Monograph

No 13

DNA and Protein Adducts: Evaluation of their use in Exposure Monitoring and Risk Assessment

October 1989

ISSN-0773-6347-13

MONOGRAPH No. 13

DNA AND PROTEIN ADDUCTS: Evaluation of their use in Exposure Monitoring and Risk Assessment

ECETOC Monograph No. 13

FOREWORD

During the last decade there has been considerable research interest in the potential for using DNA adducts or protein (more specifically haemoglobin) adducts as an indicator of potential carcinogenic risk to man. ECETOC is conscious of the implications of these technologies. The objective of this monograph is to explore the capabilities of the technology and make reference to the clinical and ethical issues. Beyond this stage there is a need for a detailed review of the medical issues involved and an exploration of their resolution; it would be a new departure for ECETOC to undertake such a task. Indeed it may be premature to undertake such a review since many of the issues will disappear as understanding and experience increase. It is worthwhile noting that in the past many improvements in preventative or therapeutic health measures have posed similar ethical difficulties and initially generated anxiety. Others addressing these problems are asked to take note that ECETOC would wish to co-operate but not initiate discussion on the topic.

R R Knowland

President

CONTENTS

SUMMARY AND CONCLUSIONS

Most genotoxic chemicals form covalent bonds with DNA or proteins thereby forming adducts. Analytical methods have been developed for the quantitative determination of the amount of chemical which binds to the DNA or protein. These methods include physico-chemical and immunochemical assays for both DNA and protein adducts and postlabelling assays for DNA adducts. Similar analytical sensitivity can be attained with DNA and protein (haemoglobin) adducts. The sensitivity is sufficient for measuring both environmental and occupational exposures.

Two applications have been discussed: human population exposure monitoring (which includes occupational, environmental and medicinal exposure) and health risk assessment.

In estimating exposure, distinction is made between external dose (environmental monitoring), internal dose (biological monitoring) and tissue dose (biochemical effect monitoring). The tissue dose can be assessed from the amount of DNA adduct in tissue. Target tissue is not usually accessible in occupational settings and as a surrogate the amount of DNA adduct in a non-target tissue, invariably white blood cells, or the amount of haemoglobin or protein adduct in blood can be determined. Animal experiments with a variety of covalently reacting compounds have shown that the DNA adduct level in target and non-target tissues and the haemoglobin adduct level were directly proportional to the external dose; furthermore the extent of haemoglobin and DNA binding were quantitatively related. A different ratio may exist between DNA and haemoglobin binding level for each chemical. Haemoglobin adducts are stable and the level of adduct probably reflects exposures over the lifetime of the erythrocyte while some DNA adducts are chemically more labile and also subject to DNA repair. Accurate measurement of the tissue dose by the measurement of DNA adducts is therefore more dependent on the time at which a sample is taken than in the case of haemoglobin adducts.

Determination of haemoglobin adducts has proved to be a sensitive method for measuring levels of occupational exposure[s] to ethylene and propylene oxides, aromatic amines and chromium (VI) well below established occupational exposure limits. DNA adduct levels in white blood cells have been measured in workers occupationally exposed to high levels of polycyclic hydrocarbons (PAH) and in patients who underwent drug therapy with *cis* diamminedichloroplatinum (II). The amounts of covalent binding were related to the extent of exposure of groups of people, but considerable variation was found between individuals. Appreciable amounts of DNA and haemoglobin adducts have been found in non-occupationally exposed populations. Smoking of tobacco has proved to be a major contributor to these background levels, however, such adducts have also been found in non-smoking subjects.

DNA adducts are likely to be of prime importance in tumour initiation and induction of mutation but their relevance to the later stages of carcinogenesis (promotion, progression) is unknown. DNA adduct levels produced by aromatic amines, for example, did not correlate with organ susceptibility to tumour induction in experimental animals. DNA binding alone cannot therefore be expected to be predictive for risk estimation for such chemicals. The "radiation-equivalence" approach has been used to relate target dose to adverse biological effects produced by low levels of exposure to alkylating agents such as ethylene oxide. The general applicability of this approach has yet to be proven for other genotoxic chemicals.

Risk assessment by extrapolating between experimental species and man using mathematical models is speculative; most models do not take into consideration existing knowledge of mechanisms of cancer initiation and promotion. Notwithstanding the difficulties, the use of DNA adducts to indicate the target dose in such models provides a more rational basis for extrapolation between animals and man.

It is concluded that the techniques for the determination of DNA or haemoglobin adducts are very promising for measurement of low levels of exposure to genotoxic chemicals in human populations but they cannot be used confidently to predict health risk. The measurement of haemoglobin adducts seems preferable for determining exposure to alkylating agents, aromatic amines and chromates and

appears more accurate than the measurement of external dose or urinary metabolite levels.

The possible use of DNA and haemoglobin adducts in monitoring human exposure to substances with carcinogenic potential is discussed. Use of protein adduct measurement for monitoring exposure to non-genotoxic chemicals which bind to macromolecules is also discussed. The many ethical implications of such human studies need to be considered before applying the techniques.

It is recognised that further information will be required before full use can be made of adduct levels in exposure monitoring and risk assessment. The areas of research which may provide such information are described.

1. INTRODUCTION

The ability of chemicals to bind to macromolecules is well recognised. Many genotoxic chemicals can cause structural alterations in the DNA of cells by the formation of covalent adducts with DNA. Analysis of DNA and/or protein adducts can provide evidence that a chemical or its metabolites has bound to these macromolecules. This information can be useful in two contexts. Firstly, DNA binding studies may complement the biological assays commonly used in experimental toxicology to predict genotoxic potential (eg. bacterial mutation and cytogenetics). Such studies can be particularly useful in determining the efficiency of adduct formation in cells or organs from different species after the same applied (external) dose. Secondly the exposure to man of potentially genotoxic chemicals can be determined. Such monitoring may eventually be of value in obtaining improved information on the exposure of individuals and in helping to assess the risk to populations from exposure to genotoxic chemicals.

During the last 10 years analytical methods have become available which enable the measurement of the amount of adduct formed between a chemical and DNA and/or haemoglobin. The use of these methods has been the subject of several scientific conferences (Berlin et al, 1984, Bartsch et al, 1988, Aitio et al, 1988). The increasing interest and experience in measuring adducts was considered to be of importance to ECETOC which set up a Task Force with the following terms of reference:

1. to review critically the present status of the use of DNA and protein adducts as an indicator for measuring chemical exposure and its relevance for human health risk assessment.

2. to review the significance of the tissue dose concept for carcinogens/mutagens in relation to the mathematical models currently used for extrapolation to low doses.

3. to advise on expected developments and on the need for further research in this area.

2. PRINCIPLES AND FUNDAMENTAL ASSUMPTIONS

A primary objective in toxicological and occupational health investigations is the study of dose-response relationships. Establishment of an appropriate estimate of dose is recognised as an important requirement if a biological event seen in one species is to be extrapolated to another.

2.1. External Dose

All living systems exist in an external environment; the measurement of a chemical in the external environment, ie. in the atmosphere, soil and water, allows estimation of the concentration of chemical to which an organism may be exposed (ie. the external dose).

2.2. Internal Dose

The physico-chemical properties of the chemical and the permeability of the organism's external membranes determine how much, if any, of the external dose actually enters the organism, ie. the received or absorbed dose. The amount of a chemical which is absorbed into an organism or which is already stored in the organism is the internal dose (Lauwerys, 1983; Neumann, 1987). The internal dose is modulated by the processes of biotransformation and elimination. The exposure of individual tissues and cells to the chemical or its metabolites is additionally modified by factors such as tissue distribution, vascular perfusion, diffusion within the different tissues and differential partitioning into hydrophilic and hydrophobic sites. The internal dose depends upon the balance of the rate of absorption, biotransformation, distribution and excretion of a chemical.

The determination of exposure by estimating the amount of chemical and/or its metabolites in biological materials such as urine and plasma is referred to as biological monitoring.

Correlations between external dose and biological response have often been employed in models used for estimating risk although species differences

in the kinetics of absorption, biotransformation, distribution or excretion show that linear pharmacokinetics may apply only within a particular range of concentrations (ECETOC, 1982). The use of correlations between internal dose and biological response compensates for such species-dependent factors and improves the estimation of risk based on correlation of human exposure with dose-response studies in experimental animals.

2.3. Target Dose

The concept of target or molecular dose is central to the application of DNA binding to human monitoring (cf. 3.3 and 7.3). A target can be defined as either a target molecule, eg. DNA or a target tissue. Target tissue is defined as "that in which DNA binding may lead to pathological abnormalities".

The target dose of a genotoxic chemical has been defined as "the dose, expressed as time integral concentration, of the ultimate genotoxic agents (which may be systemically generated), which evades metabolic detoxification and penetrates to the biologically significant site in DNA" (Ehrenberg et al, 1974; Farmer et al, 1987). Its importance lies in the fact that mutagenicity or carcinogenicity have been postulated to be initiated by chemical modification of the DNA by a covalent interaction between DNA and chemical or metabolite (Lutz, 1979).

The interaction of reactive chemicals with a non-target molecule eg. haemoglobin, an easily accessible protein available in relatively large amounts in the body has been suggested as a means of estimating indirectly the dose on DNA (Osterman-Golkar et al, 1976). It would be unwise to assume that there is a single ratio between DNA binding and haemoglobin binding which applies for all chemicals. For example the amount of haemoglobin adduct formed with 4-aminobiphenyl, benzidine and aniline were the inverse of the amounts of DNA adduct (Ashby, 1988).

Organisms have mechanisms for repairing macromolecular adducts. Consequently the level of any macromolecular adduct is determined by the

balance between its rates of formation and repair. It is most probable that there is inter- and intra-species variation in these modulating factors. In addition the determination of macromolecular adducts is complicated by background levels. The lack of specificity of the adduct used to determine the dose is a confounding factor in these cases.

Estimation of dose at a relevant target site takes a better account of the variation of pharmacokinetic factors between and within species which affect the doses of chemicals which react at the cellular and molecular target sites (Wright, 1981). Determination of the target dose provides a better measure of exposure of the target tissue to a chemical.

Currently several levels of monitoring chemical exposure can be considered, they are summarised in Figure 1.

2.4. Adduct Formation as an Indicator of Risk

It has been argued that evidence of macromolecular binding is, of itself, an indication of carcinogenic or mutagenic risk to an individual exposed to a genotoxic chemical. The underlying assumption in this argument is that the formation of a macromolecular adduct is the rate limiting step in the carcinogenic process. Adduct formation is, however, only one component of this process and subsequent events, eg. DNA repair, promotion and progression may modify the risk of developing disease. Evidence of DNA binding may allow the conclusion to be reached that the risk is increased but the magnitude of this will not be known.

2.5. Use, Validity and Ethical Considerations

Detection of macromolecular adducts provides a method of determining the dose of a reactive molecule delivered to a tissue. If the techniques are to be applied to man the source of the biological material used for analysis has to be readily available. Realistically only blood or urine would be available. Analysis of haemoglobin-bound adducts can readily be made from red blood cells (cf. 6.2.2) and analysis of DNA-bound adducts from the nucleated (white) cell fraction (cf. 6.2.1). It is doubtful

whether cells from other tissues or organs, eg. skin or liver obtained by biopsy, would be available. It must be questioned whether studies of adducts in cells remote from the known (or possible) site of cancer induction are relevant to that cancer eg. whether adducts in white blood cells caused by the bladder carcinogen 2-naphthylamine are relevant to bladder cancer. To be so it would have to be confirmed by experiment that there is a constant relation between adducts in the DNA of cells from different organs from several individuals.

Adducts that have been liberated from DNA as the result of DNA repair can be detected in urine (cf. 6.3.1). This approach to the detection of adducts has the advantage of using a non-invasive technique but the derived data provide no information about the level or types of adducts remaining in the DNA of target or other tissues.

The study of adducts produced by genotoxic agents is a relatively young and developing science and the biological significance of the majority of adducts is not understood. Much consideration has to be given to how the techniques are to be used. They have already been used in studies of cohorts of workers occupationally exposed to genotoxic chemicals (cf. 6.2) and in patients treated with genotoxic anti-neoplastic agents (cf. 6.4).

If a population has been exposed to a defined external dose of a chemical, the techniques can indicate whether all individuals have received the same target dose. Nevertheless variations in the pharmacokinetic parameters may influence the degree of macromolecular binding observed in any individual. For example the rate of uptake of chemical into the body (which in turn may be influenced by different work practices), tissue or organ distribution, the rate of metabolism leading to generation of reactive derivatives and the half lives of the target molecules may influence the degree of adduct formation. In addition DNA adducts may be chemically labile and subject to DNA repair. Thus the level of DNA adducts detected will also depend on the period of time between exposure and sample collection. In contrast haemoglobin adducts, being more stable, can give an indication of the integrated dose.

There is some evidence that the level of DNA adducts in white blood cell increases with the age of an individual (Phillips et al, 1986). Personal habits, eg. tobacco smoking, also lead to increased levels of DNA and haemoglobin adducts. These observations raise the questions of what quantities and types of DNA adduct are acceptable as safe and at what point should the level of adduct become a cause for concern. In order to address these questions the determination of adducts from man should be carried out to assess levels of exposure to genotoxic chemicals; individuals need to be informed about the correlation of adducts measured and atmospheric concentrations of the chemical. At this stage no forecast can be given about the potential health status of an individual demonstrating adduct formation. Clearly the ethical implications of studies on individuals need to be considered before embarking on such studies.

3. MECHANISMS

3.1. Introduction

Biotransformation of chemicals by oxidation, reduction, hydrolysis followed by conjugation is especially active in the liver, although many other organs have this capability. In the majority of cases the conjugated compounds are less reactive, less toxic and more water-soluble than the parent compounds. Metabolic conjugation and oxidation can also lead to more reactive forms.

Reactions producing metabolites of greater reactivity are in general oxidation reactions such as N-hydroxylation, epoxidation or reduction reactions eg. nitro reduction to N-hydroxy compounds. Such reactive metabolites may bind covalently to various endogenous molecules including proteins and DNA. Free radicals may also be formed during metabolism and may cause oxidative damage to cellular components (Wogan et al, 1979; Beland and Kadlubar, 1985; Fowler, 1987).

Conjugation eg. glutathione conjugation, glucuronidation or acetylation, normally serve to detoxify substances or their active metabolites. Such conjugates may be cleaved in other tissues and release toxic agents as in the case of carcinogenic aromatic amines in the human bladder (Jakoby, 1980; Jakoby et al, 1982; Beland and Kadlubar, 1985). These processes vary between individuals because of genetic differences (Jakoby, 1980; Boobis et al, 1985; Rüdiger and Lehnert, 1988).

3.2. Principle of Adduct Formation

Genotoxic chemicals cause structural alterations in the DNA of target and non-target cells and in protein. Although some groups of chemicals, such as metals and intercalating agents, form non-covalent bonds, most genotoxic compounds form covalent adducts with DNA and proteins because they are electrophilic per se or are converted metabolically to ultimate electrophilic reactants (Miller and Miller, 1977). These electrophiles react with various nucleophilic centres in DNA or protein to form the

chemical bond. Examples of directly acting or metabolically activated xenobiotics are given in Figure 2.

3.2.1. Adduct Formation with Electrophiles: When an electrophile (R^+X) reacts with a nucleophilic centre (Y^-), the reaction follows the general scheme

$$R^+X^- + Y^- \longrightarrow RY + X^-$$

Electrophilic chemicals generate a variety of DNA, RNA and protein adducts. The nucleophilic centres in biological macromolecules are thiol and thioether sulphurs, nitrogen atoms in amino groups and heterocylic rings and oxygen atoms within carboxylate ions and hydroxyl groups. Nucleophilic reaction sites in haemoglobin include the N- and N- atoms of histidine, S in cysteine and the free amino group of N-terminal valine; in nucleic acids reaction sites include the N^2, O^6, N7 and C8 atoms of guanine, N1 and N3 of adenine and the oxygen atom of the phosphate diesters.

Studies with alkylating agents (C_1-C_4 chain length) have demonstrated that reaction occurs on more than a dozen different nucleophilic centres in the DNA (Hemminki et al, 1986, 1987). In addition, some xenobiotics are metabolised into more than one electrophilic reactant (Anders, 1985) or may form cyclic adducts resulting in an even more complex pattern of adducts (Singer and Bartsch, 1986).

The amounts of specific DNA or protein adduct formed depend on factors such as the concentration of the electrophilic agent, steric availability and the reactivity (nucleophilic strength) of the nucleophilic atoms. The relative reactivity of nucleophilic groups towards electrophilic agents is mainly determined by the polarisibility of the atoms and this relative reactivity decreases in the order S>N>O (Ross, 1962). The nucleophilic strength (n) has been established on an arbitrary scale: n = 0 for H_2O (low nucleophilicity), n = 0 - 4 for oxygen atoms, 3.5 - 5 for nitrogen atoms and 5 - 7 for sulphur atoms (Swain and Scott, 1953). Studies with simple monofunctional alkylating

agents, eg. methyl methanesulphonate, have shown that most adduct is formed by reaction with the most reactive nucleophilic centre in DNA, the N7 atom of guanine. A minor amount of adduct is formed by reaction with the less reactive O^6 atom of guanine. The degree of reaction with O^6-guanine is relatively higher with compounds of lower electrophilicity. Thus the rate of reaction with specific nucleophilic centres in macromolecules depends on the nature of the electrophilic chemical.

3.2.2. Adduct Formation by Other Factors: DNA and protein adducts may also arise from free radicals and metals. Free radicals can add to double bonds, remove a hydrogen atom or react with oxygen to form reactive oxygen species (Anders, 1985). The targets for such reactive species include histidine, methionine, tryptophan and cysteine in proteins, although in the case of cysteine these effects are normally readily reversible (Sies, 1985).

Oxidation of DNA bases by free radicals seems to occur randomly; in contrast, alkylation reactions by electrophiles occur predominantly at guanine in DNA (Guengerich and Liebler, 1985). To a marked extent free radical reactions can be the cause of depurination and depyrimidation. An end product is thymine glycol which is excreted in urine and might be an index for DNA damage by reactive oxygen in man (Cathcart et al, 1984; Bridges, 1987). Ionising radiation also produces DNA damage probably by generation of reactive oxygen species (hydroxyl radicals).

Metals and inorganic compounds may also be metabolised to reactive species which can form complexes with DNA and proteins. In plasma and erythrocytes chromium is reduced from the hexavalent to the trivalent state which then forms stable complexes with proteins (Aaseth et al, 1982; Wiegand et al, 1984a; Lewalter et al, 1985). Metals such as chromium, nickel, cadmium, beryllium etc. diminished the fidelity of DNA replication in vitro (Sirover and Loeb, 1976). Only cytotoxic amounts of chromates induced DNA-protein crosslinks and unscheduled DNA

synthesis in cultured cells (Cupo and Wetterhahn, 1985; Fornace et al, 1981; Whithing et al, 1979).

