens/mutagens were found to form covalent adducts with hemoglobin, but the ability to do so varied over a wide range (binding index from 10 to 3322). It should be emphasized that the magnitude of this index is not reflective of potency as carcinogens for the rat, but when compounds were administered at more than one dose, the hemoglobin binding index was also dose related.

In order for protein alkylation to be useful as a monitoring procedure, reliable dose–response relationships between exposure dose and production of alkylated amino acids must be established. This requirement has been satisfied for the exposures studied to data, all of these experiments having been carried out in animals rather than in man. Thus, GC-MS determination of the production of *S*-methylcysteine in rat hemoglobin, following IP treatment with methyl methanesulfonate, showed that the alkylated amino acid was linearly related to dose (*27*). As noted above, experiments by Segerback et al. (*24*) showed that the degree of alkylation of hemoglobin in mice exhibited a linear dependence on the quantity of methyl methanesulfonate injected. For ethylene oxide a virtually linear relationship was observed between dose and production of *N*-3′-(2-hydroxyethyl)histidine in hemoglobin, in experiments in which the alkylating agent was administered by inhalation at doses of 1 to 100 ppm (30 hr/week for 2 yr) (*28*).

Other agents for which dose-response relationships have been established include the following: 4-aminobiphenyl (*29*), which displayed a linear hemoglobin binding response from 0.5 to 5000 μg/kg; *trans*-4-dimethylaminostilbene (*30*), where total binding to rat hemoglobin increased linearly over a 10^5-fold dose range; chloroform (*31*), which showed a linear binding from 0.1 to 100 mmole/kg in rat and mouse hemoglobin. Only for dimethylnitrosamine (*27*) was the dose response nonlinear over an IP dose range of 0 to 400 mmole/kg in rats.

It can thus be concluded that the amount of alkylated hemoglobin will be directly related to the erythrocyte dose of active alkylating agent, and the observed relationship between hemoglobin alkylation and exposure dose in animal experiments has also indicated that erythrocyte dose and exposure dose are directly related. The observation of hemoglobin alkylation can be considered an indication of genotoxic risk, however, only when it has been shown that such alkylations correlated with reactions at the target DNA site, i.e., that the erythrocyte dose is directly related to the target dose.

Comparison of the amounts of DNA and hemoglobin binding products have been made for a variety of radioactive alkylating agents. Vinyl chloride (*23*) and dimethylnitrosamine (*22*) have relatively short-lived metabolites, so the doses received by organs located distant from the liver represented only a small fraction of the liver dose, whereas ethylene oxide was more uniformly distributed. In the rat, observed levels of alkylation of guanine in liver DNA and testis DNA by ethylene oxide were about 150% and 50%, respectively, of values expected from the degree of alkylation of hemoglobin (*28*). Similarly, in the mouse (*32*) the extent of guanine alkylation in liver, spleen and testis deviated by no more than twofold from the amount expected from hemoglobin alkylation. Thus the degree of alkylation of DNA could be estimated approximately from the erythrocyte dose for ethylene oxide.

Further support for the use of hemoglobin (or other protein) alkylation as an indication of DNA-binding has come from the work of Neumann (*30*) who showed that the dose response of binding of *trans*-4-dimethylaminostilbene to plasma proteins and to hemoglobin was proportional to the dose-response for binding to liver DNA. Similarly, Pereira et al. (*33*) have illustrated that the dose-response curves for the binding of 2-acetyl-

aminofluorene to hemoglobin and rat liver DNA are closely related over a large dose range.

Thus in some instances, it seems possible to predict DNA-binding by means of determinations of protein binding, and therefore detection of the latter may be taken as an indication of a genotoxic risk. OPne of the most important questions remaining is how risk is quantitatively related to extent of DNA binding.

DNA Adducts

Two experimental avenues are available to obtain information on levels of DNA adducts formed through carcinogen activation. Measurements can be made of DNA adducts in cells of an accessible tissue (e.g., white blood cells, biopsy, or autopsy samples). Providing that the chemical nature and stability of the DNA adducts for the compound of interest had been fully characterized, qualitative as well as quantitative identification of adduct levels could provide for that individual not only an indication of exposure history but also of his capability to activate the carcinogen to DNA-binding forms.

It is well established that nearly all carcinogens form complex spectra of DNA adducts, involving covalent binding to various nucleophilic sites on all four DNA bases as well as the phosphate residues of DNA. Thus, from a qualitative viewpoint, detection of all DNA adducts derived from even a single carcinogen presents a very complex analytical challenge. Quantification of adduct levels is complicated further by the removal of adducts from DNA by chemical or enzymatic processes at different rates, even within the same cell. These rates can also vary substantially from one cell type to another.

Most available information of DNA adducts in experimental systems has been obtained through the use of physicochemical or radiochemical methods of detection. Usefulness of these methods for detection in human monitoring is limited by their relative insensitivity and inapplicability, respectively. However, immunological techniques are being developed which have promise of utility in detecting DNA adducts in people exposed to environmental carcinogens under ambient conditions. The determination of carcinogen–DNA adducts by immunologic procedure has certain advantages over other techniques. The sensitivity is frequently better than that obtainable with radiolabeled carcinogens (which are useful only for experimental purposes in any event). Antibodies are specific for particular three-dimensional structures and can be used to probe the conformation of unknown adducts on DNA. Immunological assays are rapid, highly reproducible and can be used in situations where the cost or availability of radiolabeled carcinogens would be prohibitive. The high sensitivity and capability of detecting nonradioactive adducts would therefore suggest a use in monitoring of human tissues. In addition, immunologic techniques can be applied together with morphologic procedures (electron microscopy and immunofluorescence) to localize adducts in particular cells, subcellular compartments, or DNA molecules. Current methods for detecting adducts in DNA are summarized below.

A second approach to monitoring DNA adducts is also being explored, taking advantage of the fact that adducts removed from cellular DNA (and also from RNA) are excreted in urine. Their detection and measurement of excretion rates would, in principle, provide information on (recent) exposure history of the subject, and possibly also indications of that individual's capability for DNA repair. Thus, studies of urinary excretions of adducts would provide data complementary to measurement of adduct levels in cellular DNA in the same individual.

The value of DNA adduct measurements in the dosimetry of genotoxic exposure will depend upon the fidelity with which they reflect exposure levels. Available information suggests that, in some experimental animal models, levels of DNA adducts in tissues are indeed reflective of carcinogen doses. Pegg and Perry (*34*) determined levels of 7-methylguanine in DNA of liver (the target organ) in rats killed 3 hr after a single dose of dimethylnitrosamine. They found that levels of this methylated base were linearly related to intraveneous or oral doses of the carcinogen over a dose range of 1 to 10^5 μg/kg body weight. Similarly, Appleton et al. (*35*) found that total AFB_1 adduct levels in DNA (and RNA) of the livers of rats injected with the carcinogen and killed 1 hr later were linearly related to AFB_1 dose over the range of 10 to 1000 ng/kg body weight.

The binding of benzo(a)pyrene (BaP) to nucleic acids of target and nontarget tissues has been studied by a number of investigators. Lutz et al. (*36*) administered the carcinogen by single injection to rats over the dose range 40 μg/kg to 4 mg/kg body weight. The animals were killed 50 hr after dosing and total carcinogen binding to liver DNA was determined. The dose–response relationship was found to be linear up to 1 mg/kg and nonlinear at higher doses. Dunn (*37*) also measured levels of DNA adducts derived from BaP in the DNA of target (stomach) and nontarget (liver) organs of mice, and found direct proportionality between binding level and dose over a wide dose range (10^{-8} to 10^{-3} g/mouse). The relationship appeared to be linear over most of the dose range, but Stowers and Anderson (*38*) report nonlinearity in other experimental models.

Relationships between concentrations of covalently bound ^{14}C-formaldehyde in the DNA of the respiratory epithelium and air levels of the carcinogen have been investigated in rats exposed at levels of 0.3 to 15 ppm (*39*). At concentrations of 2 ppm or higher, the level of DNA modification was directly proportional to the airborn carcinogen level, but below 2 ppm the amount of DNA binding was less than proportional to dose.

Collectively, these data suggest that DNA adduct levels can reflect carcinogen exposure, although the quantitative relationships between the two parameters will need to be established in a larger number of experimental models before generalizations can be drawn.

DNA Adducts and Carcinogenesis

Replication of DNA soon after carcinogen-induced DNA damage is important for mutagenesis, cell curvival (*40*), and *in vitro* transformation (*41*). Available data suggest that DNA replication after carcinogen treatment *in vivo* is also important in the development of cancer (*42–44*). It is possible that the initiation of carcinogenesis may result from replication of carcinogen-damaged DNA, which presumably fixes a lesion in DNA as a mutation. Thus, to exert its mutagenic and possibly a carcinogenic effect, a DNA adduct may need to remain in DNA until DNA replication occurs. Consistent with this idea, the persistence in DNA of some adducts (which may be premutagenic lesions) correlates with the induction of cancer, as indicated by several lines of evidence.

People who suffer from syndromes characterized by deficiencies in DNA repair are prone to the development of cancers (*45*). Examples of such syndromes include xeroderma pigmentosum (*46*), Fanconi's anemia (*47*), and ataxia telangiectasia (*48,49*). Cultured cells from people with DNA repair deficiencies do not remove DNA modifications and are hypersensitive to the induction of mutation after chemical treatment (*40,50,51*). The time interval between carcinogen treatment and DNA synthesis is inversely related to the induced mutation frequency in repair-efficient chemically treated cells, whereas this interval is inconsequential to induced mutation frequency in repair-deficient cells (*50*).

Species susceptible to the tumorigenic effects of a chemical and species resistant to the same effects form qualitatively similar DNA-adduct profiles in many organs. Examples include DNA adducts formed after treatment with aflatoxin B_1 (in male rat and mouse, kidney and liver) (*52,53*), dimethylbenzanthracene (DMBA) in female Swiss and C57BL mouse skin (*54*) and benzo(a)pyrene in male Swiss mice and Wistar rat skin (*55*). Among all adducts formed, specific DNA modifications can persist in DNA. While additional sites in DNA are modified, persisting small and bulky lesions form predominantly at the O^6, N^2, and C^8 atoms of guanine. Although N^7-guanine modification generates a positive charge in the imidazole ring, rendering the glycosidic bond labile, neutralization of the positive charge by fission of the imidazole ring stabilizes the modified guanine in DNA. Therefore, the respective formamidopyrimidine derivatives of N^7-modified guanine may also persist in DNA.