3.3. Reaction of Chemicals with DNA and Protein

Protein adducts may provide an index of exposure which takes individual variations into account but their occurrence should not necessarily be taken as evidence of a reaction with DNA, eg 2,5 hexanedione, acrylamide (Hashimoto and Aldridge, 1970; Aldridge, 1986). Nevertheless all agents so far studied in animals and cell systems which react with DNA also react with haemoglobin (Farmer et al, 1987). Binding to DNA and haemoglobin in rats has been studied on a number of chemicals. A covalent haemoglobin-binding index (HBI) which is analogous to the covalent DNA-binding index (CBI) (cf. 5) has been calculated for such chemicals. The relationship between the CBI and the HBI is different for different chemicals and in contrast to the CBI, the HBI does not reflect the genotoxic potency of the compound (Lutz, 1986; Farmer et al, 1987; Neumann, 1988). The extent of DNA binding in tissues is related to the extent of haemoglobin binding and to the administered dose if first order kinetics apply (Ehrenberg and Osterman-Golkar, 1980).

All the above factors form the basis for approaches to the development of molecular dosimetry, eg. the calculation of the dose of the reactive agents in the surroundings of the tissue cell DNA from DNA or Hb binding.

Serum albumin adduct levels may show a similar dose dependency to that of haemoglobin and consequently may be used for exposure monitoring. Because of the close contact of albumin with liver cells, this protein may trap active electrophilic metabolites produced in the liver more effectively than haemoglobin. Haemoglobin adducts are believed to be stable over the life of the erythrocyte and therefore accumulate with multiple exposure (Segerbäck et al, 1978; Wogan et al, 1979; Beland and Kadlubar, 1985; Fowler, 1987). As human albumin has a much shorter half-life (about 20 days) than haemoglobin the steady state adduct level is expected to be

lower than with haemoglobin. Some DNA adducts are chemically more labile than protein adducts and, in addition subject to DNA repair.

DNA and Hb adducts have been studied for over 30 compounds in animals. Measurement of adducts of several of these chemicals can be used for the monitoring of human exposure to them.

3.3.1. Alkylating Agents: These are classified according to the alkyl group, (normally methyl or ethyl), which reacts with nucleophilic centres in proteins and DNA. Examples include diazoalkanes, alkyl methane-sulphonates, dialkylsulphates, alkyliodides, trialkylphosphates, certain ring-strained lactones and nitroso derivatives, as well as more complex molecules such as *bis*-(chloroethyl)-nitrosourea (CNU) (Farmer et al, 1973). Bifunctional alkylating agents may produce inter- and intrastrand crosslinks which enhance the DNA damaging effects, for instance some cytostatic compounds (Tong et al, 1982).

3.3.2. Aldehydes: Inhalation of simple aldehydes such as formaldehyde has been shown to cause nasal cancer in animals at high doses. Besides the formation of Schiff bases by the reaction with amino groups in proteins and nucleic acids, stable DNA-protein cross links may be generated (Heck and Casanova, 1987). The contribution of these compounds to tumour initiation remains to be established.

3.3.3. Vinyl chloride: This compound is metabolised to the highly electrophilic chloroethylene oxide, which is responsible for the alkylation of proteins and DNA. The major adduct in haemoglobin is 2-oxoethyl-histidine (Scherer et al, 1981; Osterman-Golkar et al, 1977) and in DNA is N-7-(2-oxoethyl)guanine (Laib et al, 1981). Chloroethylene oxide is a potent mutagenic agent and is possibly responsible for the carcinogenic activity of vinyl chloride (ECETOC, 1988).

Part of the generated chloroethylene oxide undergoes rearrangement to chloroacetaldehyde. The reaction of this with adenosine and cytosine bases has been demonstrated in vitro (Barrio et al, 1972) and hydroxy-

etheno derivatives have been found in DNA (Laib et al, 1981; Kusmierek and Singer, 1982). The low activity of chloroacetaldehyde in mutation assays (500 to 1000 times lower than for chloroethylene oxide) leads to the conclusion that chloroacetaldehyde does not contribute to the genotoxic activity of vinyl chloride (ECETOC, 1988).

3.3.4. Epoxides: The simplest epoxide is ethylene oxide (EO) which is a direct alkylating agent. The reaction of EO with proteins and DNA has been studied in the rat (Osterman-Golkar et al, 1983). The major products were S-(2-hydroxyethyl)cysteine, N^{τ} and N^{π}-(2-hydroxy-ethyl)histidine and N-terminal 2-hydroxyethylvaline in haemoglobin (Osterman-Golkar, 1983) and N-7-(2-hydroxyethyl)guanine in DNA (Bolt et al, 1988). Other epoxides which have been found to react with haemoglobin and DNA include butadiene diepoxide, 2,3-epoxybutane, styrene oxide (Hemminki and Hesso, 1984) and trichloropropylene oxide. The last was found to inhibit epoxide hydrolase resulting in decreased hydrolysis and increased reactivity with DNA and haemoglobin (Hemminki et al, 1980).

3.3.5. Hydrazines: Hydrazine can react with endogenous methyl groups, derived from the 1-carbon pool eg. endogenous formaldehyde, to yield formaldehyde hydrazone. (Alternatively hydrazine can be methylated by the methyl group of s-adenosylmethionine). Analogy to the alkyl hydrazines suggests that this intermediate can be activated enzymatically to the methyldiazonium ion, which may methylate DNA bases (Lambert and Shank, 1988). The specific base adducts, N-7- and O^6-methylguanine (7-medGua and O^6-medGua) (Roggers and Pegg, 1977; Becker et al, 1981) and O^6-methylthymidine (Swenberg et al, 1985) have been isolated. Studies with haemoglobin and other proteins have demonstrated that proteins can also be alkylated during hydrazine metabolism. The nature of these adducts is not known (Prough and Moloney, 1985).

3.3.6. Arylating Agents: The principal reactive intermediates formed by metabolism of benzene and substituted benzenes are thought to be arene oxides (Jung et al, 1981). Benzene oxides are very labile and are not

always the ultimate electrophiles. In a microsomal system benzene oxide did not bind to macromolecules to the same extent as benzene derived phenols. This implies that a metabolite of phenol is the ultimate reactive agent (Tunek et al, 1978). Subsequent studies suggest that semiquinone and benzoquinone are important DNA damaging metabolites of benzene (Pallak-Walker et al, 1985, Snyder et al, 1987).

Halogenated benzenes such as PCB's bind covalently to proteins of liver but not of kidney or lung microsomal preparations (Shimada and Sato, 1978). Bioactivated 4-chlorobiphenyl becomes covalently bound to protein, DNA and RNA; the specific binding to DNA is 3.5 times greater than that to protein (Wong et al, 1979).

The amount of protein binding of the halogenated chemicals o-bromobenzonitrile, bromobenzene, o-bromotoluene and o-bromobenzotrifluoride did not correlate with their hepatotoxicity. The most extensive protein binding was found with o-bromotoluene which showed a relatively weak hepatotoxicity (Wiley et al, 1979).

3.3.7. Polycyclic Aromatic Hydrocarbons: Oxidative metabolism of these compounds leads to formation of polar metabolites which are eliminated by conjugation or further activated to diol epoxides (Miller, 1961; Alexandrov et al, 1982). The same oxidative enzymes also produce "Bay region" diol epoxides in more than a dozen PAH's such as benzo(a)pyrene (B(a)P), benz(a)anthracene and chrysene.

Sims et al (1974) demonstrated that B(a)P is converted into the ultimate carcinogen 7,8-diol-9,10-epoxide (BPDE) by a sequence of metabolic reactions. Further studies showed that BPDE exists in 4 stereoisomeric forms. All isomers react with DNA to form covalent adducts, predominantly the guanine-N^2 derivative (Kapitulnik et al, 1978; Hogan et al, 1981; Alexandrov et al, 1982). Further alkylation occurs at the O^6 and the N-7 position of guanine, the exocyclic amino group of adenine and cytosine (Straub et al, 1977; Osborne et al, 1978) and with phosphate diesters (Gamper et al, 1978). Carcinogenicity studies on the four diol epoxides in the mouse demonstrated that the

carcinogenicity of B(a)P is associated with the (+) anti BPDE isomer. This is probably the result of specific steric alignments within the double helix structure of the DNA which favours DNA reaction (Kapitulnik et al, 1978, Brookes et al, 1982; Stowers and Marshall, 1985).

3.3.8. Aromatic amines and nitroaromatics: In the 1960s Miller and Miller pioneered the concept of metabolic activation by studying the metabolism of 2-acetylaminofluorene (2-AAF) (Miller and Miller, 1974). They demonstrated that aromatic amines are activated in two steps: N-oxidation and conjugation of the N-oxidation product to yield a reactive metabolite. Aromatic amines are acetylated in vivo and excreted in the urine. The greater the degree of acetylation the greater is the amount of acetylated product (acetamide) in the urine. Acetamides can also be deacetylated and usually an equilibrium exists between the aromatic amine and its acetylated metabolite (Figure 3). The amount of acetylation is strongly influenced by the acetyl-transferase activity. In man a genetic polymorphism for N-acetyl-transferase has been established (Glowinski et al, 1978; Weber and Hein, 1985). In slow acetylators more of the aromatic amine is available for N-oxidation and this may influence to some degree the initiation of tumours in certain tissues following exposure to carcinogenic aromatic amines. (Poupko et al, 1983; Lower et al, 1979; Mommsen et al, 1985).

It has been speculated that after exposure to carcinogenic aromatic amines fast acetylators might be at increased risk of developing tumours in the liver, mammary gland and intestine (Lang et al, 1986; Ilett et al, 1987), while slow acetylators might be at increased risk of developing bladder tumours (Lower et al, 1979). N-oxidation yields an aromatic-N-hydroxylamine whereas N-oxidation of the equivalent acetamide yields an aromatic-N-hydroxyacetamide (hydroxamic acid) (Kiese, 1974; Kiese and Traeger, 1976; Eyer and Ascher, 1987). Both kinds of N-oxidation products are not reactive enough to bind with macromolecules but conjugation, for instance with glucuronides, provides a compound which spontaneously decomposes to yield the

electrophilic nitrenium ion (cf. Figure 3) (Poupko et al, 1979; Radomski, 1979; Neumann, 1986). Under acidic conditions in urine N-hydroxylamines may be protonated and then split off water to yield a nitrenium ion without further conjugation (Kadlubar et al, 1977, 1981).

The electrophilic nitrenium ions (mesomeric carbocations) react with various nucleophilic centres of DNA-bases, predominantly with C-8 in guanine (Kadlubar et al, 1981; Shut et al, 1984). N-acetylated C-8 guanine adduct of 2-aminofluorene imposes a greater conformational strain on DNA and therefore is more rapidly repaired than non-acetylated adduct. It could thus be of importance whether the ultimate electrophile is acetylated or not (Hanna et al, 1985).

The critical metabolic pathway of the human carcinogen benzidine involves acetylation of one of them and N-oxidation of the other. The resulting electrophile then reacts with C-8 of guanine (Martin et al, 1982, 1983; Kennelly et al, 1984; Jeffrey, 1985). Another human carcinogen, 2-naphthylamine, forms adducts mainly at N-6 of adenine and to a lesser extent at C-8 and N-2 of guanine in dogs (Kadlubar et al, 1981). Initially the pattern of adducts is similar in the liver (non-target tissue) and the urothelium (target tissue) but the level of adducts decreases more rapidly in liver due to the greater repair capacity of this organ (Kadlubar et al, 1980, 1981). The probable role of N-oxidation is underlined by the fact that 1-naphthylamine, which is not readily N-oxidised, is not carcinogenic (Purchase et al, 1981; Dooley et al, 1984).

Aromatic-N-hydroxylamines also give rise to a major haemoglobin binding metabolite (Bryant et al, 1987; Neumann, 1988) and this represents the key link between the DNA-damaging and haemoglobin-binding pathway, the latter for use as a dose monitor (cf. Figure 3).

Aromatic-N-hydroxylamines are formed by oxidation of aromatic amines (predominantly in the liver) or by reduction of nitroaromatics (Howard et al, 1983). They can be further oxidised to nitroso aromatics in erythrocytes in a co-oxidation reaction with oxyhaemoglobin. This

yields methaemoglobin (Kiese, 1974). Nitrosoaromatics are either converted back enzymatically to the N-hydroxylamine, which may initiate another round of methaemoglobin formation, or react with a nucleophile such as cysteine in glutathione or proteins (Neumann, 1984b; Bryant et al, 1987). Reaction of the SH-group of cysteine with the nitroso-group yields an intermediate which rearranges to give stable sulphinic acid amide (Doelle et al, 1980; Eyer and Lierheimer, 1980). The sulphinic acid amide readily hydrolyses under alkaline or acidic conditions and releases the parent aromatic amine (Albrecht and Neumann, 1985; Lewalter and Korallus, 1985; Bryant et al, 1987). This is the basis for biomonitoring of exposure to aromatic amines and nitroaromatics; the haemoglobin adduct in blood samples can be hydrolysed and the amine extracted and quantified (cf. 4.2.2).

One study has shown an excess of slow acetylators among persons with high levels of Hb adducts following exposure to non-carcinogenic aromatic amines, aniline and nitrobenzene (Lewalter and Korallus, 1985; Farmer et al, 1987).

3.3.9. Metals: Chromium and nickel are taken as examples of adduct formation with metals. Cr(VI) ion penetrates the erythrocyte membrane followed by intracellular reduction to Cr(III) by GSH and ascorbic acid and the haemoglobin binding (Figure 4). The amount of erythrocyte binding can be used for a quantitative measure of occupational exposure to Cr(VI) compounds (Aaseth et al, 1982; Wiegand et al, 1984b; Lewalter et al, 1985). These concepts have been accepted in the Federal Republic of Germany for the establishment of a Biologische Arbeitsstoff Toleranzwerte (BAT) for occupational exposure to Cr(VI).

If nickel compounds enter the target cells DNA-protein cross-links are produced in the cell nucleus. Nickel compounds could cause, either directly or indirectly, free radical reactions in target tissues, producing lipid peroxidation products that can interact with DNA. In addition, the possibility exists that Ni^{++} substitutes for Zn^{++} in finger-loop domains of growth regulating proteins resulting in alteration of gene regulation by oncogene proteins (Sunderman, 1988).

3.4 DNA Adducts and Biological Effects

A great step forward in understanding chemical carcinogenesiss was the finding that many potent carcinogens are mutagenic by virtue of their inherent or metabolically acquired reactivity towards DNA. Test systems were developed which demonstrated this interaction using different endpoints for mutagenicity. This genotoxic activity could be related in many instances to the formation of DNA base adducts. There is good evidence that many of the adducts represent promutagenic lesions in microbial systems. In case of base substitution mutations the same base transitions have been found in microorganisms, rodents and man.

It is essential to distinguish between total DNA binding and the formation of critical adducts at particular sites on DNA which may be related to cell survival and normal replication (Bichara and Fuchs, 1985) and may lead to a mutation which is widely accepted as the crucial step in the initiation of carcinogenicity (Miller and Miller, 1977).

Formation of adducts resulting from methylation and ethylation of ring nitrogen (ie. N-7-deoxyguanosine, N-3-deoxyadenosine) shows little correlation with tumour induction and are not considered as miscoding lesions (Singer and Gruneberger, 1983). Less information is available on the biological outcome of O^2-deoxycytosine and DNA alkylphosphodiesters adducts but these also do not appear to contribute to mutagenicity and carcinogenicity (Beranek et al, 1980; Brennard et al, 1982). In contrast, alkylation of the exocyclic oxygens especially O^6-deoxyguanosine (Singer and Gruneberger, 1983) and O^4-deoxythymidine (Swenberg et al, 1985; Dyroff et al, 1986) has received most attention recently because of their obvious promutagenic nature (Saffhill et al, 1985).

The biological consequences of adducts of DNA with large (aromatic) carcinogens may be due distortion of the helical structure as a consequence of intercalation, followed by mispairing in the distorted area to stabilise the DNA helix (Hogan et al, 1981).

Adduct formation is not a biological but a biochemical lesion which needs to be "fixed". After two or more cell replications a stable mutation may be generated if the promutagenic DNA lesion is unrepaired or misrepaired. Stable alterations in DNA may remain silent and without detrimental consequences for a somatic cell. Nevertheless some of them may lead to the formation of a mutated gene product with new properties or to altered expression of genetic information if regulatory sequences are involved, events which are regarded to be associated with tumour formation (Stowers et al, 1987).

Results of studies on cells in culture and in experimental animals suggest that there is a good correlation between levels of the critical DNA adduct and the frequency of induced mutations where that DNA adduct has been determined and measured (Lawley, 1984; Heflich et al, 1986; Vogel et al, 1986). Limited data on alkylating agents, benzo(a)pyrene and the N-oxidised aromatic amines suggests that the ratio of adducts to mutation is similar in several test systems for the same chemical or class of chemicals. With monofunctional alkylating agents the mutagenic activity in plants and bacteria is proportional to the degree of reaction with nucleophilic centres which have a nucleophilic strength of about n = 2, eg. O^6-guanine (Osterman-Golkar et al, 1970). However, the ratio of adduct to mutations varies significantly between chemical classes or agents.

Adduct formation and concurrent effects on different genetic endpoints like point mutations, chromosome aberrations, dominant or sex-linked recessive mutations and sister chromatid exchanges have been investigated. The induction frequency of point mutations induced by ethyl nitrosourea (ENU) and EMS in Drosophila correlated well with the degree of O^6-alkylation of guanine while the frequencies of chromosome aberrations and sister chromatid exchanges did not, indicating that different lesions must be responsible for these effects (Vogel et al, 1982). Van Zeeland et al (1985) have also used a series of ethylating agents to study the consequences of DNA adduct formation. In several mutagenicity test systems (E. coli, Drosophila, cultured mammalian cells) it was shown that the effectiveness of ethylating agents varied considerably but that

mutation induction for these compounds was similar at equal levels of O^6-ethylguanine in DNA.

The relationship between DNA damage and mutation frequency was shown with EMS and ethylene dibromide; the degree of alkylation they produced in germ cells of Drosophila melanogaster could be correlated with the frequency of sex-linked recessive lethals (Lee, 1983).

Willems et al (1987) found that levels of 2-AAF which induced DNA adducts in the liver of rats (target organ) resulted in excretion of measurable amounts of mutagenic activity in urine and faeces. However, DNA adducts were not detected in the spleen and peripheral lymphocytes and no increased incidence of sister chromatid exchanges (SCE) was observed in lymphocytes.

The relationship between DNA adducts and carcinogenicity has also been investigated but this situation is more complicated. It may be because carcinogenesis is multistage process in which both the initiation and a promotion action may be necessary for tumour formation. DNA damage as measured by adduct formation does not appear to correlate well with the tumour susceptibility of different tissues or species, eg. a comparable amount of DNA adduct is formed in rat liver after treatment with 2-AAF, 4-acetylaminophenanthrene or *trans*-4-acetylaminostilbene, but only the first of these produces tumours in this organ (Neumann, 1987),(cf. 5.1.5). The role of post-initiation promotional events has been emphasised by Hilpert et al (1983).