Although qualitatively similar adducts form in sensitive and resistant species, in many cases a greater total amount of adducts is found in target organ DNA of sensitive species. Persistent adducts and the respective target organs are summarized in Table 1. Target organs in which more adducts persist and nontarget organs where fewer adducts persist are listed in Table 2.

The quantitative difference in adduct persistence in target organ DNA of sensitive and resistant species may be attributable to a number of possible mechanisms. Different organs in one animal or the same organ in different species or even different cell types in one organ might possess different capacities for specific repair processes; those organs or cells with minimal repair capacities would be least efficient in removing certain adducts. Indeed, greater persistence of DMH-induced O^6-methylguanine in DNA has been demonstrated in nonparenchymal cells (*70*) that are deficient in repair activity for this lesion (*69*) and in which DMH-induced tumors arise.

Furthermore, different organs in one animal or the same organ in different species may vary in metabolic activation capacity (including types and extents of reaction). Therefore, target organ DNA may be modified to a greater extent due to relatively high activating enzyme levels or relatively low levels of enzymes for competing detoxification pathways. If adducts are removed at a constant rate, greater initial adduct levels would be reflected in longer adduct persistence. Alternatively, high initial amounts of adduct might saturate constitutive repair processes, leading to enhanced persistence.

Although a positive correlation is often observed between adduct persistence and sensitivity to tumor induction for different species, differences in adduct persistence do not correlate well with the differences in carcinogenicity of one compound in different strains of the same species. Instances where more adducts form or persist in resistant rather than sensitive species or strains are noted in Table 3. In some of these studies covalent modification was monitored for a relatively short interval after dosing, especially since the level of modification is still increasing at 24 hr after dosing (*78*). It is also significant that DNA was analyzed for total covalent modifications instead of for specific lesions. Measurement of persistence of total lesions may not detect the persistence of a minor but important lesion (as would be the case with O^6-alkylguanine). Finally, the relative rate of specific adduct removal rather than the absolute amount of persistent adduct may be important for susceptibility to tumor formation. For instance, although higher levels of adducts form in the liver (a nontarget organ) compared to the mammary gland (the target organ), Daniel and Joyce (*77*) observed 70% DMBA adduct removal from liver DNA but essentially total persistence of DMBA adducts in the rat mammary gland.

In addition to indicating potential target organs for tumorigenesis, the quantity of adducts in DNA may also indicate the relative carcinogenic potency of different chemicals. The extent of covalent binding of polycyclic aromatic hydrocarbons to DNA in mouse skin correlates in a positive manner with carcinogenic potency (*79*) and with tumor initiating activity (*80*). Covalent binding of PAH to DNA in mammalian cells also correlates with carcinogenic potency (*81*).

Lutz (*82*) reviewed the correlation between covalent modification of DNA and carcinogenicity for a wide range

of chemical classes, and discusses the covalent binding index (CBI), defined as

$$\text{CBI} = \frac{\text{damage to DNA}}{\text{dose}} = \frac{\text{micromole chemical bound per mole nucleotides}}{\text{millimole dose/kilogram body weight}}$$

The CBI is determined when rodents are treated with radiolabeled chemicals, liver DNA is isolated at the time of maximum binding to DNA and covalent modification is quantified by liquid scintillation counting. The CBI is correlated with the rodent hepatocarcinogenicity of at least 16 chemicals from different classes.

The value of the CBI as a quantitative index of general carcinogenicity is, however, limited by a relatively high rate of false positives, especially with direct acting alkylating agents such as sulfonates and some nitroso derivatives. The weakness of this index may be explained by the fact that there are many factors which do not enter into the CBI calculations but on which the CBI depends. These factors, such as species, strain, dose response, time between dose and DNA isolation, method of DNA isolation, metabolic state of test animals, target organ, and cell specificity, probably vary with each chemical. The value of the CBI as an index of carcinogenicity is compromised for two additional reasons in particular. First, this index reflects total covalent modification of DNA. It is possible that only specific DNA modifications, as discussed above, are biologically relevant to carcinogenesis, a relationship that the CBI

Table 1. DNA adduct persistence associated with target organ sensitivity.

Carcinogen[a]	Persistent adduct[a]	Species and organ in which adduct persists and tumor develops	Doses		Persistence[b]	References
			Number	Route		
AFB_1	AFB_1-FAPy[c]	Rat liver	10	IP	2 weeks	(*53,56*)
Aromatic amines						
N-OH-AAF	AF-C^8-dG	Rat mammary gland	1	IP	4 weeks	(*57*)
	AF-C^8-dG					
	AAF-N^2-dG[d]	Rat liver	1–4	Oral	8 weeks	(*58*)
N-OH-1-NA	1-NA-O^6-dG	Rat skin	1	SC	7 days	(*59*)
2-NA	2-NA-C^8-dG	Dog urinary bladder	1	Oral	7 days	(*60*)
BZ	ABZ-C^8-dG	Mouse liver	7 days	In water	7 days	(*61*)
DMAB or AB	AB-C^8-dG	Mouse liver	1	IP	21 days	(*62*)
BaP	BPDE-I-N^2-dG	Swiss mouse[e] epidermis	1	Topical	3 weeks	(*55,63*)
		BALB/c mouse skin	1	Topical	4 weeks	(*64*)
		SENCAR mouse epidermis and basal epidermal cells	1	Topical	72 hr	(*65*)
(+)BPDE – I	BPDE-I-N^2-dG					
MCPP	MCPP-N^2-dG, MCPP-dA	Mouse skin	1	IM	14 days	(*66,67*)
3-MC	(total)	A/J and C3H mouse[f] lung	1	IV	28 days	(*68*)
Alkylating agents						
DMH	Me-O^6-G	Rat liver nonparenchymal cells	2	Oral	48 hr	(*69*)
			28	Oral	4 weeks	(*70*)
MNU	Me-N^7-FAPy	Rat bladder	1	IU	21 days	(*71*)
ENU	Et-O^6-G	Rat brain	1	IP	10 days	(*72*)
DEN	Et-O^4-T	Rat hepatocytes	77	Oral	11 weeks	(*73*)

[a] AAF-N^2-dG, 3-(deoxyguanosin-N^2-yl)-acetylaminofluorene; AB, aminoazobenzene; AB-C^8-dG, *N*-(deoxyguanosin-8-yl)-4-aminoazobenzene; ABZ-C^8-dG, N-(deoxyguanosin-8-yl)-N′-acetylbenzidine; AFB_1, aflatoxin B_1; AFB_1-FAPy, 2,3-dihydro-2-(N^5-formyl)-2′,5′,6′-triamino-4′-oxo-N^5-pyrimidyl-3-hydroxyaflatoxin; AF-C^8-dG, *N*-(deoxyguanosin-8-yl)-2-aminofluorene; BaP; benzo(a)pyrene; (+)BPDE-I, (+)−7R,8S-dihydroxy-9R,10R-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene; BPDE-I-N^2-dG, 10β-(deoxyguanosin-N^2-yl)-7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene; BPDE-I-tetrol, (±)-7β,8α,9α,10(α or β)-tetrahydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene; BZ, benzidine; DEN, diethylnitrosamine; DMAB, *N,N*-dimethyl-4-aminoazobenzene; DMBA, dimethylbenzanthracene; DMH, 1,2-dimethylhydrazine; ENU, ethylnitrosourea; Et-O^6-G, O^6-ethylguanine; Et-O^4-T, O^4-ethylthymidine; MCPP, 15,16-dihydro-11-methyl-cyclopenta[a]phenanthren-17-one; MCPP-dA, an unidentified adduct between an MCPP metabolite and deoxyadenosine; MCPP-N^2-dG, 1-(deoxyguanosin-N^2-yl)-1,2,3,4-tetrahydro-2,3,4-trihydroxy-15,16-dihydro-11-methylcyclopenta(a)phenanthren-17-one; Me-O^6-G, O^6-methylguanine, Me-N^7-FAPy, N^5-methyl-N^5-formyl-2,5,6-triamino-4-hydroxypyrimidine; MNU, methylnitrosourea; N-OH-AAF, *N*-hydroxy-acetylaminofluorene; N-OH-1-NA, *N*-hydroxy-1-naphthylamine; PAH, polycyclic aromatic hydrocarbon; 1-NA-O^6-dG, N-(deoxyguanosin-O^6-yl)-1-naphthylamine; 2-NA, 2-naphthylamine; 2-NA-C^8-dG, *N*-(deoxyguanosin-C^8-yl)-2-naphthylamine; 3-MC, 3-methylcholanthrene.

[b] Persistence indicates time from the first dose. Time noted was the last time point in the study.

[c] The adduct in equilibrium with AFB_1-FAPy is also persistent.

[d] This adduct is detected only in male rat liver, is found in increasing amounts with continued dosing, and persists only as long as dosing continues.

[e] In this case, the strain of mouse is important. See Table 3.

[f] In this case, the strain of mouse is important. See Table 2.

Table 2. DNA adduct levels and persistence associated with target organ sensitivity.

Carcinogen[a]	Target tissues or organ where adducts persist[a]	Nontarget tissues or organ where fewer adducts form and/or persist	References
AFB_1	Male rat liver	Male rat kidney	(*53,56*)
	Male rat liver	Male mouse liver	
N-OH-AAF	Male rat liver	Female rat liver, rat kidney	(*58*)
2-NA	Dog urinary bladder	Dog liver	(*60*)
DMAB and AB[b]	Mouse liver	Rat liver	(*62*)
BaP	Male or female mouse skin	Male rat skin	(*55*)
MCPP	TO mouse skin	TO mouse liver	(*66*)
3-MC	A/J and C3H mouse lung	DBA and C57BL mouse lung or A/J and C3H mouse liver	(*68*)
DMH	Male rat nonparenchymal cells	Male rat hepatocytes	(*70*)
ENU	Rat brain	Rat liver	(*72*)
DEN	Male rat hepatocytes	Male rat nonparenchymal cells	(*73*)

[a] See Table 1 for abbreviations and the corresponding persistent DNA adduct.

[b] AB and its *N*-methyl and *N,N*-dimethyl derivatives are tumorigenic in mice while only the methylated derivatives are hepatocarcinogenic in rats. AB-C^8-dG is the major adduct formed and it persists in mouse liver DNA after AB or DMAB treatment. The same adduct forms only in very low amounts in rat liver DNA after DMAB or MAB treatment.

would obscure. Second, the CBI is based on the initial formation of adducts whereas initiation of carcinogenesis may be a consequence of adduct persistence, also as discussed above.