On the other hand a good correlation exists between the ability of some polycyclic aromatic hydrocarbons, N-nitrosamines, mycotoxins, and hydrazines to form covalent DNA adducts and their carcinogenic potency (Lutz, 1979; 1986; Pelkonen et al, 1980). For example, the amount of total and specific DNA adduct and mouse skin tumour incidence associated with benzo(a)pyrene giving an approximate parallel dose-response curve, suggesting a possible causal relationship (Ashurst et al, 1983).

It should be noted that there is no evidence of DNA binding with a number of animal carcinogens (Hemminki et al, 1986; Sterzel et al, 1985), (cf. 5.1.8). In some cases this may be attributable to limitations in the studies but in others the carcinogenicity apparently does not involve formation of DNA adducts.

DNA adducts have been implicated in activation of oncogens. Examples are mutations of the h-ras proto-oncogene in the 12th codon (associated with the promutagenic O^6-guanine adduct), or in the 61st codon (associated with binding of dimethylbenzanthracene (DMBA) with adenine) leading to the synthesis of altered proteins which make cells more susceptible to growth stimuli (Stowers et al, 1987). Alternatively increased expression of the c-myc proto-oncogene can be the result of translocation from the human chromosome 8 to chromosome 14 which brings the gene into an actively transcribed region (Croce et al, 1984). Activation of oncogenes may also occur by the loss of tumour suppressor genes (Varmus, 1984).

The data on the biological relevance of specific DNA adducts are derived from animal models and in vitro investigations and evaluation of their significance to man still requires more detailed investigation.

4. METHODS FOR DETECTING ADDUCTS

Exposure to genotoxic electrophilic chemicals may be monitored by measuring haemoglobin or DNA adducts isolated from peripheral blood cells. DNA adducts have also been analysed in urine, placental and mucosal cells but blood is best used for routine biological monitoring. DNA isolation from white blood cells from 1 ml blood results in a yield of 1-4 µg DNA whereas it yields about 100 mg haemoglobin. Since relatively larger amounts of protein are usually available a lower sensitivity in the analytical methods for adduct determination can be obtained with protein than with DNA. Currently available methods for the detection and quantification of DNA and haemoglobin adducts are reviewed in this section.

4.1. Detection of DNA Adducts

4.1.1. [^{32}P]Postlabelling: DNA (up to 25 µg) is digested enzymatically to the deoxynucleotide-3′-monophosphates. The nucleotides then are [^{32}P]phosphorylated with carrier free [gamma-^{32}P]ATP catalysed by T4 polynucleotide kinase to yield the [5′-^{32}P]deoxynucleotide-3′,5′-bisphosphates. The nucleotide adducts are purified by reverse phase TLC followed by contact transfer on polyethylenimine coated cellulose sheets and two dimensional chromatography. The adducts are localised by autoradiography and quantified by Cherenkov counting (Randerath et al, 1981; Gupta et al, 1982; Reddy et al, 1984; Randerath et al, 1985). This method is sufficiently sensitive to detect about 10 fmol adduct/mg DNA and requires only 20 µg DNA. The sensitivity can be enhanced to a limit of detection of about 1 fmol/mg DNA by incubation of the DNA digest with nuclease P1 before [^{32}P] labelling. Nuclease P1 cleaves the phosphate from normal nucleotides to yield nucleosides which do not serve as substrates for the polynucleotide kinase. Modified nucleotides containing bulky or aromatic adducts are resistant to the dephosphorylating activity of nuclease P1 (Reddy and Randerath, 1986).

The application of the postlabelling method is limited to the detection of adducts from aromatic or bulky carcinogens because nucleotides bearing smaller adducts cannot be readily separated from the natural

nucleotides and they are sensitive to the nuclease P1 action. In addition it must be shown for each DNA adduct that the phosphorylation by the polynucleotide kinase takes place with reasonable efficiency and the adduct is resistant to nuclease P1 degradation (Gupta and Early, 1988). Nevertheless, this method has been used to detect DNA adducts of unknown origin in man (Phillips et al, 1986; Everson et al, 1986; Watson, 1987), although techniques to determine the structure of the adducts are still lacking.

4.1.2. Fluorimetry: This method has only been applied for measurement of PAH adducts. PAH modified DNA is hydrolysed with 0.1 N HCl to liberate the adducts in the form of the isomeric tetrols. These have fluorescence quantum yields in deoxygenated solutions which are similar to those of the parent PAH. DNA hydrolysates from up to 200 µg DNA are separated by reverse phase HPLC. The fluorescence of the eluant is continuously monitored at optimal excitation and emission wavelengths. By using a photon counter coupled to the fluorimeter a limit of detection of around 1000 fmol adduct/mg DNA could be achieved for various PAHs [Rahn et al, 1982; Shugart and Kao, 1985).

Conventional excitation and emission spectra are often complex with many peaks. By scanning excitation and emission wavelength using a fixed wavelength difference (synchronous fluorescence spectroscopy, SFS), often only one peak emerges. The DNA samples (up to 100 µg) have to be hydrolysed to eliminate quenching of the signal by the double helical structure of DNA. The hydrolysates can be analysed by SFS without further purification. Using this method 300 fmol adduct/mg DNA is detectable (Vahakangas et al, 1985; Harris et al, 1985). An interesting improvement of this method is the three dimensional scanning by which the best wavelength differences for scanning of new adducts might be found. A three dimensional contour map might even provide "fingerprints" for the detection of unknown adducts (Vahakangas et al, 1985b).

4.1.3. Gas Chromatography-Mass Spectrometry: During the past years GC-MS techniques have been developed which have the same sensitivity as the fluorimetric methods. The DNA has to be hydrolysed to liberate the base adducts which are analysed by GC-MS after solvent extraction (Bolt et al, 1988). A limit of detection for 7-medGua of 100,000 fmol/mg DNA can be achieved (Föst, 1987) but the sensitivity could be improved by the use of electrophore labelling (Mohamed et al, 1984; Adams et al, 1986; Minnetian et al, 1987). In the gas phase electrophore derivatives readily accept low energy electrons which are easily detectable by GC-MS using negative ion detection (NICI). With this method the limit of detection for O^4-etThy was 100 fmol/mg DNA. Selective enrichment of adducts could also enhance the sensitivity of the GC-MS methods, eg. N7-alkylated guanine is released by heating alkylated DNA in water (Watson et al, 1987).

4.1.4. Immunological methods: DNA adducts have been detected using polyclonal or monoclonal antibodies. The methods include competitive radio-immunoassays and solid phase assays, such as enzyme linked immuno-sorbent assay (ELISA) or ultrasensitive enzyme radioimmuno assay (USERIA) (Adamkiewicz et al, 1984). Antibodies can be isolated from animals immunised with the hapten-coupled DNA adduct either directly (polyclonal antibodies) or after fusion of spleen cells with a myeloma cell line (monoclonal antibodies). The modified DNA is bound to a solid surface, treated with the antibody, and a species-specific second antibody conjugated to an enzyme or a fluorescent dye added. Determination of the amount of bound fluorescence or enzyme activity is used to quantify the amount of the DNA adduct. About 30 fmol adduct/mg DNA may be detected using 20 µg DNA (Haugen et al, 1986; Baan et al, 1985b; Perera et al, 1986; Shamsuddin et al, 1985). A list of available antibodies against DNA adducts is presented in Table 1. Sensitivity of the assay can be enhanced by hydrolysis of DNA and separation of the adduct from the normal nucleotides. Despite the high sensitivity and specificity of this method it should not be overlooked that the production of an antibody with the required specificity and sensitivity is difficult and time-consuming. Thus the structure of the adduct must be determined, the corresponding hapten synthesised and

coupled to a carrier protein and the antibodies selected and characterised with respect to sensitivity and specificity. Analysis of unknown adducts is therefore not possible. In addition, the quantification of an immunoassay may depend on the adduct level of the DNA in that adducts are detected more efficiently at higher rather than lower levels (Santella et al, 1988).

4.2 Detection of Protein Adducts

4.2.1. Fluorimetry: This method can only be used for the measurement of PAH protein adducts or other adducts which exhibit strong fluorescence (Shugart, 1985). Haemoglobin adducts of PAH can be cleaved from cysteine by mild acid or alkaline hydrolysis to yield the free tetrols. After extraction these tetrols can be separated by HPLC and quantified by fluorescence spectroscopy (cf. 4.1.2). The sensitivity of detection is about 0.01 nmol adduct/g Hb using 5-10 ml blood.

4.2.2. Gas Chromatography: Aromatic amines react with cysteine of haemoglobin to form an adduct which may be hydrolysed to regenerate the original amine (cf. 3.3.8). After extraction the amine can be separated and analysed by GC using N-FID (Lewalter et al, 1985) or after derivatisation with perfluorinated acylating agents, using ECD (Green et al, 1984; Skipper et al, 1986). With N-FID 0.5 nmol adduct/g Hb can be detected whereas after derivatisation and ECD the limit of detection is about 0.01 nmol adduct/g Hb using 5-10 ml blood. The sensitivity of this method can be enhanced up to 0.001 nmol/g Hb using mass spectroscopy for the detection of aromatic amines (Stillwell et al, 1987). This relatively easy and fast technique is applicable only if the metabolites can be cleaved from amino acids.

Amino acid adducts which resist mild hydrolysis, such as those resulting from alkylation of histidine, N-terminal valine or cysteine, can be detected by GC-MS after protein hydrolysis and derivatisation. To determine the amount of alkylated amino acids of haemoglobin, the protein is hydrolysed with 6 N HCl. The hydrolysate is separated by

HPLC or ion exchange chromatography and the fractions containing the alkylated amino acids are derivatised with perfluoroacetylating agents. These derivatives can be detected by GC-MS with a sensitivity of 0.01 nmol adduct/g Hb using NICI detection and starting from 5-10 ml blood (van Sittert et al, 1985; Farmer et al, 1986; Bailey et al, 1987). This method is complex and time-consuming, Törnqvist et al (1986a) developed a modification in which alkylated N-terminal valine residues are cleaved by a modified Edman sequencing reagent, pentafluorophenyl isothiocyanate, in the neutral-alkaline coupling medium; non-alkylated N-terminal valine and the other amino acids are resistant to cleavage. The pentafluorophenylhydantoin derivative can be determined by GC-MS after extraction from the derivatisation mixture. A limit of detection of 0.01 nmol adduct/g Hb can be achieved.

These techniques also have the potential to detect haemoglobin adducts after exposure to unknown chemicals or to a mixture of different chemicals.

4.2.3. Immunological Methods: Monoclonal or polyclonal antibodies have been developed for the measurement of haemoglobin adducts (Santella, 1986; Wraith et al, 1988). As described earlier (cf. 4.1.4) antibodies obtained from immunised animals can be used in radioimmunoassay (RIA) or ELISA techniques. A possible problem for the isolation of antibodies against protein adducts is their lower accessibility for antibody binding if the adduct is hidden in hydrophobic regions (Santella, 1986). Hydrolysing the protein before reaction with antibodies may improve sensitivity. Recently, Wraith et al (1988) have developed an immunoassay against a heptapeptide containing N-terminal 2-(hydroxyethyl)valine obtained by trypsin degradation of haemoglobin. A limit of detection for the hydroxyethylated peptide of about 0.001 nmol/g Hb can be achieved. It should be kept in mind that the production of antibody with an appropriate specificity and sensitivity involves a number of difficult and time-consuming steps.

4.2.4. Atomic Absorption Spectroscopy (AAS): Biomonitoring for protein-bound metal ions, such as chromium for example, can be performed by AAS. Blood samples or isolated erythrocytes are solubilised with aqueous surfactants and applied directly to the graphite furnace. A three-step temperature program is normally used for the mineralisation of the sample. The chromium content is measured by atomic absorption at 357.9 nm. A limit of detection of 0.6 µg chromium/l blood, corresponding to about 0.1 nmol adduct/g Hb, has been achieved using 2.5 ml blood (Lewalter et al, 1985b; Angerer et al, 1987).

4.3. Comparisons of Sensitivity, Cost and Time

Methods used for monitoring DNA and haemoglobin adducts are summarised in Tables 2 and 3. Special attention is drawn to the time required for analysis and the cost of the equipment used.

5. DNA AND PROTEIN ADDUCTS IN ANIMALS

Experimental animal investigations have identified a large number of chemicals which react covalently with haemoglobin and DNA (Hemminki, 1983; Farmer et al, 1987).

5.1. DNA-adducts

Animal experiments have demonstrated adduct formation with DNA using a variety of covalently reacting compounds (approx. 100 chemicals). Those tested include simple alkylating agents [methyl methanesulphonate, 1,2-dimethylhydrazine, ethyl carbamate and various n-nitroso compounds], aromatic amines [N-hydroxy-4-(acetylamino)biphenyl, 2-AAF, N-hydroxy-4-acetylamino-4'-fluorobiphenyl, N-methyl-4-aminoazobenzene, 4-nitro-quinoline-1-oxide], epoxides and epoxide forming compounds [2,3-epoxy-butane, vinyl chloride] and miscellaneous compounds like aflatoxin B_1, 1-hydroxyestragole and 1-hydroxysafrole. The DNA adducts were determined predominantly in the liver of the exposed animals. Most of the earlier studies had difficulties with the quantification of DNA adducts and gave no information on the relative DNA binding potency of the investigated chemicals (Hemminki, 1983).

5.1.1. The covalent binding index (CBI): To compare the DNA binding capacity of compounds Lutz and Schlatter (1977), Lutz (1979, 1984, 1986) proposed use of a covalent binding index (CBI: defined as µmol chemical bound/mol DNA nucleotide/mmol chemical administered/kg body weight). CBI's have been calculated from animal experiments for approximately 100 chemicals (Lutz, 1979). Most data are available for liver DNA at the time of maximum DNA damage following single administration of the test material by various exposure routes to rats.

The authors claim that determination of liver DNA binding can be used as a semi-quantitative short-term assay to predict the carcinogenic potency of chemicals, although the liver was not necessarily the target organ for tumour formation. The authors claimed that strong carcinogens had a CBI above 1,000, moderate carcinogens of the order of

100 and weak carcinogens of less "than 10". Examples are aflatoxin B_1 (CBI > 10,000), dimethylnitrosamine (CBI > 5,000), vinyl chloride (CBI > 500), benzene (CBI = 1.7) and di-(2-ethylhexyl)-phthalate (CBI < 0.02) (Lutz, 1979; Däniken et al, 1984). The significance of low CBI values in relation to non-carcinogens was not resolved.

5.1.2. Experimental Findings on the Relationship between Dose and DNA Binding: Many DNA-binding chemicals require metabolic activation by enzymatic processes and diffusion processes as well as nucleophilic substitution reactions are involved in the formation of DNA adducts. The rate of all these steps is probably proportional to the concentration, even in the low dose range, thus a linear relationship between dose and DNA binding could be postulated.

This was verified in studies on a number of compounds including benzo(a)pyrene (Dunn, 1983), 2-AAF (Driver et al, 1987), dimethylnitrosamine, *trans*-4-dimethylaminostilbene (Neumann et al, 1978, 1980), EMS, aflatoxin B_1 (Lutz, 1987) benzidine (Talaska et al, 1987) and EO (Potter et al, unpublished). All studies demonstrated a linear relationship. The administered doses predominantly covered 4 orders of magnitude and the lowest dose used was 1 ng/kg for aflatoxin B_1 (Appleton et al, 1982; Lutz, 1986, 1987).

5.1.3. DNA binding after Repeated Dosage: Most studies have concentrated on the level of DNA binding after a single dose while it would be more appropriate to detect DNA adducts after repeated (chronic) exposure so that the competing processes of DNA binding and DNA repair, cell division or cell death could be taken into account.

Oral administration of aflatoxin B1 to rats for 10 consecutive days (Caviezel, 1984) produced a linear relationship between dose and DNA binding over 4 orders of magnitude. Recently Richardson and Swenberg (1987) found a linear relationship for diethylnitrosamine (DEN) given as 0.4 - 40 ppm solution. The level of DNA binding appeared to be no longer linear proportional to dose at the highest level following administration of *trans*-4-dimethylaminostilbene (Neumann, 1980),

aflatoxin B_1 (Caviezel, 1984), vinyl chloride (Watanabe et al, 1978) and DEN (Richardson and Swenberg, 1987), probably due to saturation phenomena.

5.1.4. Non-linear Relationships: Non-linear dose response relationships for DNA binding were shown after i.p. injection of dimethylnitrosamine in rats. Whereas the 7-medGua content in liver increased linearly with dose, O^6-medGua levels were lower than expected at low doses. This was attributed to more efficient repair in the low dose range (Pegg and Hui, 1978). Similar results were obtained in hamster liver (Stumpf et al, 1979). In a study with benzo(a)pyrene the amounts of DNA adducts increased more than proportional at high oral dose levels in mouse liver and lung (Adriaenssens et al, 1983).

5.1.5. Specificity of DNA binding (Organ Susceptibility): DNA binding can be detected at dose ranges which are orders of magnitude below those required to increase tumour incidence in long term bioassays.

Recently Swenberg et al (1985) compared DNA binding in target and non-target cell populations within rat liver. Chronic exposure to methylating hepatocarcinogens predominantly induce haemangiosarcomas, whereas ethylating agents cause hepatocellular carcinomas. This cell specificity correlated well with the presence of promutagenic DNA adducts. In the case of methylating agents (eg 1,2-dimethylhydrazine), the non-parenchymal cells accumulated O^6-medGua, whereas the hepatocytes did not. Exposure to ethylating agents (eg DEN) lead to accumulation of O^4-ethyldeoxythymidine, but not O^6-ethyldeoxyguanosine in rat hepatocytes. Thus, the different ability of the two cell populations to repair the promutagenic O^6-alkylguanine, is strongly associated with the carcinogenic response of target and non-target cells.

In contrast organotropic carcinogens that require metabolic activation, like 1,2-dimethylhydrazine give rise to 6 times less DNA-binding in the colon, the target organ, as compared to liver (Lutz, 1979).

DNA binding of carcinogenic aromatic amines has been measured in target and non-target tissues of rats. DNA adduct levels in liver did not correlate with their hepatocarcinogenic potential. With *trans*-4-aminostilbene derivatives, which almost exclusively produce ear duct tumours in rats, the highest levels of adducts were found in liver and kidney (Baur and Neumann, 1980). However, the pattern of adducts seems to be qualitatively similar in most tissues. The highest level of DNA binding (in liver) differed from the lowest level (in mammary gland) by a factor of 10, 3 days after a single dose [5 µmol/kg] or 3 days after the last of 12 doses (Neumann, 1984a). *Trans*-4-acetylaminostilbene and 2-acetylaminophenanthrene do not produce liver tumours but generate a level of DNA damage similar to that produced by as 2-AAF which is a complete carcinogen for this organ (Neumann, 1986).