Methods for Detecting Carcinogen–DNA Adducts

Methods of varying sensitivities exist to measure DNA-carcinogen adducts. Many of these methods are applicable for the detection of adducts in DNA from human tissues, as discussed below. To facilitate comparison of the various techniques, assays are presented for detection of the major BaP-DNA adduct, 10β-(deoxyguanosin-N^2-yl)-7β,8α,9α-trihydroxy-7,8,9-tetrahydrobenzo(a)pyrene (BPDE-I-N^2-dG), since most available assays have been used to detect this PAH-DNA adduct. A summary of the assays and their sensitivities is found in Table 4. It is important to note that the sensitivity of an assay as defined here depends on the amount of DNA assayed as well as the rate of modification of that DNA. In other words, the most sensitive assay requires the least DNA, modified at the lowest level. Both factors must be evaluated for each method.

Ultraviolet absorbance (at 254 nm or other wavelengths, depending on the modifying carcinogen) in line with RP HPLC is sensitive to the detection of approximately hundreds of picomoles (generally less than 1 μg) of adduct. This approach is generic for many carcinogen–DNA adducts due to the strong absorbance of DNA bases at 254 nm. Although this method is in principle applicable to the detection of adducts in DNA from people, compared with other available methods, it is relatively insensitive.

Rahn and his colleagues (*84*) developed an assay for the detection of BaP–DNA adducts by fluorescence. They hydrolyze BPDE-DNA in 0.12 N HCl for 6 hr at 80°C to release (±)-7β,8α,9α,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo(a)pyrenes (BPDE-I-tetrols), and then measure the released tetrols by fluorescence in line with RP HPLC and estimate the amount of adduct from the amount of tetrols recovered. Although fluorescence of the tetrols is due to the same intact pyrene moiety found in covalently bound BPDE, the interaction of the DNA bases with covalently bound BPDE strongly quenches the fluorescence of the pyrene moiety. Thus, indirect measurement of adducts using tetrols is necessary to achieve sensitivity with this technique. Application of this approach to the detection of other carcinogens in DNA clearly depends on the presence and the intact release of a fluorescent moiety. Under the HPLC and detection conditions of Rahn and co-workers (*84*), as little as 31 fmole of BaP tetrols or one BP modification per 10^7 bases in 100 μg DNA is theoretically detectable.

Advantage has been taken of the specificity and sensitivity of antibody–antigen reactions to detect DNA

Table 3. Experimental models in which DNA adduct levels and persistence do not correlate with target organ specificity.

Carcinogen[a]	Tumor-"resistant" organ where adducts persist[b]	Tumor-"sensitive" organ where fewer adducts form and/or persist	Persistence	References
BaP	C57BL/6 and DBA/2 mouse skin[c,d]	Swiss mouse skin	48 hr	(*74*)
			24 hr	(*75*)
MCPP	DBA/2 mouse skin[d]	TO and C57/2 mouse skin	24 hr	(*76*)
3-MC	C57BL/6 and DBA/2[d] mouse skin	Swiss mouse skin	48 hr	(*74*)
DMBA	C57BL/6 and DBA/2[c,d] mouse skin	Swiss mouse skin	8 days	(*74*)
	C57Bl mouse skin	Swiss mouse skin	48 hr	(*54*)
DMBA	Sprague-Dawley rat liver	Sprague-Dawley rat mammary gland	12 days	(*77*)

[a] Abbreviations given in footnote to Table 1.

[b] Total covalent modification of DNA is measured to determine this relationship; in addition, for MCPP (see Table 1) and DMBA, but only in Dipple et al. (*54*), the persistence of individual adducts was measured.

[c] Similar levels of adducts persisted in organs which are resistant and sensitive.

[d] DBA/2 mice are more resistant to the tumorigenic effects of polycyclic aromatic hydrocarbons than are C57BL mice.

Table 4. Assays for the detection of carcinogen–DNA adducts.

Method	Limit of detection, fmole	Amount of DNA used per analysis, μg[a]	Modification of analyzed DNA, adducts/base
UV in line with HPLC [major benzo(a)pyrene-DNA adduct[b]]	100,000[c]	2600	2×10^{-5}
Fluorescence in line with HPLC (BPDE-I-tetrol)	31[d]	100	1×10^{-7}
Photon counting synchronous scanning fluorimetry[e]			1×10^{-6}
Immunoassays			
Polyclonal rabbit antibodies against BPDE-I-DNA[f]			
Competitive assays			
RIA	5300[g]	1	1.7×10^{-3}
ELISA	55[g]	1	1.8×10^{-5}
USERIA	12[g]	1	3.9×10^{-6}
USERIA	10	25	1.4×10^{-7}
Noncompetitive assays			
USERIA	3	0.01	9.7×10^{-5}
Monoclonal antibodies against BPDE-I-DNA[h]			
Competitive ELISA	19[g]	0.005	1.2×10^{-3}
Noncompetitive ELISA	3	0.0002	4.9×10^{-3}
^{32}P-postlabeling[i]	0.03–0.3[j]	1	1×10^{-7}–10^{-8}

[a] As a guideline, 10–100 μg DNA is recoverable from 0.2-1 g tissue or the buffy coat of 25–50 mL human blood.
[b] Benzo(a)pyrene is used as an example in this table since these techniques have been applied to the detection of its major adduct, 10-(deoxyguanosin-N^2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene.
[c] Data of Weinstein (*83*).
[d] Data of Rahn et al. (*84*).
[e] Data of Vahakangas (*85*).
[f] Data of Hsu et al. (*86*).
[g] This amount of adduct shows 50% inhibition.
[h] Data of Santella et al. (*87*).
[i] Data of Gupta (*88*).
[j] Theoretical limit of detection.

adducts. Polyclonal rabbit antisera have been raised and monoclonal antibodies produced against carcinogen-modified DNA and modified DNA and RNA components (Table 5). Both small alkylations and bulky modifications are included in this list. Available antibodies specific for carcinogen-modified DNA components have been reviewed elsewhere (*103,104*).

These antisera and antibodies have been used in three types of immunoassays: radioimmunoassays (RIA) including the radioimmunosorbent technique (RIST), enzyme-linked immunosorbent assays (ELISA), and ultrasensitive enzymatic radioimmunoassays (USERIA). As is evident from the summaries below, all of these assays could be applied to the detection of chemical–DNA adducts in human tissues.

Immunoassays may be executed in a competitive or noncompetitive fashion. RIA are competitive assays whereas solid phase assays may be performed according to a competitive or noncompetitive protocol. In competitive immunoassays, two chemically identical haptens, one the tracer and the other an inhibitor, compete under equilibrium conditions for the same antibody binding site. When the concentration of the tracer is constant, the concentration of the inhibitor is reflected in the degree to which it inhibits binding of the tracer to the antibody. Quantitation is based on the comparison of unknowns with an inhibition curve generated by competing increasing concentrations of standard unlabeled hapten (*104*).

In RIA, although the haptens are chemically identical, one hapten is radiolabeled (the tracer) while the other one is unlabeled (the inhibitor). In the conventional RIA, performed in tubes, the antigen–antibody complex is separated from the whole reaction mixture by precipitation. After centrifugation or filtration (*89*), the precipitate is counted to determine the antigen level.

In solid phase assays (including ELISAs, USERIAs, and RISTs), where the antigen is coated on a solid plastic surface, antibody binding to the antigen is monitored by binding a second antibody. The second antibody is either linked to an enzyme which can cleave a chromogen (*p*-nitrophenylphosphate) or a radioactive sub-

Table 5. Monoclonal antibodies or polyclonal antisera directed against carcinogen-modified DNA or DNA components.

Antigen or carcinogen	Nature of antibody	References
O^6-Methyldeoxyguanosine[a]	Rabbit polyclonal	(*89*)
	Rabbit polyclonal	(*90*)
	Monoclonal	(*90*)
O^6-Ethyldeoxyguanosine[a]	Rabbit polyclonal	(*91*)
	Monoclonal	(*92*)
O^6-isopropyldeoxyguanosine[a]	Monoclonal	(*92*)
O^6-butyldeoxyguanosine[a]	Monoclonal	(*92*)
	Monoclonal	(*93*)
O^4-ethyldeoxythymidine[a]	Monoclonal	(*92*)
O^4-butyldeoxythymidine[a]	Monoclonal	(*93*)
O^2-butyldeoxythymidine[a]	Monoclonal	(*93*)
AAF-C^8-dG[b]	Rabbit polyclonal	(*94*)
AAF-DNA	Rabbit polyclonal	(*95*)
		(*96*)
BPDE-I-DNA	Rabbit polyclonal	(*97*)
BPDE-I-DNA	Monoclonal	(*87*)
BPDE-I-N^2-dG[b]	Monoclonal	(*87*)
AFB_1-DNA	Monoclonal	(*98*)
		(*99*)
Cis-diamminedichloro-platinum (II)-DNA	Rabbit polyclonal	(*100*)
Thymine dimer-DNA	Monoclonal	(*101*)
Thymine glycol-DNA	Monoclonal	(*102*)

[a] The immunogen was the corresponding riboside complexed to various carrier proteins.

[b] These haptens were complexed to various carrier proteins to elicit an immune response.

strate (^{3}H-adenosine monophosphate) (*105*), or the second antibody is labeled with ^{125}I (e.g., ^{125}I-labeled goat antirabbit IgG) (*91*). The initial antibody–antigen reaction is amplified in the first two cases because one molecule of enzyme can react with many substrate molecules; in the third case, amplification is due to the high specific activity of the iodinated second antibody. Bound enzymatic activity or radioactivity is thus determined by spectrophotometric assay of p-nitrophenol or by radiometric assay of ^{3}H-adenosine or ^{125}I-antibody–antigen complexes.

In a noncompetitive assay, antiserum is reacted with standard or unknown antigen samples only. The assay is completed as described above and, finally, bound enzymatic activity or radioactivity is determined.

The sensitivity of different types of immunoassays depends in part on the characteristics of the antiserum or monoclonal antibody. Solid phase immunoassays are generally more sensitive than, but at least as sensitive as, RIA (*86,91,105*). Of the different solid phase immunoassays, USERIA is at least as sensitive as, if not more sensitive than, ELISA (*86,99,105*).