The levels of DNA adducts derived from benzo(a)pyrene in different tissues of A/HeJ mice varied within one order of magnitude. The total binding and the level of specific adducts was highest in forestomach after oral administration (Stowers and Anderson, 1984).

These data were corroborated by Gupta and Dighe (1984) using rats administered (i.p.) N-hydroxy-2-acetylaminobiphenyl, N-hydroxy-2-acetyl aminophenanthrene (carcinogenic aromatic amines which do not produce liver tumours in rats), and N-hydroxy-2-acetyl-aminofluorene (a liver carcinogen). The persistence of DNA adducts was measured in the liver by the ^{32}P-postlabelling method. The persistence decreased in the order listed, the hepatocarcinogen being least persistent. The authors considered that persistence of DNA adducts, when measured as an average of the events over the entire genome, does not in itself explain organ susceptibility to tumour induction.

In contrast, in liver DNA of animals treated with 250 mg/kg AAF a level of 30,000 fmol adducts/mg DNA were detected by immunological methods 48 h after oral administration. No adducts were detectable in DNA isolated from the non-target tissues (spleen and nucleated blood cells) at the limit of detection of 60 fmol adducts/mg DNA (Baan et al, 1985a). These data were confirmed by Willems et al (1987) after

treating rats with 200 mg/kg AAF, using the ^{32}P-postlabelling method for the detection of DNA adducts in liver, spleen and nucleated blood cells.

The fact that all the carcinogens bind to DNA of non-target tissue cells without inducing tumours in them strongly suggests that DNA binding alone is not sufficient for carcinogenesis. The rate of cell division (rates of DNA replication), distribution of adducts in the genome and localisation of adducts in different cell types may all have an influence on whether covalent DNA binding eventually results in tumour formation.

5.1.6. Stability of DNA Adducts: The formation of DNA adducts appears to represent only the first in a sequence of events and several possibilities exist by which the lesion can be modified before it has biological consequences. The persistence of an adduct depends primarily on its chemical stability and how it is recognised by repair enzymes. The glycosidic bond of the modified base may be chemically labile so the base is split off or, in case of guanine adducts, the five membered ring opens and a rather persistent adduct results. This is exemplified by the N-7-guanine-aflatoxin adduct. Glycosylase enzymes are able to cleave off adducts as a first step in one of several repair processes. Excision repair, postreplication repair and the enzymatic removal of alkyl groups from O-alkylated bases are other ways of reducing the life time of primary lesions.

Although these mechanisms have been studied in experimental systems, particularly in microbial systems, only limited information exists about the elimination rate of adducts *in vivo*. The elimination of C-8-guanine adducts of 2-aminofluorene of rat liver DNA is biphasic. This is consistent with a model in which there are two genomic regions, one readily accessible to repair and one relatively persistent. It is, however, also conceivable that these regions represent different cell types within the organ, or separate compartments within the same cell, such as linker and core regions, euchromatin and heterochromatin, or nuclear and mitochondrial DNA (Poirier *et al*, 1984). The removal rate

for the 2-aminofluorene adduct has been determined as 0.213 day $^{-1}$ for the rapid and 0.026 day $^{-1}$ for the slow process.

Biphasic removal has also demonstrated with DNA adducts formed with benzidine, 1'-hydroxysafrole, and benzo(a)pyrene. With most of the so-called bulky adduct-forming chemicals rather persistent adducts have been found. The picture is more complex with small alkylating chemicals which generally lead to many different adducts, some of which are readily repaired and others are more persistent. Thus within 48 h after administration of ethylnitrosourea the level of the adduct O^4-ethylthymine remained constant in liver, kidney, lung and brain of rat; in contrast O^6-ethylguanine decreased rapidly in liver, lung and kidney but not in brain (Müller and Rajewsky, 1983).

5.1.7. Background Levels of DNA Adducts: DNA preparations from liver, kidney, lung and heart derived from untreated rats of different ages were analysed for the presence of DNA adducts using the ^{32}P-postlabelling method. Specific patterns of DNA adducts were detected in adult animals. These adducts were not detected in newborn rat DNA but increased markedly with age. The authors were not able to identify the structure of the adduct but estimated the degree of alkylation to be one adduct in 10^8 - 10^9 DNA nucleotides or 3-30 fmol adducts/mg DNA (Randerath et al, 1986).

This estimation was confirmed by Gupta et al (1987) who investigated liver DNA of rats. Aromatic DNA adduct lesions were revealed at levels which were at the limit of detection using the ^{32}P-post-labelling method (1 adduct per 0.5 - 3 x 10^9 nucleotides, ie 6-100 fmol adducts/mg DNA).

5.1.8. Lack of covalent binding to DNA: Investigations on the potential of di-(2-ethylhexyl)-phthalate (v. Däniken et al, 1984), clofibrate/fenofibrate (v. Däniken et al, 1981) and oestrone/oestradiol (Caviezel, 1984) to bind to DNA after oral administration to rats revealed practically no binding (CBI < 0.02), yet all chemicals investigated

were carcinogenic in rats in long-term bioassays. This strongly indicates that covalent interaction of these compounds with DNA is unlikely to be the mechanism of tumour induction.

5.2. Protein Adducts

Animal experiments have been carried out with a variety of covalently reacting compounds including unsaturated hydrocarbons, epoxides, aromatic amines, aromatic nitro compounds, benzene, polycyclic aromatic hydrocarbons, aflatoxin B_1, nitrosamines (Ehrenberg and Osterman-Golkar, 1980; Pereira and Chang, 1980; Green et al, 1984; Neumann, 1984b). Several of the studies indicate that the haemoglobin adduct levels are linearly proportional to the administered dose over a wide range of doses, eg. EO (Ehrenberg et al, 1974), *trans*-4- dimethylaminostilbene (Neumann et al., 1978; Neumann et al, 1980), 2-AAF (Pereira et al, 1981), EMS (Murthy et al, 1984), 4-aminobiphenyl (Skipper et al, 1984), benzo(a)pyrene (Shugart, 1985), and chromates (Wiegand et al, 1984, 1985).

Pereira and Chang (1980) examined 15 compounds, mostly nitrosamines and aromatic hydrocarbons. The efficiency of binding to haemoglobin (HBI) in rats ranged from 0.007 to 2.3 % of an oral dose. Methyl methanesulphonate a direct acting mutagen and weak carcinogen, was bound to the greatest extent (3,230 pmol/g Hb/mol dose, HBI = 222), followed by dimethylnitrosamine (HBI = 47), a strong indirect acting carcinogen. In contrast to the CBI, the haemoglobin binding index (HBI) does not appear to correlate with carcinogenic potency when considering diverse classes of chemicals. It was suggested by the authors that the extent of haemoglobin binding may be correlated with genotoxic activity within structurally-related groups of chemicals.

5.2.1. The Relationship of Haemoglobin Adducts to DNA Adducts: Examples of this relationship are given in the following sections for three mono-functional alkylating agents and one aromatic amine.

5.2.1.1. Methyl methanesulphonate (MMS): The directly alkylating agent, methyl methanesulphonate (MMS) was administered (i.p.) to male CBA mice at dose levels of 0.537, 11.1 and 132 mg/kg. Covalent binding to DNA in liver and testes and to haemoglobin was determined after 5 h by measuring 7-medGua and S-methylcysteine respectively (Segerbäck et al, 1978). The degree of alkylation of haemoglobin was linearly correlated to dose, while alkylation of N-7-guanine in DNA of liver and testes showed a slight deviation from linearity at the high dose level.

The rate constants for the reaction of MMS at N-7 of guanine and S of cysteine were determined in vitro and used with the degree of alkylation in the animal experiments to calculate tissue doses (Osterman-Golkar et al, 1976, Ehrenberg et al, 1983). Comparison of in vivo alkylation of DNA and haemoglobin showed a slightly higher tissue dose in red blood cells than in the compartments of DNA (testis, liver). S-Methylcysteine accounted for about 50% of the total radioactivity in haemoglobin. The persistence of the S-methyl-cysteine adduct was followed over a period of 30 days. The observed linear decrease of S-methylcysteine which reached zero after 40 days corresponded exactly to the life-time of erythrocytes in mice. This demonstrated that haemoglobin adducts are sufficiently stable to use as an integral dose monitor. The authors drew the conclusion that approximately the same tissue dose is obtained in all parts of the body if a direct alkylating agent is sufficiently long lived and consists of small, uncharged molecules sufficiently soluble in lipid and water.

5.2.1.2. Ethyl methanesulphonate (EMS): Ethylation of guanine at the N7 position in liver and kidney, and of amino acids (N-2-ethylvaline, S-ethylcysteine and N^{τ} and N^{π}-ethylhistidine) in haemoglobin was determined 12 and 20 h after i.p. administration of 0.04 - 446 mg/kg radiolabelled EMS to male CBA mice. At doses up to 7 mg/kg the degree of ethylation in both haemoglobin and DNA increased in proportion to the administered dose (Murthy et al, 1984). The rate

constants for the reaction of EMS with guanine at the N7 position and with the amino acids of haemoglobin were determined in vitro and used with the degree of alkylation found in the animal experiments in order to calculate tissue doses in the cellular DNA and in the red blood cells. At doses above 7 mg EMS/kg (70 and 446 mg/kg) a relatively higher degree of alkylation occurred than would have been expected if alkylation was directly proportional to dose. The ratio between covalent binding to haemoglobin and to DNA was approximately constant over the range of doses with the exception of a higher degree of alkylation in DNA of liver at the highest dose level (446 mg EMS/kg). To explain these deviations from linearity the authors suggested that the detoxification mechanism was progressively saturated leading to accumulation of the compound in the liver at the high dose levels. Because of the proportionality of haemoglobin and DNA binding over a wide range of doses the authors were convinced that haemoglobin alkylation could be a valid indicator of DNA alkylation.

5.2.1.3. Ethylene Oxide (EO): Radiolabelled ethylene oxide (EO) was administered by i.p. injection to male Fischer 344 rats at dose levels of 2.77 and 20.4 mol/kg. The degree of alkylation of N histidine of haemoglobin (N^{τ}-(2-hydroxyethyl)histidine) and at the N7 position of guanine (N-7-(2-hydroxyethyl)guanine) in DNA from livers and testes were determined (Osterman-Golkar et al, 1983).

The rate constants for the reaction of EO with N-7-guanine in calf thymus DNA (Ehrenberg et al, 1974; Segerbäck, 1985), and DNA in mouse spleen cells (Ehrenberg et al, 1974) and of N^{τ}-histidine of haemoglobin in red blood cells were determined in vitro and used with the in vivo alkylation data of haemoglobin and DNA in rats to calculate tissue doses (Osterman-Golkar et al, 1976; Ehrenberg et al, 1983). Comparison of in vivo alkylation of DNA and haemoglobin showed that the tissue dose in liver DNA was higher (by 50 %) and in testicular DNA lower (by 50 %) than the values calculated in red blood cells.

These results were consistent with the mouse where the relative tissue doses to liver DNA, haemoglobin and testis DNA ratios were 1.3, 1, and 0.6 (Segerbäck, 1983). The authors concluded that the dose of EO was approximately the same in the different tissues studied. Thus the in vivo dose of EO determined from data on haemoglobin alkylation might give a suitable approximation of the DNA dose.

The studies of Wright (1983) supports this view. Rats exposed by inhalation to 1, 10 and 33 ppm of (^{14}C)EO demonstrated a relatively uniform level of alkylation of DNA in a wide range of tissues at each exposure level. Such homogeneous distribution in tissues, ostensibly possessing different capacities to metabolise EO, suggests that it is unlikely that there will be any significant variation in the correlation between the haemoglobin dose of EO and the tissue DNA dose of EO in mammalian species (ECETOC, 1984; Potter et al, 1989).

After repeated (long-term) exposure at atmospheric concentrations of 10, 33, 100 ppm EO, 6h/d, 5d/w for 2 years the degree of N^{τ}-2-(hydroxyethyl)histidine/g Hb exhibited a clear linear relationship with external dose. The degrees of alkylation found were 1.3 and 2.8 nmol/g Hb in two groups of nonexposed rats and 14, 34, and 82 nmol N^{τ}-2-(hydroxyethyl)histidine/g Hb) respectively at the three air levels of EO (Osterman-Golkar et al, 1983). Thus a linear relationship between dose and haemoglobin adducts occurs with chronic as well as a single exposure.

5.2.1.4. *Trans*-4-dimethylaminostilbene (*trans*-DAS): Radiolabelled ^{3}H-*trans*-4-dimethylaminostilbene (*trans*-DAS) was administered orally to female Wistar rats at doses ranging from 5×10^{-10} to 1.8×10^{-4} mol/kg and covalent binding in liver, kidney and blood was determined after 24 h (Neumann, 1980). Adduct formation was monitored in the liver (which controls the entry of the chemical into the organism and modulates metabolite formation), the kidney (an extra-hepatic non-target tissue) and blood proteins and haemoglobin (a monitor of the distribution of reactive metabolites).

Total radioactivity increased in a linear dose-dependent manner in these tissues. Deviations from linearity were seen only at the highest level. This was close to the LD_{50} and delayed absorption and a decrease in the ability of the liver to clear material from blood at first-pass may have occurred leading to less activation of *trans*-DAS. Liver macromolecules (proteins, RNA, DNA) were labelled in a dose dependent manner but again deviations from linearity were observed with the highest dose used. The total binding to proteins, RNA, and DNA was approximately three times lower than in liver. Covalent binding with plasma proteins and haemoglobin was linear and parallelled that with DNA. This indicated the presence of active metabolites in blood after activation in the liver. It was claimed that the results provided experimental evidence for the occurrence of first-order kinetics even at low doses. Thus metabolic activation, distribution of reactive metabolites and their reaction with cellular macromolecules appeared to occur even at very low doses, consequently no threshold dose at which no DNA or haemoglobin binding occurred could be demonstrated for *trans*-DAS in experimental animals.

5.2.1.5. Specificity of Haemoglobin Adducts: Almost all animal studies conducted so far have included exposure of rats or mice and in vitro determination of rate constants for haemoglobin binding in their blood (Farmer et al, 1987). It has been demonstrated that the alkylation pattern of amino acids of haemoglobin is different in man compared to rats and mice at similar tissue doses of EO (Segerbäck, 1985; Potter et al, 1989). The reactivity towards cysteine varies by two orders of magnitude between species, the rate of reaction increasing in the order human, mouse, rat, reflecting the amount of in vivo alkylation of cysteine of haemoglobin. Reactivity towards the N-atom of the terminal valine and the N^{π}-atom of histidine were highest in the rat, but it was highest towards the N^{τ} - atom of histidine in man (Segerbäck, 1985). Cross species studies with s-triazines showed specific pathway binding to cysteine ß-125 of rat haemoglobin, but not to the usually more reactive cysteine ß-93, which is the only cysteine present in most haemoglobins. Therefore

haemoglobin adducts could not be detected in mammalian species other than the rat (Hamboeck et al, 1981).

5.2.2. Stability of Haemoglobin Adducts: Several studies demonstrate the stability and persistence of haemoglobin adducts. The decrease of *trans*-4-dimethyl-aminostilbene adducts in rat blood was measured over a period of 4 weeks (Neumann, 1979), and benzidine adducts over 10 weeks (Neumann, 1984c); half-lives of 14 and 11 days respectively were found. After long-term administration of 4-aminobiphenyl haemoglobin adduct levels accumulated in the rat and after cessation of dosing they declined at a rate of 2.5 % per day. After 60 - 65 days the labelled adducts were cleared from the blood (Skipper et al, 1984).

Haemoglobin adducts from benzo(a)pyrene declined linearly as the erythrocytes aged in mice and were cleared from the blood by 40 days (Segerbäck, priv. commun.). Similarly, methylated haemoglobin resulting from treatment with MMS decreased linearly in mice and reached zero values after about 40 days, the life-span of erythrocytes in the mouse (Segerbäck et al, 1978). The results are all consistent with the concept that the haemoglobin adducts are stable in vivo and that their elimination is related to the life-span of erythrocytes.

5.2.3. Background levels of haemoglobin adducts: In two independent groups of non-exposed rats (Osterman-Golkar et al, 1983) the background levels of haemoglobin adducts was found to be 1.3 and 2.8 nmol N^{τ}-2-(hydroxyethyl)histidine/g Hb. The source of such background alkylations is unclear and more work is required to discover their origin. It has been suggested that endogenous ethene production could give rise to the observed background levels of 2-hydroxyethyl adducts in Hb (Törnqvist et al, 1986a; Fu et al, 1979; Filser and Bolt 1983).

5.3. Summary

Animal experiments have been carried out with a variety of covalently reacting compounds including epoxides, aromatic and polycyclic aromatic compounds and nitrosamines. For the four chemicals discussed above,

haemoglobin adduct and DNA adduct level in the target and non-target tissues were directly proportional to the administered (external) dose over a wide range of doses; deviations from linearity have occasionally been observed at high dose levels. The ratio between haemoglobin and DNA adducts remained constant even at low doses (5×10^{-10} mol/kg) for *trans*-DAS. Thus for a given chemical, the extent of haemoglobin binding is quantitatively related to DNA binding. The relationship between haemoglobin and DNA adduct ratio and between dose and adducts needs to be determined individually for each compound.

Haemoglobin binding appeared to persist over the life-span of red blood cells in rats and mice, indicating a sufficient stability for its suitability as an integral dose monitor.

The stability of DNA adducts is more variable than haemoglobin adducts and depends upon several factors, eg. stability of lymphocytes in circulation, repair mechanisms and life-span of the lymphocytes.

6. DNA AND PROTEIN ADDUCTS IN MAN

6.1. Introduction

A large number of chemicals react covalently with DNA and proteins in experimental models (cf. 5) and several adducts have been used for monitoring human exposure. Examples described here come from subjects exposed to DNA and/or protein binding chemicals originating from occupational exposure, life-style (dietary, tobacco use), medicine and the environment.

6.2. Occupational Exposure

Most studies on macromolecular adducts in occupationally exposed workers measure adducts to haemoglobin. The best examined chemical is EO but others include propylene oxide, vinyl chloride, chromium (VI), aromatic amines and some phenol-containing pesticides. DNA binding has been reported in blood cells of workers exposed to polycyclic aromatic hydrocarbons (PAHs) and to styrene.

6.2.1. DNA adducts

6.2.1.1. Polycyclic Aromatic Hydrocarbons: In a series of studies DNA adducts were measured in workers exposed to PAH's, including benzo(a)pyrene (B(a)P). B(a)P is activated to the ultimate carcinogenic metabolite 7-ß,8-alpha-dihydroxy-(9-alpha,10-alpha) epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDE) which forms adducts with DNA, predominantly with N^2-guanine (Kapitulnik et al, 1978).

Shamsuddin et al (1985) measured the amount of BPDE-DNA adducts in peripheral white blood cells of roofers and foundry workers using the USERIA technique. BPDE-DNA adducts were detected in only seven of 28 samples from roofers and 7 of 20 samples from foundry workers (median: 200; range: 40 -2,400 fmol BPDE/mg DNA). Levels of PAH's in air were not determined. In a control group, two cigarette smokers had detectable amounts of BPDE-DNA adducts; the levels were

approximately 1000 fmol BPDE/mg DNA. Harris et al (1985) determined BPDE-DNA adducts in mononuclear cells from peripheral blood of coke oven workers. Using the SFS technique, 31 of 41 workers had detectable amounts of BPDE-DNA adducts but the levels were not quantified. USERIA was carried out on DNA samples from 27 workers; 18 had detectable amounts of adducts (median: 2,500; range: 40-34,000 fmol BPDE/mg DNA). The amount of DNA adduct was related to PAH exposure. In the highest exposure category 6 of 7 workers were positive. No DNA adducts were detected in 9 non-smoking controls.

In 30 aluminium plant workers, a sample from only one contained an amount of BPDE-DNA adduct in peripheral blood lymphocytes detectable using SFS (Vahakangas et al, 1985a). Haugen et al (1986) found one-third of a group of coke-oven workers positive using USERIA (median: 500; range: 100-13,700 fmol/mg DNA), but only 10% using SFS (median: 450; range: 380-2,200 fmol/mg DNA). The positive samples using SFS had the highest levels using USERIA. The 80% of workers who wore full face masks received mean PAH air concentrations of 110 $\mu g/m^3$ whilst those who did not were exposed to 266 $\mu g/m^3$. Corresponding B(a)P exposures were 3 $\mu g/m^3$ and 8 $\mu g/m^3$ respectively. The median values of BPDE-DNA adducts were similar for both groups.

It should be noted that BPDE-DNA antibodies used in the USERIA technique were shown to cross-react with DNA modified by other PAHs, eg. benzanthracene, chrysene. (Perera et al, 1987). Thus the "BPDE-DNA" adducts detected in the above studies may have been multiple PAH-DNA adducts.

Using the ^{32}P post-labelling procedure, an average level of 2 fmol/mg DNA (range: 0-20) was found in a group of 16 foundry workers exposed to B(a)P concentrations of <0.05 $\mu g/m^3$ in air. In the medium and high exposure groups (>0.05 $\mu g/m^3$) the average level of adducts was 50 fmol/mg DNA (range 0-300). Most of the DNA adducts detected were not BPDE-DNA adducts (Phillips et al, 1988). Different adduct levels in the exposure groups were were also demonstrated

with the ELISA technique. The level of BPDE-DNA adduct in each group was much higher than with the ^{32}P post-labelling procedure, probably reflecting differences in the PAH-DNA adducts detected by these assays (Perera et al, 1988).

6.2.1.2. Styrene: A single determination on one worker showed covalent binding of styrene oxide with lymphocyte DNA following occupational exposure to about 100 ppm styrene (8 hour TWA) using the ^{32}P post-labelling technique. No adduct levels were observed in a control worker (Liu and Rappaport, 1988).

6.2.2. Haemoglobin Adducts

6.2.2.1. Ethylene Oxide (EO): In a number of studies haemoglobin adducts have been measured in workers occupationally exposed to EO. Calleman et al (1978) measured the binding of EO with the amino acid histidine in seven hospital sterilisation workers. The amount of N^{τ}-(2-hydroxyethyl)histidine determined by GC-MS after complete hydrolysis of haemoglobin ranged from 0.4-13.5 nmol/g Hb (median: 3.4 nmol/g Hb). Two persons with no occupational exposure to EO showed levels below 0.05 nmol/g Hb.

The above GC-MS method was also used in a study of 32 workers involved in the manufacture of EO and of 31 controls (Van Sittert et al, 1985). The control subjects had detectable amounts of histidine adducts (mean 1.59 $\pm$ 0.18 nmol/g Hb). The mean level in manufacturing workers was not statistically significantly different from the controls (2.08 $\pm$ 0.24 nmol/g Hb). All values are high compared with the results of more recent analyses which have used improved methods. Ethylene oxide concentrations in air were generally below 0.05 ppm.

With a more rapid and accurate GC-MS method based on a modified Edman degradation technique (Törnqvist et al, 1986a) the degree of 2-hydroxyethylation of N-terminal valine of haemoglobin was

determined in 10 employees (seven exposed, three controls) from an EO gas bottling establishment (Farmer et al, 1986). An exposure-related increase of 2-(hydroxyethyl)valine was found (median: 0.98, range: 0.02-7.7 nmol/g Hb); the level in controls was 0.14 nmol/g Hb, range 0.03 - 0.93 nmol/g Hb. Similar levels of 2-(hydroxyethyl) histidine adducts were obtained.

Wraith et al (1988) determined 2-(hydroxyethyl)valine adducts in haemoglobin of 17 sterilisation workers and 14 control subjects using a radioimmunological method. The former group had statistically significantly higher concentrations (mean: 0.58 $\pm$ 0.37 nmol/g Hb) than the control group (mean: 0.25 $\pm$ 0.09 nmol/g Hb). Ethylene oxide air concentrations for the exposed group ranged from < 0.1 - 0.51 ppm (mean: 0.15 ppm) (Jongeneelen, unpublished data). Similar adduct levels were obtained using the GC-MS method. These studies have shown that measurement of 2-hydroxyethylation of N-terminal valine and histidine of haemoglobin are sensitive methods of monitoring internal exposure to EO at air levels below 1 ppm (8 h TWA). Background levels of these amino acid adducts were found in control subjects without occupational exposures to EO. This has been confirmed in separate studies of members of the general population (Osterman-Golkar, 1983; Törnqvist et al, 1986b, Passingham et al, 1988).

6.2.2.2. Propylene Oxide (PO): In one study human exposure to propylene oxide was assessed by determination of 2-hydroxypropyl levels of histidine of haemoglobin (Osterman-Golkar et al, 1984). The level of 2-hydroxypropylation (median: 5.6; range: 0.85-13 nmol/g Hb) was related to propylene oxide exposure. In control subjects the levels were below the limit of detection (0.10 nmol/g Hb).

6.2.2.3. Vinyl Chloride: In preliminary studies 2-oxoethylhistidine adducts of haemoglobin have been measured in workers exposed to vinyl chloride monomer (Svensson and Osterman-Golkar, 1987). No difference was observed in the level of 2-oxoethylhistidine between

control subjects and workers exposed to 800-1200 ppm.h/wk, possibly due to high and variable background levels (0.1-1.5 nmol/g Hb).

6.2.2.4. Aromatic Amines: There have been several studies on the determination of haemoglobin adducts in man exposed to aromatics amines, both carcinogenic and non-carcinogenic (Lewalter and Korallus, 1985, 1986; Bryant et al, 1987; Stillwell et al, 1987). Taking exposure to aniline as an example, the determination of the amount of aniline binding with haemoglobin was a much better indicator of internal dose of aniline than the determination of metabolites in urine or blood (Lewalter and Korallus, 1985). This was particularly so in workers categorised as "slow acetylators". At similar absorbed-dose levels the amount of aniline-haemoglobin adduct was much higher in the "slow" than in the "fast" acetylators. A BAT value has been established of 100 µg aniline liberated from haemoglobin/l blood, based on extensive studies of workers exposed to aniline. At this level, methaemoglobin concentrations in blood did not exceed 5% (Bolt et al, 1985; Lewalter, 1986).

Haemoglobin adduct determinations have also been carried out for monitoring exposure to the aromatic amine herbicides amitrole and diuron, the first by measuring the parent compound and the second by measuring 3,4-dichloroaniline liberated from haemoglobin. For both compounds methaemoglobin levels above 5% have not been reported at levels below 100 µg/l blood (Lewalter and Korallus, 1986).

6.2.2.5. Other Aromatic Chemicals: Occupational exposure to the aromatic compounds propoxur, triadimefon and bitertanol has been assessed by the measuring their haemoglobin adducts 2-isopropoxyphenol, p-chlorophenol and 4-phenylphenol (Lewalter and Korallus, 1986). The mechanism of haemoglobin binding has not yet been ascertained.

6.2.2.6. Chromium: Lewalter et al (1985) reported on the measurement of chromium bound to erythrocytes as a means of monitoring exposure of water-soluble chromium (VI) substances using atomic absorption spectroscopy. Individuals varied in their ability to reduce Cr(VI)

into Cr(III) in plasma. At similar Cr(VI) absorbed-dose levels, "slow" reducers showed much higher binding of chromium to erythrocytes than "fast" reducers. Measurement of chromium levels in erythrocytes is, therefore, a much more reliable indicator of internal dose than measurement of urinary chromium concentrations.

6.3. Life-style exposures

6.3.1. Dietary Exposures: The best documented genotoxic contaminant in food is Aflatoxin B_1 (AFB). A recent study in a population of Guang Xi Province (Republic of China) showed the presence of AFB-albumin adducts in the blood of 42 residents. The concentration of adducts was statistically significantly correlated with AFB ingested (Liang-Shang Gan et al, 1988). Dietary exposure to AFB has also been measured in a population in Kenya by determining the urinary aflatoxin-guanine adduct using HPLC and fluorescence spectroscopy (Autrup et al, 1983). In a total of 983 individuals from various parts of Kenya 12.6% were positive (Autrup et al, 1987). The results may indicate a possible association between exposure to AFB and liver cancer incidence.

6.3.2. Tobacco Use: 2-Hydroxyethylation of both histidine and N-terminal valine of haemoglobin were seen in individuals without known occupational exposures to EO (cf. 6.2.1.1). The concentrations of 2-(hydroxyethyl)valine adducts of haemoglobin were determined in 11 smokers (more than 20 cigarettes/day) and 14 non-smokers in a Swedish study in order to determine the influence of tobacco smoke on background levels. Mean levels in non-smokers and smokers were highly significantly different (0.058 and 0.389 nmol/g Hb respectively). Since the amounts of 2-hydroxyethylation of histidine and valine in selected samples were similar, it was suggested that EO may be the hydroxyethylating agent in cigarette smoke (Törnqvist et al, 1986b). Similar findings have been reported in a UK study (Passingham et al, 1988).

In another study, levels of 4-aminobiphenyl (ABP) haemoglobin adducts were determined in smokers (20-50 cigarettes/day) and non-smokers. The

mean level in smokers was 154 pg 4-ABP/g Hb compared to 28 pg/g Hb for non-smokers (Bryant et al, 1987). Adducts of other aromatic amines (aniline, toluidines and 2-naphthylamine) have also been detected in the blood of smokers and non-smokers (Stillwell et al, 1987). BPDE-DNA adducts were measured in white blood cells in the same groups using the ELISA technique (Perera et al, 1987). Detectable adduct levels occurred in 5 of 22 smokers and in 7 of 24 non-smokers. In subsequent samples taken 48 hours later, adducts were detectable only in 4 of 21 smokers and 4 of 21 non-smokers. Mean BPDE-DNA levels were higher in smokers (128 $\pm$ 54 fmol/mg DNA) than in non-smokers (65 $\pm$ 26 fmol/mg DNA) but the difference was not statistically significant.

6.4. Medicinal Exposure

Reactivity towards DNA is the basis of the anti-neoplastic activity of genotoxic agents used in cancer chemotherapy. The measurement of DNA adducts in patients treated with such agents offers an opportunity to study the relation between the formation of DNA adducts and clinical response.

Studies have been initiated among cancer patients receiving treatment with the antitumour drug *cis* diamminedichloroplatinum (II) (*cis* DPP). *Cis* DPP reacts with DNA by forming intrastrand crosslinks on neighbouring bases (Fichtinger-Schepman et al, 1985). Poirier et al (1985) and Reed et al (1986) showed a positive correlation between the formation of *cis* DPP-DNA adducts in white blood cells and response to drug therapy. No adducts were detected in controls not treated with *cis* DPP. There were major differences in *cis* DPP-DNA adduct levels in the blood cells of patients receiving similar doses of the drug. Ovarian cancer patients, who formed a high level of adduct, showed a high rate of response to treatment. In contrast, patients who did not respond had low amounts of *cis* DPP-DNA adduct (Reed et al, 1986; Reed et al, 1987). The marked individual differences in *cis* DPP-DNA adduct levels in the white blood cells of cancer patients were confirmed by Fichtinger-Schepman et al, (1987).

6.5. Environmental Exposure

Background levels of some DNA and haemoglobin adducts (BPDE-DNA; 4 ABP-Hb; 2-hydroxyethylations of Hb) have been reported to occur in those not occupationally exposed to chemicals and non-smokers (cf. 6.2. & 6.3.2). The origin of these adducts is unknown.

In other studies, using the ^{32}P-post-labelling technique, adducts with aromatic compounds were detected in the DNA of bone marrow cells and white blood cells of healthy smoking and non-smoking individuals (Phillips et al, 1986) and in the DNA of colonic mucosa cells of colorectal cancer patients (Phillips et al, 1987). The origin and identity of these adducts is unknown and were not detected in foetal bone marrow or foetal colonic DNA. Using the same technique human placental DNA adducts of aromatic compounds were detected, of which one was probably related to smoking (Everson et al, 1986).

Perera et al (1982) detected BPDE-DNA adducts in lung tissue and white blood cells from some lung cancer patients using immunochemical methods, but adducts were not detected in non-cancer patients. O^6-medGua was detected in oesophageal DNA from people in Linxian County (Republic of China) who underwent surgery for cancer of the oesophagus. Comparable tissues samples from Europeans showed lower levels of O^6-medGua adducts in DNA. No O^6-etdGua adducts were detected in any of the samples (Umbenhauer et al, 1985).

The DNA and haemoglobin adducts reported in healthy and diseased human beings possibly result from presently unrecognised environmental exposures and their effects on health are unknown. The detection of DNA adducts in cancer patients does not prove that the adducts caused the cancer and current adducts may not necessarily be relevant to current cancers.

6.6. Summary

The determination of haemoglobin adducts has proved to be a sensitive method for measuring occupational exposures to ethylene and propylene

oxides, aromatic amines and chromium (VI) at levels below the established occupational exposure limits. DNA adducts in white blood cells have been measured in workers occupationally exposed to high levels of PAH and in patients treated with the anti-tumour drug *cis* DPP. The amount of haemoglobin or DNA binding with these chemicals is generally related to the extent of exposure when groups of people are considered as a whole. However, major differences are seen between individuals in the formation of adducts with all of these chemicals. No studies have been reported in which haemoglobin and DNA adducts have been determined simultaneously. Background haemoglobin and DNA adducts have been found in human beings not occupationally exposed to chemicals and not smoking. The origin of these adducts is unknown. So far, it has been demonstrated that smoking of cigarettes is related with increased background levels of 2-hydroxyethyl adducts of valine and histidine of haemoglobin, and 4-aminobiphenyl-haemoglobin adducts.

7. EVALUATION OF DNA AND/OR PROTEIN ADDUCTS AS MEASURES OF EXPOSURE AND FOR RISK ASSESSMENT

7.1. Introduction

The control of exposure is one of the corner stones in the prevention of adverse health effects of chemicals. Exposures to chemicals can be determined by measuring air concentrations of the chemical (environmental monitoring), by the determination of the chemical or metabolites in body fluids (biological monitoring) or by assessing the amount of adduct formation of the chemical with DNA and/or haemoglobin (biochemical effect monitoring). Distinction is drawn between biochemical effect monitoring and biological effect monitoring. Biochemical effect monitoring involves measuring changes at the biochemical level, eg. formation of DNA or haemoglobin adducts, the biological consequences of which may not be known. Biological effect monitoring, in the context of this document, would involve measuring a biological effect, eg. gene mutation.

This chapter evaluates the use of DNA and haemoglobin adduct measurement as a method for estimating exposure to potentially genotoxic chemicals and for risk assessment.

7.2. DNA and Protein Adducts as Measures of Exposure

Experience is increasing in the use of DNA and haemoglobin adducts for monitoring occupational exposure to genotoxic chemicals in man, for example ethylene and propylene oxides, aromatic amines, hexavalent chromium and PAH's (cf. 6). The results show that:

a. the determination of haemoglobin adducts is a sensitive method for measuring internal exposures to ethylene and propylene oxides, aromatic amines and hexavalent chromium at atmospheric levels below occupational exposure limits. In contrast DNA binding of PAHs was only found in white blood cells of coke oven and foundry workers with high PAH exposures.

b. in cohorts exposed to these chemicals the amount of adduct formed is related to the degree of exposure. However major differences are seen between individuals with similar exposures in the amount of adducts measured. This may be due to differences in work practices, variations in the toxicokinetics of the compound in different individuals, or to other factors. More specifically the determination of adducts provide a more accurate estimate of the tissue dose for each individual in that it takes into account genetic variability in enzyme activities etc. It may thus assist in identifying individuals at higher risk.

c. background levels of DNA and haemoglobin adducts have been identified to most of these chemicals. High background levels are particularly associated with exposure to tobacco smoke. There are also other, as yet unknown, sources which may contribute to the amounts of background adducts. Exposure to chemicals below occupational exposure limits may produce haemoglobin adduct levels in excess of background concentrations eg. EO; in other cases, eg. vinyl chloride, high background levels may at present preclude the measurement of low levels of exposures.

Haemoglobin adducts in red blood cells or DNA adducts in white blood cells have been used as surrogates for target tissue DNA adducts because in most cases target tissue is inaccessible for biomonitoring. Surrogate data can be used, provided a quantitative relationship exists, over a wide range of dose levels, between binding to haemoglobin or to white blood cell DNA and binding to DNA in target tissue. Direct proportionality between the external dose and the amount of DNA adducts in tissues and haemoglobin adducts has been demonstrated in experimental species, particularly with simple alkylating agents, a series of aromatic amines (cf. 5) and benzo(a)pyrene (Shugart and Kao, 1985). The results demonstrate that first order kinetics control the amount of DNA and haemoglobin binding over a wide range of dose levels.

Haemoglobin adducts are more stable than most DNA adducts. Studies in experimental animals have shown that haemoglobin adducts are removed from

the blood when the red blood cells degenerate. Thus it can be assumed that the amount of haemoglobin adduct is the result of exposure extended over the life of the red blood cell (4 months in man). In contrast DNA adducts tend to be chemically more labile and are also subject to DNA repair. The time of sampling of DNA adducts is thus more critical than haemoglobin adducts and should take account of the elimination rate of the specific DNA lesion.

The determination of the haemoglobin binding level is preferable for exposure control because of the higher stability of haemoglobin adducts and greater sensitivity of their detection compared with DNA adducts. However, the amount of haemoglobin binding is not a parameter of genotoxic risk in itself. The determination of DNA adducts may provide more information on possible risks of exposure to DNA binding chemicals than the determination of haemoglobin adducts (cf. 7.3).

Current experience in the use of haemoglobin adducts for monitoring occupational exposure to genotoxic agents is that for the chemicals investigated (EO, PO, Cr(VI), aromatic amines) the determination of haemoglobin adducts is a more accurate measure of individual internal dose, and therefore possible health risk, than the measurement of environmental air concentrations or urinary metabolite levels.