For example, a typical RIA with polyclonal antisera may detect tens of nanograms of adducts in 2 to 20 μg of DNA (*94,97*). In competitive assays, using 1 μg of DNA, the amounts of BaP-DNA adducts that showed 50% inhibition were 5300 fmole in RIA, 55 fmole in ELISA, and 12 fmole in USERIA. As little as 3 fmole adduct in 10 ng were detected by noncompetitive USERIA, and as little as 10 fmole adduct in 25 μg DNA were detected by competitive USERIA (approximately 1.3 modifications in 10^7 bases) (*86*). Therefore, USERIA is approximately 500-fold more sensitive than radioimmunoassay and 5-fold more sensitive than ELISA for detection of BaP–DNA adducts (*86*).

By application of highly sensitive instrumentation, for example for the measurement of fluorescent products in ELISA assays, the sensitivity of the immunoassays currently in use can be increased to theoretical limits of detection of one DNA adduct per cell or less (Poirier, personal communication).

The advantages of using immunoassays as opposed to other methods to detect carcinogen-DNA adducts include the ability to recognize adducts at levels probably found in human DNA. Theoretically, the specificity of the antigen-antibody reactions should permit detection of one type of chemical adduct in DNA where many types of adducts may persist. This is especially true of monoclonal antibodies which are specific for usually a single antigenic determinant. The reproducibility and rapidity of performing immunoassays is also advantageous for monitoring exposure to carcinogens in large numbers of tissue samples. Perera et al. (*106*) demonstrated that adducts present at low levels in human tissues are detectable by immunoassay.

Alternative methods for adduct detection which do not involve the use of radioactive carcinogens include methods where nucleic acid monomers are chemically or enzymatically derivatized with a radioactive reagent, chromatographically separated, and fractions are assayed for radioactivity. The chromatogram is compared with chromatograms of standard derivatized adducts to identify modifications in a DNA sample. When the ribosyl or deoxyribosyl moiety is derivatized, these methods are generally applicable to the detection of many carcinogen-DNA adducts for which standards are available. These methods in principle also circumvent the need for time-consuming development of special assay reagents, such as antibodies, for each carcinogen.

One method of this type is ^{32}P-postlabeling, described initially by Randerath and colleagues (*107*) and later by Haseltine and coworkers (*108*), and discussed in detail elsewhere in this issue (*109*). In this procedure, the digestion of DNA to deoxynucleoside 3′-monophosphates precedes enzymatic phosphorylation of the 5′-hydroxyl group with [gamma-^{32}P]-ATP, chromatographic separation of the labeled products, and assay for radioactivity.

Randerath and his colleagues analyze the [5′-^{32}P]-deoxynucleoside-3′,5′-bisphosphates (^{32}pdNps) by thin-layer chromatography (TLC). Unmodified ^{32}pdNps were resolved by one- or two-dimensional TLC on a PEI–cellulose plate. To resolve the modified ^{32}pdNps, this group adopted a four-dimensional thin-layer chromatography procedure on PEI-cellulose layers (*88*) or PEI-cellulose in combination with C_{18} reverse-phase layers (*110*). Chromatography in buffer or buffer-organic mixtures (directions 1 and 2) afforded separation of inorganic ^{32}P-phosphate (a product of the unreacted [gamma-^{32}P]-ATP) and unmodified ^{32}pdNps from the modified ^{32}pdNps. Carcinogen-DNA adducts were finally re-

solved by chromatography in high salt, high urea solvents (directions 3 and 4).

The advantages of postlabeling include: the obvious lack of requirement for radioactive carcinogens; any animal or human DNA sample can be screened; many samples can be analyzed simultaneously; and the sensitivity of the assay is, in theory, the highest of available adduct assays. The sensitivity of the assay is based on the availability of [gamma-^{32}P]-ATP with a specific activity much higher than the specific activity of available carcinogens: as high as 8000 Ci/mmole. In addition, since ^{32}P emits high energy beta rays compared to the lower energy rays emitted during ^{14}C and ^{3}H decay, disintegration can be easily detected on X-ray film. Since films exposed to samples containing ^{32}P may be developed for extended periods and exposure may be enhanced with an intensifying screen and/or in the cold, as little as 10 dpm in a 10 m^2 area are detectable (*111*).

Using only PEI-cellulose TLC in the protocol described above, Randerath and coworkers recovered approximately 50% of chromatographed adduct and have detected approximately one aromatic adduct in 10^7 bases using 1 ug of DNA (*88*). When inorganic phosphate and unmodified nucleotides were initially removed from the postlabeling reaction mixture by RP-TLC, background radioactivity was reduced further and, although recoveries were generally less than 50%, the lower limit of detection calculated by these researchers was one adduct in about 10^8 bases (*110*).

DNA modified *in vitro* and *in vivo* by different types of carcinogens has been subjected to postlabeling analysis (*64,88,110,112*). This method can successfully detect adducts of aromatic amine and amides, polycyclic aromatic hydrocarbons and methylating agents.

In conclusion, there are a number of methods available to detect DNA adducts but only some of them are applicable in their current forms to the detection of adducts in human DNA samples. Each technique has its own advantages and limitations; depending on the specific objectives of monitoring projects, different methods may prove to be advantageous for specific purposes.