In addition the technique is a continuous monitor of exposure to the genotoxic chemical for a prolonged period due to the stability of the formed haemoglobin adducts during the life of the red blood cell. Thus the amount of adduct also includes unexpected exposures during non-routine conditions in the plant. Such exposures will not readily be measured by conventional methods (atmospheric or urinary metabolite monitoring), unless this monitoring is carried out daily, which is impractical.

7.3. DNA and/or Haemoglobin Adducts as a Measure for Risk Assessment

Currently the carcinogenic risk of a chemical in man is assessed by determination of the quantitative relationship between external dose and

tumour incidence in human epidemiological studies or, in its absence, in carcinogenicity bioassays. The most common "short-comings" of human epidemiological studies are the lack of accurate exposure data and the low statistical power due to limited population size. These factors usually preclude a reliable assessment of carcinogenic risk at current occupational exposure levels.

The assessment of carcinogenic risk of a chemical to the human population from toxicological studies proceeds in the following stages:

a. determination of the quantitative relationship between external dose of the test chemical and tumour incidence in an appropriate carcinogen bioassay. The lower dose levels in the test are usually much higher than those encountered in the occupational situation or by the general public.

b. estimation of the carcinogenic risk for the experimental species by extrapolation from the high experimental doses to the relatively low doses which result from workplace exposure.

c. extrapolation of cancer risk estimated in the experimental species, to man.

Various mathematical models have been used to extrapolate from high dose bioassay results to the lower doses associated with human exposure. The use of models that do not take existing knowledge of mechanisms into consideration makes extrapolation from experimental species to man speculative. The simultaneous use of several models results in estimates which show large variation and illustrates the uncertainty in the risk estimates generated. The determination of the tissue dose from measurements on the amount of DNA or haemoglobin adduct, may provide a more rational basis for risk extrapolation from high to low doses and between experimental species and human. Differences in toxicokinetics between species exposed to similar external dose levels of a chemical may result in different tissue doses. Such differences may also occur in man, eg. caused by genetically determined polymorphism of enzymes and

lifestyle. Human genotoxic risks might eventually be assessed by comparison of tissue doses of chemicals in an appropriate animal species, for which tumour incidence is established, with tissue doses in a human population. This would imply that in carcinogenicity bioassays the tissue doses in selected major organs should be determined. Analytical techniques for the determination of DNA and haemoglobin adducts may not have yet been developed for the chemical under investigation. In that case major organs should be retained for later analysis of adducts especially if tumours are found.

Most genotoxic chemicals react at more than one centre on the DNA molecule, producing a spectrum of DNA adducts (cf. 3). In experimental systems some of these adducts are more pro-mutagenic than others. The primary critical DNA lesions for tumour initiation are, however, not yet defined. The quality of risk assessment of exposure to genotoxic chemicals could be improved if more were known about critical DNA lesions and about their repair, particularly those occurring at low doses.

The radiation equivalence approach has been advocated as a way of compensation for modifiers of genotoxicity such as repair. This prospective risk model proposed by Ehrenberg et al (1974) is based on determination of the capacity of low doses of the chemical (defined in terms of tissue dose) and gamma radiation to induce defined genetic damage, ie. a specific gene mutation in a particular experimental species. In this way the capacity of a defined tissue dose of the genotoxic chemical to induce genetic damage can be expressed in terms of rad-equivalents, ie. the number of rads giving the same effect. For EO, a tissue dose of 1 mmol.h was equal to 80 rad of low LET radiation in a variety of species and in vitro test systems, suggesting that the radiation equivalence value of EO may have a similar numerical value in man. Radiation equivalence values have also been determined in Chinese hamster ovarian (CHO) cells and in E.coli for a range of other genotoxic chemicals (Couch et al, 1978; Hussain, 1981).

Haemoglobin adduct levels have been used to make predictions of the risk of leukemia by the application of the radiation equivalence concept in

cohorts that had exposure to EO in common (Calleman et al, 1978; Hogstedt et al, 1984). However, the radiation equivalence concept requires more investigation if it is to be used for risk assessment. For example evidence may be provided by validation against other models for genotoxic risk assessment, such as somatic cell gene mutation assays eg. the HRPT-assay in peripheral lymphocytes and assays for haemoglobin mutants (Tates et al, 1989) which are being developed to monitor mutational events in human beings. It also requires validation for other chemicals with more complicated metabolism and also with promotional properties.

At the present time risk estimates based on the amounts of ultimate carcinogens in target tissue are not possible. This is not only due to the lack of knowledge about the ultimate extent of genetically relevant DNA lesions produced by that tissue dose, but also to the multistage nature of tumour formation. The tumour incidence produced by chemicals of either low genotoxic potential or low level of exposure may depend more on secondary events, for instance exogenous promoting action. The steep slope observed for the tumour incidence resulting from formaldehyde exposures between 5.6 and 14.3 ppm could be explained by exacerbation of weak genotoxicity by severe tissue irritation (Lutz, 1987). Other examples of complex correlations between DNA adduct and tumour incidence were reported for O^4-etdThy generated from diethylnitrosamine and the incidence of rat liver tumours (Swenberg et al, 1987) and DNA adducts generated by 2-AAF and mouse liver tumours (Beland et al, 1988) (cf. 5.1.5.).

Some chemicals produce DNA damage in tissues without occurrence of tumours. However, tumours may then be produced in specific tissues following proper promoting treatments (Burns et al, 1983; Hilpert, 1983; Neumann, 1986; Denk et al, 1988). For such chemicals the extent of DNA damage (initiation) does not correlate well with tumour formation if the tissue is not treated with exogenous promoting agents. Thus DNA binding cannot be expected to be the sole predictor of risk for these chemicals. Tissue specificity of tumour formation seems to be related to tumour specific promotive action, either associated with the chemical itself or with general exogenous promoting actions. The estimation of promotional

pressure is an important component of risk evaluation. The radiation equivalence concept seeks to compensate for generic promoting pressures (Kolman et al, 1988, 1989).

A deeper insight into the mechanism of carcinogenesis is required before the measurement of DNA and haemoglobin adducts can be used confidently for human risk assessment; this should not preclude their use in exposure assessment (cf. 7.2).

7.4 General Conclusions on the use of Adducts

The great potential of these techniques should be exploited bearing in mind certain reservations (Ashby, 1988). The method may be used for established and suspected carcinogens. On the other hand the measurement of protein adducts could also be used for monitoring exposure to non-genotoxic chemicals which may only bind to protein.

At present the measurement of adducts should be seen in the light of monitoring exposure to chemicals. Current experience shows that the measurement of haemoglobin adducts is more accurate and more relevant than conventional exposure monitoring because of the greater resolving power in terms of individual exposure. This is the result of compensation for individual differences in metabolism and related toxicokinetic factors that determine the quantitative relationship between external dose and the dose of the ultimate toxicant delivered to the target molecule. Interpretation of adduct measurements can be made by translation of the amount of adduct into atmospheric levels of the chemical. This requires that correlations have been established connecting the adduct level with the external dose. Once this relationship has been established then the measurement of adducts gives a more reliable means of assessing exposure (cf. 7.2). The amount of adduct can then give an indication as to whether the current exposure limits have been exceeded.

Adduct levels should where possible be assessed on an individual basis with the aim of identifying increased susceptibility due to genetic polymorphism for instance, or to become aware of unknown exposures.

Historical control data on adduct levels do not exist in most cases but control populations should be examined in order that proper account of background or non-occupational exposures can be taken. The influence of confounding factors (eg life style factors) on adduct levels need to be assessed before reliance can be placed on the use of adducts for the measurement of occupational exposure. A more rational basis for setting occupational exposure limits will then be obtained as such data become available.

It may be regarded as good practice if individuals are informed of the results of health surveillance tests. In the case of adducts such information can only be given in terms of exposure monitoring. In this respect it is comparable to the measurement of urinary metabolites or atmospheric concentrations. If a positive result is obtained personal hygiene and/or work practices may need improvement. Subject to relevant national legislation individuals may have right of access to the results of health surveillance (EEC/80/1107). As little is known about the long term effects of adducts this will require explanation by the occupational physician and implies appropriate understanding by the recipient.

Due to factors such as repair mechanisms, progression and promotion no indication can be given as to the health interpretation of any determined adduct level. Currently the only possible model for risk assessment is the comparison of adduct levels with radiation equivalence. The radiation equivalence needs further evaluation and more work is needed to explore use of tests for monitoring early biological effects such as gene mutation and enzyme assays (Tates et al, 1989), DNA repair assays (Bhattacharyya et a 1988) and assays for enzyme polymorphism (Harris et al, 1987) which are poss health risks.

8. RECOMMENDATIONS FOR FUTURE RESEARCH

These recommendations should be read in conjunction with the preceding section (cf. 7). The following research would assist in the development of the field.

8.1. Exposure Measurement

a. Methods should be developed for measuring the amount of DNA and haemoglobin adducts in order to obtain reliable estimates of exposure of target tissue produced by specific substances in experimental animals. The quantitative relationship between the amount of DNA adduct in non-target tissue, eg. peripheral white blood cells, the amount of haemoglobin adduct and the amount of DNA adduct in target tissue (where known) should be investigated at several dose levels. [see 8.2]

b The DNA and haemoglobin adduct levels should be studied in those who have been exposed to carcinogens; these should be related to data on external and internal exposure.

c. Carry out an interlaboratory study to examine the consistency of results from the different methods for analysing specific DNA and haemoglobin adducts, and to examine the consistency of the individual methods when used in different laboratories.

d. The kinetics of formation and repair of specific DNA adducts in target and non-target tissues and the stability of haemoglobin adducts should be investigated in order that reliable sampling strategies can be defined.

e. Studies on appropriate control populations should be conducted to obtain data on inter- and intra-individual variability of the amounts and ratio of background DNA and haemoglobin adducts and the nature and influence of confounding factors (eg. tobacco smoking, use of alcoholic beverages). Such data will assist in establishing criteria for control of exposure.

f. The mechanism of formation of background adducts should be investigated to identify the sources responsible for the adducts.

8.2. Risk Assessment

a. Where experimental animal studies are carried out to clarify the carcinogenic or mutagenic potential of chemicals, greater attention should be given to the possible value of relating biological endpoints (cancer or mutation) not only to the administered (external) dose but also to the amounts of DNA adducts in target and non-target tissues and to the amount of haemoglobin adduct, to the study of the rate of formation, repair and persistence of DNA adducts in different tissues.

b. Prospective epidemiological studies should be conducted in which DNA and haemoglobin adduct levels are related to early biological markers of genetic response, eg. somatic cell gene mutation assays. This would enhance the predictive value of DNA and haemoglobin adducts in terms of biological effects of exposure to genotoxic agents in man. Such data may assist in determining the level of DNA lesions and/or haemoglobin adducts which should be considered to represent an acceptable risk.

c. In prospective epidemiological studies of worker populations exposed to potentially genotoxic chemicals it would be of value to obtain data on DNA and haemoglobin adduct levels in individuals and correlate these with eventual disease.

d. Research to identify "critical" DNA adducts in order to obtain a better understanding of which DNA lesions lead to increased health risk.

e. Other biochemical tests should be developed which assist the prediction of the individual risk of genotoxic exposure such as DNA repair assay and enzyme polymorphism.

REFERENCES

- Aaseth, J., Alexander, J. and Norseth, T.(1982). Uptake of ^{51}Cr-chromate by human erythrocytes - A role of glutathione. Acta Pharmacol. et toxicol., 50, 310-315.
- Adamkiewicz, J., Dorsdziok, W., Eberhardt, W., Langenberg, U. and Rajewsky, M.F. (1982). In: Bridges, B.A., Butterworth, B.E. and Weinstein, I.B. (eds), 'Indicators of genotoxic exposure (Banbury Report 13), Cold Spring Harbor, N.Y., 265-276.
- Adamkiewicz, J., Nehls, P. and Rajewsky, M.F. (1984). in: Berlin, A., Draper, M., Hemminki, K., Vainio, H. (eds.) Monitoring human exposure to carcinogenic and mutagenic agents. IARC Sci. Publ., 59, 199-216.
- Adams, J., David, M., and Giese, R.W. (1986) Pentafluorobenzylation of O^4-ethylthymidine and analogues by phase transfer catalysis for determination by gas chromatography with electron capture detection. Anal. Chem., 58, 345 - 348.
- Adriaenssens, P.I., White, C.M., and Anderson, M.W. (1983). Dose-response relationships for the binding of benzo(a)pyrene metabolites to DNA and protein in lung, liver and forestomach of control and butylated hydroxyanisol-treated mice. Cancer Res., 43, 3712 - 3719.
- Aitio, A., Becking, G. Berlin, A., Bernard, A., Foa, V., Kello, D., Krug, E., Leonard, A., Nordberg, G., eds, (1988) Indicators for assessing exposure and biological effects of genotoxic chemicals, Consenses and technical reports. Commission of the European Communities.
- Albrecht, W., Neumann, H.-G. (1985). Biomonitoring of aniline and nitrobenzene. haemoglobin binding in rats and analysis of adducts. Ach. Toxicol., 57, 1-5.
- Aldridge, W.N. (1986). The biological basis and measurement of threshold. Am. Rev. Pharmacol. Toxicol., 26, 39-58.
- Alexandrov, K., Bekcer, M., Frayssinet, C., Dubowska, W. and Guerry, R. (1982). Persistence of benzo(a)pyrene diol-epoxide DNA adduct in mouse skin. Cancer Lett., 16, 247-251.
- Anders, M.W. (ed). (1985). Bioactivation of foreign compounds. Acad. Press, Orlando, Florida.
- Angerer, J., Amin, W., Heinrich-Rahm, R., Szadkowski, D. and Lehnert, G. (1987): Occupational chronic exposure to metals. I. Chromium exposure of stainless steel welders - biological monitoring. Int. Arch. Occup. Environ. Health, 59, 503-512.
- Appleton, S.B., Goetchius, M.P., and Campbell, T.C. (1982). Linear dose-response curve for the hepatic macromolecular binding of aflatoxin B1 in rats at very low exposures. Cancer Res.,42, 3659 - 3662.
- Ashby, J. (1988). Comparison of techniques for monitoring human exposure to genotoxic chemicals. Mutat. Res., 204, 543-551.
- Ashurst, S.W., Cohen, G.M., Nesnow, S., Di Giovanni, J. and Slaga, T.J. (1983). Formation of benzo(a)pyrene/DNA adducts and their relationship to tumour initiation in mouse epidermis. Cancer Res., 43, 1024-1029.
- Autrup, H., Bradley, K.A., Shamsuddin, A.K., Wakhisi, J. and Wasunna, A. (1983). Detection of putative adduct with fluorescence characteristics identical to 2,3-dihydro-2-(7'-guanyl) - 3 - hydroxyaflatoxin B1 in human urine collected in Murang' a district, Kenya. Carcinogenesis, 4, 1193.
- Autrup, H., Seremet, T., Wakhisi, J. and Wasunna, A. (1987). Aflatoxin exposure measured by urinary excretion of aflatoxin B1-guanine adduct and hepatitis B virus infection in areas with different liver cancer incidence in Kenya. Cancer Res., 47, 3430.
- Baan, R.A., Lansbergen, M.J., de Bruin, P.A.F., Willems, M.I. and Lohman, M.J. (1985a). The organ-specific induction of DNA-adducts in 2-AAF-treated rats, studied by means of a sensitive immunochemical method. Mutation Res., 62, 23-32.
- Baan, R.A., Zaalberg, O.B., Fichtinger-Schepman, A.M.J., Muysken-Schoen, M.A., Lansbergen, M.J., and Lohman, P.H.M. (1985b). Use of monoclonal and polyclonal antibodies against DNA adducts for the detection of DNA lesions in isolated DNA and single cells. Environ. Health Perspect., 62, 81-88.

- Bailey, E., Farmer, P.B., and Shuker, D.E.G. (1987). Estimation of exposure to alkylating carcinogens by the GC-MS determination of adducts to haemoglobin and nucleic acid bases in urine. Arch. Toxicol., 60, 187-191.
- Barrio, J.R., Secrist, J.A. and Leonard, N.J. (1972) Fluorescent adenosine and cylidine derivatives. Biochem. Biophys. Res. Comm., 46, 597-604.
- Bartsch, H., Hemminki, K., O'Neill, I.K., eds, (1988) Methods for detecting DNA damaging agents in humans : Applications in cancer epidemiology and prevention IARC Scientific publication N° 89, Lyon, International Agency for Research on Cancer.
- Baur, H. and Neumann, H.-G. (1980). Correlation of nucleic acid binding by metabolites or trans-4-aminostilbene derivatives with tissue specific acute toxicity and carcinogenicity in rats. Carcinogen., 1, 877-885.
- Becker, R.A., Barrows, L.R. and Shank, R.C.(1981). Methylation of liver DNA guanine in hydrazine hepatotoxicity : dose-response and kinetic characteristic of 7-methyl guanine and O^6-methylguanine formation and persistance in rats. Carcinogenesis, 2, 1181-1188.
- Beland, F.A. and Kadlubar, F.F.(1985). Formation and persistence of arylamine DNA adducts in vivo. Environm. Health Perspectives, 62, 19-30.
- Beland, F.A., Fullerton, N.F., Kinouchi, T., Lopez, A.T., Korfmacher, W.A. and Poirier, M.C. (1988). Macromolecular adduct formation in relation to tumorigenesis in mice continuously fed 2-AAF at multiple dose levels. Proceedings of the 79th Annual Meeting of the Americal Association of Cancer Research, Abstract 377.
- Beland, F.B., Fullerton, N.F., Kinouchi, T., Poirier, M.C. (1988). DNA adduct formation during continuous feeding of 2-AAF at multiple concentrations. In: Bartsch, H., Hemminki, K., O'Neill, J.K. (eds.). methods for detecting DNA damaging agents in humans. IARC Scientific Publications No.89, IARC, Lyon, pp. 175-180.
- Beranek, D.T., Weis, C.C. and Swenson, D.H., (1980). A comprehensive quantitative analysis of methylated and ethylated DNA using high pressure liquid chromatography. Carcinogen., 1, 595-606.
- Berlin, A., Draper, M., Hemminki, K., Vaino, H., eds (1984). Monitoring of human exposure to carcinogenic and mutagenic agents IARC Scientific Publications N°. 59, Lyon, International Agency for Research on Cancer.
- Bhattacharyya, D., Boulden, A.M., Foole, R.S. and Mitra, S. (1988). Effect of polyvalent metal ions on the reactivity of human O^6-methylguanine-DNA methyl transferase. Carcinogenesis, 9, 683 - 685.
- Bichara, M. and Fuchs, R.P.P. (1985) DNA binding and mutation spectra of the carcinogen N-2 aminofluorene in E. coli: a correlation between the conformation of the pre-mutagenic and mutation specificity. J. mol. Biol., 183, 341-351.
- Bolt, H.M., Neumann, H.G., Lewalter, J. (1985). Zur Problematik von BAT-Werten für aromatische Amine. Arbeitsmed. Sozialmed. Präventivmed., 20, 197-202.
- Bolt, H.M., Peter, H. and Foest, U. (1988). Analysis of macromolecular ethylene oxide adducts. Int. Arch. Occup. Environ. Health, 60, 141-144.
- Boobis, A.R., Caldwell, J., DeMatteis, F. and Elcombe, C.R. (eds) (1985). Microsomes and drug oxidation, Taylor & Francis, London.
- Bosan, W.S., Lambert, C.E. and Shank, R.C. (1986). The role of formaldehyde in hydrazine-induced methylation of liver DNA guanine. Carcinogenesis, 7, 413-418.
- Brennard, J., Saffhill, R. and Fox, M. (1982). The effects of methylated thymidines upon cultures of V79 cells and the mechanism of incorporation of O^4-methylthymidine into their DNA. Carcinogen., 3, 219-222.
- Bridges, J.W. (1987). Metabolism and molecular interactions related to toxicity, in : Mechanisms of cell injury : implications for human health. ed. B.A. Fowler, 353-382, John Wiley & Sons.
- Brookes, P and Osbourne, M.R.(1982). Mutation in mammalian cells by sterioisomers of anti-benzo(a)pyrenediolepoxide in relation to the extent and nature of DNA reaction products. Carcinogenesis, 3, 1223-1226.

- ECETOC, European Chemical Industry Ecology and Toxicology Centre (1982): Hepatocarcinogenesis in Laboratory Rodents: Relevance to Man. Monograph No. 4.
- ECETOC (1984) European Chemical Industry Ecology and Toxicology Centre: Ethylene oxide toxicology and its relevance to man. Technical Report No. 11.
- ECETOC (1988) European Chemical Industry Ecology and Toxicology Centre: The Mutagenicity and Carcinogenicity of Vinyl Chloride: a Historical review and assessment; Technical Report No 31.
- EEC/80/1107, Council Directive of 27th October 1980, on the protection of workers from the risks related to exposure to chemical, physical and biological agents at work. Off. J. Eur. Commun. (l 327).
- Ehrenberg, L., Hiesche, K.D., Osterman-Golkar, S. and Wennberg, I. (1974): Evaluation of genetic risks of alkylating agents: Tissue doses in the mouse from air contaminated with ethylene oxide. Mutat. Res., 24, 83 - 103.
- Ehrenberg, L. and Hussain, S. (1981) Genetic Toxicity of some important epoxides. Mutat. Res. 86, 1-113.
- Ehrenberg, L. and Osterman-Golkar, S. (1980): Alkylation of macromolecules for detecting mutagenic agents. Teratogen. Carcin. Mut.,1, 105 - 127.
- Ehrenberg, L., Moustacchi, E. and Osterman-Golkar, S. with an Appendix(II) by Ekman, G. (1983): Dosimetry of genotoxic agents and dose-response relationships of their effects. Mutat. Res.,123, 121 - 182.
- Everson, R.B., Randerath, E., Santella, R.M., Cefalo, C.C., Avitts, T.A. and Randerath, K. (1986). Detection of smoking-related covalent DNA adducts in human placenta. Science, 231, 54 - 57.
- Eyer, P. and Ascher, L.M. (1987). Reactions of para-substituted nitrosobenzenes with human haemoglobin. Biol. Chem., Hoppe-Seyler, 368, 285-294.
- Eyer, P. and Lierheimer, E. (1980). Biotransformation of nitrosobenzene in the red cell and the role of glutathione. Xenobiotica, 10, 517 - 526.
- Farmer, P.B., Foster, A.B., Jarman, M. and Tisdale, M.J. (1973). The alkylation of 2'-deoxyguanosine and of thymidine with diazoalkanes. Biochem. J., 135, 203-213.
- Farmer, P.B., Bailey, E., Gorf, S.M., Törnqvist, M., Osterman-Golkar, S., Kautiainen, A. and Lewis-Enright, D.P. (1986). Monitoring human exposure to ethylene oxide by the determination of haemoglobin adducts using gas chromatography - mass spectromety. Carcinogenesis, 7, 637 - 640.
- Farmer, P.B., Neumann, H.-G., Henschler, D. (1987). Estimation of exposure of man to substances reacting covalently with macromolecules. Arch. Toxicol., 60, 251 - 260.
- Fichtinger-Schepman, A.J.M., Baan, R.A., Luiten-Schuite, A., Van Dijk, M. and Lohman, P.H.M. (1985). Immunochemical quantitation of adducts induced in DNA by cis diamminedichloroplatinum (II) and analysis of adduct-related DNA-unwinding. Chem. Biol. Interactions, 55, 275 - 288.
- Fichtinger-Schepman, A.J.M., Van Oosterom, A.T., Lohman, P.H., Berends, F. (1987). Cis-diamminedichloroplatinum (II)-induced DNA adducts in peripheral leukocytes from seven cancer patients: quantitative immunochemical detection of the adduct induction and removal after a usingle dose of cis diamminedichloroplatinum (II). Cancer Res., 47, 3000.
- Filser, J.G. and Bolt, H.M. (1983). Ethylation of ethylene oxide by rats on exposure to ethylene. Mutat. Res., 120, 57-60.
- Flammang, T.J., Hein, D.W.,Talaska, G. and Kadlubar, F.F. (1988). N-hydroxyarylamine O-acetyltransferase and its relationship to aromatic amine N-acetyltransferase polymorphism in the inbred hamster and in human tissue cytosol, In "Carcinogenic and mutagenic response to aromatic amines and nitroarenes", Eds King, C.M., Romano, L.J. and Schuetzlz, D. Elsevier, New York, London, Amsterdam, London. pp 137-148.
- Foiles, P.G., Chung, F.L. and Hecht, S.S. (1986). Development of a monoclonal antibody based immunoassay for cyclic DNA adducts resulting from exposure to crotonaldehyde. Cancer Res., 47, 360-363.
- Fornace, A.J., Seres, D.S., Lechner, J.F. and Harris, G.C. (1981). DNA-protein cross-linking by chromium salts. Chem.-biol. Interactions, 36, 345-354.

- Bryant, M.S., Skipper, P.L., Tannenbaum, S.R. and Maclure, M. (1987). Hemoglobin adducts of 4-aminobiphenyl in smokers and non smokers. Cancer Res., 47, 602.
- Burns, F., Albert, R., Althhuler, B. and Morris, E. (1983). Approach to Risk Assessment for Genotoxic Carcinogens based on data from the Mouse Skin Initiation-Promotion Model. Environ. Health Perspectives, 50, 309-320.
- Calleman, C.J., Ehrenberg, L., Janson, B., Osterman - Golkar, S., Segerbäck, D., Svensson, K. and Wachtmeister, C.A. (1978). Monitoring and risk assessment by means of alkyl groups in haemoglobin in persons occupationally exposed to ethylene oxide. J. Environ. Pathol. Toxicol., 2, 427.
- Cathcart, R., Schwiers, E., Saul, R.L. and Ames, B.N. (1984). Thymine glycol and thymidine glycol in human and rat urine; a possible assay for oxidative DNA damage. Proc. Natl. Acad. Sci., USA, 81, 5633-5637.
- Caviezel, M. (1984): Untersuchungen zur kovalenten Bindung an DNS und Protein in vivo durch die Steroide Cholesterin-5,6-epoxid, Oestron, Oestradiol und Trenbolon, sowie durch Aflatoxin B1. Ph.D. Thesis, Zurich, ETH Nr. 7564
- Caviezel, M., Lutz, W.K., Minini, U., and Schlatter, C. (1984): Interaction of estrone and estradiol with DNA and protein of liver and kidney in rat and hamster in vivo and in vitro. Arch. Toxicol., 55, 97 - 103.
- Connett, P.H. and Wetterhahn, K.E. (1983). Metabolism of the carcinogen chromate by cellular constituents. Structure and Bonding, 54, 93-124.
- Couch, D.B., Forbes, N.L. and Hsie, A.W. (1978). Comparative Mutagenicity of Alkyl Sulphate and alkane sulphonate derivatives in Chinese hamster ovary cells. Mutation Res., 57, 217-224.
- Croce, C.M., Tsujimoto, Y., Erikson, J., and Nowell, P. (1984). Biology of disease: Chromosome translocations in B cell neoplasia. Lab. Invest., 51, 258-267.
- Cupo, D.Y. and Wetterhahn, K.E. (1985). Binding of chromium and DNA from liver and kidney of rats treated with sodium dichromate and chromium (III) chloride in vivo. Cancer Res., 45, 1146-1151.
- v. Däniken, A., Lutz, W.K., and Schlatter, C. (1981): Lack of covalent binding to rat liver DNA of the hypolipidemic drugs clofibrate and fenofibrate. Toxicol. Letters, 7, 311 - 319.
- v. Däniken, A., Lutz, W.K., Jäckh, R., and Schlatter, C. (1984): Investigation of the potential for binding of di-(2-ethylhexyl)phthalate (DEHP) and di-(2-ethylhexyl)adipate (DEHA) to liver DNA in vivo. Toxicol. Appl. Pharmacol., 73, 373 - 387.
- Denk, B., Filser, J.G., Oesterle, D., Deml, E. and Greim, H. (1988). Inhaled ethylene oxide induces preneoplastic foci in rat liver. J. Cancer Res. Clin. Oncol., 114, 35-38.
- Dölle, B., Töpner W. and Neumann, H.-G. (1980). Reaction of arylnitroso compounds with mercaptans. Xenobiotica, 10, 527 - 536.
- Dölle, B., Töpner W., Neumann, H.-G. Shut, H.A.J., Daniel, F.B., Schenck, K.M., Loeb, T.R., and Stoner, G.R. (1984) Metabolism and DNA adduct formation of 2- acetylaminofluorene by bladder explants from human, dog, monkey, hamster and rat. Carcinogenesis, 5, 1287 - 1292.
- Dooley, K.L., Beland, F.A., Bucci, T.J. and Kadlubar, F.F. (1984). Local carcinogenicity, rates of adsorption, extent and persistence of macromolecular binding and acute histopathological effects of N-hydroxyl-1-napthylamine and N-hydroxy-2-napthylamine. Cancer Res., 44, 1172-1177.
- Driver, E.H., White, I.N.H., and Butler, W.H. (1987). Dose response relationship in chemical carcinogenesis: renal mesenchymal tumors induced in the rat by single dose dimethylnitrosamine. Br. J. exp. Path., 68, 133 - 143.
- Dunn, B.P. (1983): Wide-range linear dose-response curve for DNA-binding of orally administered benzo(a)pyrene in mice. Cancer Res., 43, 2654 - 2658.
- Dyroff, M.C., Richardson, F.C., Popp, J.A., Bedell, M.A., and Swenberg, J.A. (1986): Correlation of O4-ethyldeoxythimidine accumulation, hepatic initiation and hepatocellular carcinoma induction in rats continuosely administered diethylnitrosamine. Carcinogenesis, 7, 241 - 246.

- Föst, U, (1987) Detection of derivatised deoxynucleosides and nucleobases with GC-MS, MS Thesis, University of Dortmund.
- Fu, P.C., Zic, V. and Ozimy, K. (1979). Studies of ethylene-forming system in rat liver extract. Biochem. Biophys. Acta, 585, 427-434.
- Gamper, H., Mechan, T., Straub, K., Tung, A.S.-C. and Kelvin, M. (1978). DNA and RNA, In: Polycyclic hydrocarbon and cancer, eds H.V. Gelboin and P.O. Ts'o, Vol.2, pp.51-61. Academic Press, New York.
- Glowinski, J.B., Radtke, H.E. and Weber, W.W. (1978). Genetic variation in N-acetylation of carcinogenic arylamines by human and rabbit liver. Mol. Pharmacol., 14, 940-949.
- Green, L.C., Skipper, P.L., Turesky, R.J., Bryant, M.S., and Tannenbaum, S.R. (1984) In vivo dosimetry of 4-aminobiphenyl in rats via a cystein adduct in haemoglobin. Cancer Res., 44, 4254-4259.
- Guengerich, F.P. and Liebler, D.C. (1985). Enzymatic activation of chemicals to toxic metabolites. CRC Crit. Rev. Toxicol., 14, 259-307.
- Gupta, R.C. and Dighe, N.R. (1984). Formation and removal of DNA adducts in rat liver treated with N-hydroxy-derivatives of 2-AAF, 4-acetylaminobiphenyl, and 2-acetylaminophenanthrene. Carcinogen., 5, 343-349.
- Gupta, R.C. and Early, K. (1988) ^{32}P-adduct assay: comparative recoveries of structurally diverse DNA adducts in the various enhancement procedures. Carcinogenesis, 9, 1687-1693.
- Gupta, R.C., Reddy, M.V. and Randerath, K. (1982) ^{32}P-postlabelling analysis of non-radioactive aromatic carcinogen DNA adducts. Carcinogenesis, 3, 1081-1092
- Gupta, R.C., Early, K., Cocker, J., Lombardi, B. (1987): ^{32}P-postlabelling analysis of liver DNA adducts in rats chromically fed a choline-devoid diet. Carcinogenesis, 8, 187 - 189.
- Hamboeck, H., Fischer, R.W., Di Iorio, E.E. and Winterhalter, K.H. (1981) Binding of s-triaine metabolites to rodent haemoglobins appears irrelevant to other species. Mol. Pharmacol. 20, 579-584.
- Hanna, P.E. and Banks, R.B. (1985). Arylhydroxylamines and arylhydroxamic acids : conjugation reactions. In Bioactivation of foreign compounds, ed M.W. Anders, Academic Press, Inc, Orlando, 375-402.
- Harris, C.C., Vahakangas, K., Newman, M.J., Trivers, G.E., Shamsuddin, A., Sinopoli, N., - Mann, D.L. and Wright, W.E. (1985). Dection of benzo(a)pyrene diol epoxide - DNA adducts in peripheral blood lymphocytes and antibodies to the adducts in serum from coke oven workers. Proc. natl. Acad. Sci. USA, 82, 6672 - 6676.
- Harris, C.C., Weston, A, Willey, J.C., Trivers, G., and Man, D.L. (1987). Biochemical and molecular epidemiology of human cancer: indicators of carcinogen exposure, DNA damage, and genetic predisposition. Environm. Health. Perspect. 75, 109-119.
- Hashimoto. K. and Aldridge, W.N. (1970), Biochemical studies on acrylamide, a neurotoxic agent. Biochem. Pharmacol., 19, 2591 - 2604.
- Haugen, A., Groopman, J.D., Hau, J.C., Goodrich, G.R., Wogan, G.W. and Harris, C.C. (1981). Monoclonal antibody to aflatoxin B_1-modified DNA detected by enzyme immunoassay. Proc. Natl. Acad. Sci. (USA), 78, 4124 - 4127.
- Haugen, A., Becer, G., Benestad, C., Vahakangas, K., Trivers, G.E., Newman, M.J. and Harris, C.C. (1986). Determination of polycyclic aromatic hydrocarbons in the urine, benzo(a)pyrene diol epoxide - DNA adducts in lymphocyte DNA, and antibodies to the adducts in sera from coke oven workers exposed to measured amounts of polycyclic aromatic hydrocarbons in the work atmosphere. Cancer Res., 46, 4178 - 4183.
- Hech, H. d'A. and Casanova, M. (1987) Isotope effects and their implications for the Covalent Binding of Inhaled [^{3}H]- and [^{14}C]Formaldehyde in Rat Nasal Mucosa, Toxicol. Appl. Pharmacol, 89, 122 - 134.
- Heck, H.D'A. and Casanova, M. (1987). Isotope effects and their implications for the covalent binding of inhaled [^{3}H]- and [^{14}C] formaldehyde in the rat nasal mucosa. Toxicol. Appl. Pharmacol., 89, 122-134.
- Heflich, R.H., Fifer, E.K., Djuric, Z. and Beland, F.A. (1986) Mutation induction and DNA adduct formation by 1,8-di-nitropyrene in Chinese Hamster Ovary Cells. In, Genetic

Toxicology of Environmental Chemicals, Part A: Basic Principles and Mechanisms of action, Eds Ramel, C., Lambert, B. and Magnusson, J.; Liss, New York, pp 265-273.

- Hemminki, K. (1983): Nucleic Acid Adducts of Chemical Carcinogens and Mutagens. Arch. Toxicol., 52, 249 - 285.
- Hemminki, K. and Hesso, A. (1984). Reaction products of styrene oxide with guanosine in aqueous media. Carcinogenesis, 5, 601-607.
- Hemminki, K. and Randerath, K. (1987) Direction of genetic interaction of chemicals by biochemical methods: Determination of DNA and protein adducts. In Mechanisms of Cell injury: implications for human health. Ed Fowler, B.A., Dahlem Conference, pp 209-227.
- Hemminki, K., Paasivirta, J., Kurkirinne, T., Virkki, L. (1980). Alkylation products of DNA bases by simple epoxides. Chem. Biol. Interact., 30, 259-270.
- Hemminki, K., Forsti, A., Mustonen, R., Savela, K., (1986): DNA adducts in experimental cancer research. J. Cancer Res. Clin. Oncol., 112, 181 - 188.
- Hilpert, D., Romen, W. and Neumann, H.G. (1983) The role of partial hepatectomy and of promotors in the formation of tumours in non-target tissues of trans-4-acetylaminostilbene in rats. Carcinogenesis, 4, 1519-1525.
- Hogan, M.E., Dattagupta, N. and Whitlock, J.P. (1981). Carcinogen induced alteration of DNA structure. J. Biol. Chem., 256, 1504-1513.
- Hogstedt, C;, Aringer, L., Gustavsson, A. (1984) Ethylene oxide and cancer- a review and follow up of two epidemiological studies. Work Health, 49, 1-37.
- Howard, P.C., Neflich, R.N., Evans, F.E. and Beland, F.A. (1983) Formation of DNA adducts in vitro and in Salmonella typhimurium upon metabolic reaction of the environmental mutagen 1-nitropyrene. Cancer Res. 43, 2052 - 2058.
- Hussain, S. (1981). Mutagenic action of readiation and chemicals: Parameters affecting the response of the test system. Ph.D. Thesis, University of Stockholm.
- Ilett, K.F., David, D.M., Datchon, P., Castleden, W.M., and Kwa, R. (1987). Acetylation phenotype in colorectal cancer. Cancer Res., 47, 1466-1469.
- Jakoby, W.B. (ed) (1980). Enzymatic basis of detoxication, Vol I & II. Acad. Press, NY. Jakoby, W.B., Bend, J.R. and Caldwell, J. (eds) (1982). Metabolic basis of detoxication. Acad. Press, NY.
- Jeffrey, A.M. (1985). DNA modification by chemical carcinogens. Pharmacol. Ther., 28, 237-272.
- Jeffrey, A.M., Yeh, H.J.C., Jerina, D.M., DeMarinis, R.M., Foster, C.H., Piccolo, D.E. and Berchthold, G.A. (1974). Stereochemical course in reactions between nucleophiles and arene oxides. J. Am. Chem. Soc., 96, 6929-6937.
- Jung, R., Beermann, D., Glatt, H.R. and Oesch, F. (1981). Mutagenicity of structurally related oxiranes derivative of benzene and its hydrogenated congeners. Mutat. Res., 81, 11-19.
- Kadlubar, F.F., Miller, J.A. and Miller, E.C. (1977). Hepatic microsomal N-glucuronidation and nucleic acid binding of N-hydroxy arylamines in relation to urinary bladder carcinogenesis. Cancer Res., 37, 805-814.
- Kadlubar, F.F., Unruh, L.E., Beland, F.A., Straub, K.M. and Evans, F.E. (1980). In vitro reaction of the carcinogen, N-hydroxy-2-naphthylamine with DNA at the C-8 and N-2 atoms of guanine and at the N-6 atom of adenine. Carcinogenesis, 1, 139-150.
- Kadlubar, F.F., Anson, J.F., Dooley, K.L. and Beland, F.A.(1981). Formation of urothelial and hepatic DNA adducts from carcinogen 2-napththylamine. Carcinogenesis, 2, 467-470.
- Kapitulnik, J., Wislocki, P.G., Hevin, W., Yogi, H., Thakker, D.R., Akagi, H., Korceda, M., Jerina, D.M. and Conney, A.H. (1978). Marked differences in the carcinogenic activity of optically pure (+) and (-) trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene in newborn mice. Cancer Res., 38, 2661-2665.
- Kennelly, J.C., Beland, F.A., Kadlubar, F.F. and Martin, C.N. (1984). Binding of N-acetylbenzidine and N,N'-diacetylbenzidine to hepatic DNA of rat and hamster in vivo and in vitro. Carcinogenesis, 5, 407-412.