Use of DNA Adducts as Dosimeters: Factors Affecting DNA Adduct Levels

Analysis of human DNA for the presence of specific adducts has been successful in at least two instances using immunoassays (*106,113–115*). Using polyclonal rabbit antiserum specific for BaP covalently bound to DNA in an enzyme-linked immunosorbent assay, Perera and colleagues (*106*) established that lung DNA from BaP-treated rabbits or mice contained antigenicity. Assuming this antigenicity was due to BaP adducts in DNA, they detected as little as 0.08 to 0.1 fmole (45–57 fg) adducts/microgram DNA. In the same system with the same assumptions, these researchers detected low levels (0.14–0.18 fmole or 80–100 fg) of BaP–DNA adducts in tissues from five of 27 patients. Adducts were detected in DNA from tumorous lung tissue from four patients, in DNA from nontumorous lung or bronchus tissue from three patients, and in peripheral blood mononuclear cell DNA in three patients.

Exposure histories were available for only 15 of the 27 cases, and the five positive samples were among those cases for which histories were available. The five positive samples were from lung cancer patients who at some time in their lives had smoked cigarettes or had been exposed to smoking in the home (one individual). Only one patient had smoked within three years prior to surgery; this patient quit two months before surgery. No correlation, however, was observed between smoking and the results of the immunoassays. One positive case had been occupationally exposed to coal dust and fumes. All positive cases and almost all negative cases had been exposed to urban air pollution for most of their lives. It is impossible, unfortunately, to draw substantial conclusions from a study such as this one with a small number of unselected cases and controls. These preliminary results are encouraging, however, for the possibility of detecting adducts in human tissues in a more comprehensive study.

The feasibility of quantitatively assessing exposure to chemicals using DNA adducts is the subject of this conference. In addition to the availability of sensitive methods to quantitate adducts, factors essential to determine the usefulness of adducts as dosimeters include: the rate of modification as a function of dose (due to changes in metabolic paths with dose, induction or saturation of specific and nonspecific DNA repair processes, and similar dose-dependent processes); the ability of the modification to reflect past or recent exposures (i.e., the persistence of the modification, including chemical stability and susceptibility to enzymatic repair); and finally the ability of the particular modification to reflect acute or chronic exposure to the parent chemical (i.e., modification as a function of the number of doses).

Using techniques of molecular epidemiology, adducts have been quantified in animal DNA and related to dose, as discussed above. Quantitative adduct analysis in human DNA to measure exposure, however, is more complicated for several reasons. Individuals live in environments where hazardous chemical contamination is diverse and ill-defined, unlike controlled laboratory experiments. Thus, human doses are complex and not easily determined. Most carcinogens require metabolic activation prior to DNA modification. Metabolic capacity is determined genetically as well as environmentally and therefore varies between individuals (*116*), between tissues (*117*), and between different sections of the same tissue (*118*). The magnitude of differences is greatest between individuals, and outbred animals vary over a wider range than inbred animals (*114,119*). People, therefore, might vary extensively in adduct formation with respect to environmental dose. Variations in adduct formation has been demonstrated using organ ex-

plants: benzo(a)pyrene modification of human DNA varied 50- to 200-fold in colon (*118*), bladder (*120*), bronchi (*121*), and esophagus (*122*); other species showed less variation than humans in covalent BaP binding to DNA (*123*). Similar individual variations were observed with the binding of other carcinogens to these and additional organs in culture (*114*).

The kinetics and the extent of DNA adduct removal in human tissues *in vivo* are unknown. There is evidence for DNA repair in cultured human cells (*124*) and human tissue extracts (*125*). DNA repair capacities, however, may well vary *in vivo* in different organs and between individuals. Unpredictable and variable rates of DNA repair would make it difficult to extrapolate the amounts of adducts in DNA at one time from the amount of adducts left in DNA at some later time. Furthermore, the qualitative and quantitative profiles of chemical-DNA adducts formed in people may be different from the profiles of adducts already identified in animal DNA. Of particular importance, the biologically relevant adducts may be as yet unidentified minor modifications of DNA. If adduct profiles are qualitatively different, structural identification of adducts in human DNA will be a sizable task. Results to date indicate, however, that adducts produced in human organ culture after treatment with different carcinogens (including BaP, AFB_1, DMBA and DMH) are the same adducts identified in experimental animals (*114,118,122,126*). Assuming the profile of metabolic enzymes does not change radically early in organ culture (when the adducts in these experiments were identified), it is likely the same adducts form *in vivo* in people.

Given that adduct repair and persistence *in vivo* in humans is unknown, the interval between exposure and sampling might be critical. If human cells are highly effective in DNA repair, adducts, although formed, may go undetected. Detection of adducts in human DNA will also depend on the rate of cell turnover in the tissue used to monitor adduct formation. If cells replicate quickly, then only short-term exposures could be measured in this tissue. Both of these considerations would be strongly affected by the time interval between exposure and sampling of tissue for analysis. Finally, the usefulness of adducts a dosimeters depends on the fidelity of the surrogate cell DNA as an index of target cell exposure.

Thus, although the interpretation of adduct levels as dosimeters remains complicated, adducts can serve as useful qualitative markers of exposure. As more information is learned about adduct kinetics in DNA of human tissues as well as other factors mentioned above, DNA adducts may become more useful as dosimeters. In addition, further study of the formation of DNA adducts in human tissue will provide further insight into factors determining how people respond to exposure to chemicals.

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