- Kiese, M. (1974). Methaemoglobinaemia : A Comprehensive Treatise. CRC Press. Inc., Cleveland, Ohio.
- Kiese, M. and Taeger, L. (1976). The fate of phenylhydroxylamine in human red cells. Arch. Pharmacol., 292, 59-66.
- Kolman. A., Segerbäck, D. and Osterman-Golkar, S. (1988). Estimation of cancer risk of genotoxic chemicals by the rad-equivalence approach. IARC Sci. Publ. 89, 258 - 264.
- Kolman, A., Nöslund, M., Osterman-Golkar, S., Sealia Tomba G-P. and Meyer, A. (1989) Comparative studies of in vitro transformation by ethylene oxide and gamma radiation of C3H/10T1/2 mouse embryo fibroblasts. Mutagenesis, (in press).
- Kriek, E., Welling, M. and Van der Laken, C.J. (1984). In: 'Monitoring Human Exposure to Carcinogenic and Mutagenic Agents', eds. Berlin, A., Draper, M., Hemminki, K. and Vanio, H., IARC, 59, 297-309.
- Kusmierek, J.T. and Singer, B. (1982). Chloroacetaldehyde treated ribo- and deoxyribonucleotides. 2 errors in transcription by different polymerase resulting from ethenocytosine and its hydrated intermediate. Biochem., 21, 5723-5728.
- Laib, R.J., GWinner, L.M., and Bolt, H. (1981) DNA alkylation by vinyl chloride metabolites. Chem. Biol. Interact., 37, 219-231.
- Lambert, C.E. and Shank, R.C. (1988). Role of formaldehyde hydrazone and catalase in hydrazine-induced methylation of DNA guanine. Carcinogenesis 9, 65-70.
- Lang, N.P., Chu, D.Z.J., Hunter, C.F., Kendall, D.C., Flammang, T.J. and Kadlubar, F.F. (1986). Role of aromatic amine acetyltransferase in human colorectal cancer. Arch. Surg., 121, 1259-1261.
- Lauwerys, R. (1983). Industrial Chemical Exposure: Guidelines for biological monitoring. Biomedical Publications, Davis, California.
- Lawley, P.D. (1984) Some chemical aspect of dose relationship in alkyation mutagenesis. Mutat. Res., 23, 283-295
- Leadon, S.A. and Hanavalt, P.C. (1983). Monoclonal antibody to DNA containing thymine glycol. Mutat. Res., 112, 191-200.
- Lee, W.R. (1983). Macromolecular dosimetry. In, Developments in the Science and Practice of Toxicology, Eds, Hayes, A.W., Schnell, R.C., and Miya, T.S., pp 281-288, Amsterdam, Elsevier Science Publishers.
- Lewalter, J. (1986). Grenzwerte in biologischem Material. Aniline.
- Lewalter, J. and Korallus, U. (1985). Blood protein conjugates and acetylation of aromatic amines. New findings on biological monitoring. Int. Arch. Occup. Environ. Health, 56, 179-196.
- Lewalter, J. and Korallus, U. (1986). Erythrocyte protein conjugates as a principal of biological monitoring for pesticides. In: Bioligical monitoring of workers manufacturing, formulating and applying pesticides. Eds: Van Heemstra-Lequin, E.A.H. and Van Sittert, N.J. Toxicology Letters, 33, 153.
- Lewalter, J., Korallus, U., Harzdorf, C. and Weidemann, H. (1985). Chromium bond detection in isolated erythrocytes: a new principle of biological monitoring of exposure to hexavalent chromium. Int. Arch. Occup. Environ. Health, 55, 305-318.
- Liang-Shang Gan, Skipper, P.L., Xioocong Peng, Gropinan, J.D., Jun-shi Chen, Wogan, G.N. and Tannenbaum, S.R. (1988). Serum albumin adducts in the molecular epidemiology of aflatoxin carcinogenesis : correlation wioth aflatoxin B_1 intake and urinary excretion of aflatoxin M_1. Cancer Res., 9, 1323-1325.
- Liu, S. and Rappaport, S.M. (1988). Detection of styrene-oxide-DNA adducts in lymphocytes of workers exposed to styrene. In: Methods for Detecting DNA-Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention. IARC Scientific Publications, 89, 217-222.
- Lower, G.M. and Bryan, G.T. (1976). J. Toxicol. Env. Health, 1, 421-432.
- Lower, G.M.jr., Nilsson, T., Nelson, C.E., Wolf, H., Gamsky, T.E. and Bryan, G.T.(1979). N-acetyltransferase phenotype and risk in urinary bladder cancer : approaches in molecular epidemiology. Envrion. Health Perspect., 29, 71-79.

- Ludeke, B.I. and Kleihues, P. (1988). Formation and persistence of O^6-(2-hydroxyethyl). 2'-deoxyguanosine in DNA of various rat tissues following a single dose of N-nitroso-N-(2-hydroxyethyl)urea. An immuno-slot-blot- study. Carc., 9, 147-151.
- Lutz, W.K. (1979): In vivo covalent binding of organic chemicals to DNA as quantitative indicator in the process of chemical carcinogenesis. Mutat. Res., 65, 289 - 365.
- Lutz, W.K. (1984): Structural characteristics of compounds that can be activated to chemically reactive metabolites: use for a prediction of carcinogenic potential. Arch. Toxicol. Suppl., 7, 194 - 207.
- Lutz, W.K. (1986): Quantitative evaluation of DNA-binding data for risk estimation and for classification of direct and indirect carcinogens.J. Cancer Res. Clin. Oncol., 112, 85 - 91.
- Lutz, W.K. (1987): Quantitative evaluation of DNA-binding data in vivo for low-dose extrapolations. Arch. Toxicol. Suppl., 11, 66 - 74.
- Lutz, W.K. and Schlatter, C. (1977): Saccharin does not bind to DNA of liver and bladder in the rat. Chem. Biol. Interact., 19, 253 - 257.
- Martin, C.N., Beland, F.A., Roth, R.W. and Kadlubar, F.F. (1982). Covalent binding of benzidine and N-acetylbenzidine to DNA at the C-8 atom of deoxyguanosine in vivo and in vitro. Cancer Res., 42, 2678-2687.
- Martin C.N., Beland, F.A., Kennelly, J.C., and Kadlubar, F.F. (1983). Binding of benzidene, N-acetylbenzidene and N,N'-diacetylbenzidene and direct blue 6 in rat liver DNA. Envir. Hlth. Perspect., 49, 101-106.
- Miller, E.C. (1951). Studies on the formation of protein-bound derivatives of 3,4-benzpyrene in epidermal reaction of mouse skin. Cancer Res., 11, 100-108.
- Miller, J.A.and Miller, E.C. (1977): Ultimate chemical carcinogens as reactive mutagenic electrophiles. In: Origins of human cancer. Eds. Hiatt, H.H., Watson, J.D., Winsten, J.A., Cold Spring Harbour Laboratory, pp 605-627.
- Minnetian, O., Saha, M., and Giese, R.W. (1987) Oxidation-elimination of a DNA base from its nucleoside to facilitate determination of alkyl chemical damage to DNA by gas chromatography with electrophore detection. J. Chromatogr. 410,453-457.
- Mohamed, G.B., Nazareth, A., Hayes, M.J., Giese, R.W. and Vouros, P. (1984). Gas chromatography-mass spectrometry characteristics of methylated perfluoracyl derivatives of cytosine and 5-methylcytosine. J. Chromatogr., 314, 211-217.
- Mommsen, S., Barfod, N.M., and Aagaard,J.(1985): N-Acetyltransferase phenotype in the urinary bladder carcinogenesis of a low-risk population. Carcinogenesis, 6, 199-210.
- Müller, R. and Rajewsky, M.F. (1980) Immunological quantification by high affinity antibiodies of O^6-ethyldeoxyguanosine in DNA exposed to N-ethyl-N-nitroseurea. Cancer Res. 40, 887-896.
- Müller, R. and Rajewsky M.F. (1983) Enzymatic removal of O^6-ethyl-guanine versus stability of O^4-ethyl-thymine in the DNA of rat tissues exposed to the carcinogen ethyl nitrosourea; possible interference of guanine-O^6 alkylation with 5-cytosine methylation in the DNA of replicating target cells. Z. Naturf. 38c, 1023 - 1029.
- Murthy, M.S.S., Calleman, C.J., Osterman-Golkar, S.,Segerbäck, D. and Svensson, K. (1984): Relationships between ethylation of haemoglobin, ethylation of DNA and administered amount of ethyl methanesulfonate in the mouse. Mutat. Res.,127, 1 - 8.
- Neumann, H.-G. (1980). Dose relationships in the primary lesion of strong electrophilic carcinogens. Arch. Toxicol. (Suppl.), 3, 69-77.
- Neumann, H.-G, (1984a): Quantitative aspects in the metabolic activation of aflatoxin B1 and carcinogenic aromatic amines, in Biochemical Basis of Chemical Carcinogenesis (Greim, H., Jung, R., Kramer, H., Marquardt, H., Oesch, F. edts), Raven press, NY, 33-45.
- Neumann, H.-G. (1984b): Analysis of haemoglobin as a dose monitor for alkylating and arylating agents. Arch. Toxicol., 56, 1 - 6.
- Neumann, H.-G. (1984c): Dosimetry and dose-response relationship. IARC Scientific Publications, No. 59, pp. 115 - 126.

- Neumann, H.-G. (1986). The role of DNA damage in chemical carcinogenesis of aromatic amines. J. Cancer Res. Clin. Oncol., 112, 100-106.
- Neumann, H.-G. (1987). Concepts for assessing the internal dose of chemicals in vivo, In Mechanisms of Cell Injury: Implications for Human Health ed, B.A. Fowler, pp 241-254. John Wiley & Sons, Chichester, New York, Brisbane, Toronto, Singapore.
- Neumann, H.-G. (1988). Biomonitoring of aromatic amines and alkylating agents by measuring haemoglobin adducts. Int. Arch. Occup. Environ. Health, 60, 151-155.
- Neumann, H.-G., Gaugler, B.J.M. and Taupp, W. (1978): The metabolic activation of trans-4-dimethylaminostilbene after oral administration of doses ranging from 0.025 to 250 mol/kg, in: Plaa, G.L. and Duncan, W.A.M.: Proceedings of the first international congress on toxicology. Academic Press, New York, San Francisco, London, pp. 177 - 190.
- Neumann, H.-G., Baur, H. and Wirsing, R. (1980): Dose-response relationships in the primary lesions of strong electrophilic carcinogens. Arch. Toxicol. Suppl., 3, 69 - 77.
- Osborne, M.R., Harvey, R.G. and Brookes, P. (1978). The reaction of trans-7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetra-hydrobenzo(a)pyrene with DNA involves attack at the N7-position of guanine moieties. Chem. Biol. Interact, 20, 123-130.
- Ostermann-Golkar, S. et al (1970). Reaction kinetics and biological action in barley of monofunctional methanesulfonic esters. Radiat. Bot. 10, 303-327.
- Ostermann-Golkar, S. (1983). Tissue doses in man: Implications in risk assessment. In: Developments in the Science and Practice of Toxicology. Eds.: Hayes, A.W., Schnell, R.C. and Miya, T.S. Elsevier, Amsterdam, 289.
- Osterman-Golkar, S., Ehrenberg, L., Segerbäck, D. and Hällström, I. (1976): Evaluation of genetic risks of alkylating agents. II. Haemoglobin as a dose monitor. Mutat. Res., 34, 1 - 10.
- Ostermann-Golkar, S., Hultmark, D., Segerback, D., Calleman, C.J., Gothe, R., Ehrenberg, L. and Wachtmeister, C.A. (1977). Alkylation of DNA and proteins in mice exposed to vinyl chloride. Biochem. Biophys. Res. Commun., 76, 259-266.
- Osterman-Golkar, S., Farmer, P.B., Segerbäck, D., Bailey, E., Calleman, C.J., Svensson, K. and Ehrenberg, L. (1983): Dosimetry of EO in the rat by quantitation of alkylated histidine in haemoglobin. Teratogen. Carcin. Mut., 3, 395 - 405.
- Osterman-Golkar, S., Bailey, E., Farmer, P.B., Gorf, S.M. and Lamb, J.H. (1984). Monitoring exposure to propylene oxide through the determination of hemoglobin alkylation. Scand. J. Work Environ. Health, 10, 99.
- Passingham, B.J., Farmer, F.B., Baily, E., Brookes, A.G.F. and Yates, D.W. (1988). In: "Methods for detecting DNA and damaging agents in humans: applications in cancer epidemiology and prevention. IARC Scientific Publications. 89, 279-288.
- Pegg, A.E., and Hui, G. (1978): Formation and subsequent removal of O6-methylguanine from deoxyribonucleic acid in rat liver and kidney after small doses of dimethylnitrosamine. Biochem. J., 173, 739 - 748.
- Pelkonen, O., Vahakangas, K., and Nerbert, D.W. (1980). Binding of polycyclic aromatic hydrocarbons to DNA: Comparisons with mutagenesis and tumorigenesis. J. Toxicol. Environ. Health, 6,1009-1020.
- Pellak-Walker, P., Walker, J.A., Evans, H. and Blumer, J.L. (1985). Relationship between the oxidation potential of benzene metabolites and their inhibitory effect of DNA synthesis in L5178YS cells. Mol. Pharmacol. 28, 560-566.
- Pereira, M.A. and Chang, L.W. (1980): Binding of chemical carcinogens and mutagens to rat haemoglobin. Chem.-Biol. Interact., 33, 301 - 305.
- Pereira, M.A., Lin, L.-H.C. and Chang, L.W. (1981): Dose-dependency of 2-AAF binding to liver DNA and haemoglobin in mice and rats. Toxicol. Appl. Pharmacol., 60, 472 - 478.
- Perera, F., Poirier, M.C., Yuspa, S.H., Nakayama, K., Jaretzki, A., Curren, M.M., Knowles, D.M. and Weinstein, I.B. (1982). A pilot project in molecular cancer epidemiology.

Determination of benzo(a)pyrene-DNA adducts in animal and human tissues by immuno-assays. Carcinogenesis, 3, 1405.

- Perera, F., Santella, R., and Poirier, M. (1986). Biomonitoring of workers exposed to carcinogens: Immunoassay to Benzo[a]pyrene-DNA adducts as a prototype. J. Occup. Med. 28, 1117-1123.
- Perera, F.P., Santella, R.M., Brenner, D., Poirier, M.C., Munski, A.A., Fischmann, H.K. and Van Ryzin, J. (1987). DNA adducts, protein adducts, and sister chromatid exchange in cigarette smokers and non-smokers. JNCI, 79, 449.
- Phillips, D.H., Hewer, A. and Grover, P.L. (1986). Aromatic DNA adducts in human bone marrow and peripheral blood leukocytes. Carcinogenesis, 7, 2071 - 2075.
- Phillips, D.H., Hewer, A., Grover, P.L. and Jass, J.R. (1987). In: Intern. Conference on Detection Methods for DNA-Damaging Agents in Man: Applications in Cancer Epidemiology and Prevention, 2-4 Sept., Espoo, Finland.
- Philips, D.H., Hemminki, K., Alhonen, A., Hewer, A. and Grover, P.L. (1988). Monitoring occupational exposure to carcinogens: detection by ^{32}P-postlabelling of aromatic DNA adducts in white blood cells from iron foundry workers. Mutation Research, 204, 531-541.
- Poirier, M.C., Dubin, M.A., and Yuspa, S.H. (1979). Formation and Removal of specific acetyl aminofluorene-DNA adducts in mouse and human cells measured ny radioimmunoassay. Cancer Res., 39, 1377-1381.
- Poirier, M.C., Santella, R., Weinstein, I.B., Grunberger, D. and Yuspa, S.H. (1980). Quantitation of benzo[a]pyrene-deoxyguanosine adducts by radioimmunoassay. Cancer Res. 40, 412-416.
- Poirier, M.C., Lippard, S., Zwelling, L.A., Ushay, M., Kerrigan D., Santella, R.M., Grunberger, D. and Yuspa, S.H. (1982) Antibodies elicited against cis-diaminodichloro-platinum(II)-DNA adducts formed in vivo and in vitro. Proc. Nat. Acad. Sci. (USA), 79,6443-6447.
- Poirier, M.C., Hunt, J.M., True, D.A., Laisher, B.A., Young, J.F. and Beland F.A. (1984) DNA-Adduct formation, removal and persistence in rat liver during one month of feeding 2-acetlyaminofluorine. Carcinogensis, 5, 1591 - 1596.
- Poirier, M.C., Reed, E., Zwelling, L.A., Ozols, R.F., Litterst, C.L. and Yuspa, S.H. (1985). Polyclonal antibodies to quantitate cis diamminedichloroplatinum (II)-DNA adducts in cancer patients and animal models. Environm. Health Perspect., 62, 89.
- Potter, D., Blair, D., Davies, R., Watson, W.P. and Wright, A.S. (1989) Relationship between alkylation of Haemoglobin and DNA in Fischer 344 Rats exposed to ^{14}C-Ethylene Oxide. Arch. Toxicol.,Suppl 13, 254-257.
- Poupko, J.M., Hearn, W.L., Radomski, J.L. (1979). N-glucuronidation of N-hydroxy aromatic amines : a mechanism for their transport and bladder-specific carcinogenicity. Toxicol. Appl. Pharmacol., 50, 479-484.
- Poupko, J.M., Radomski, T., Santella, R.M. and Radomski, R.M. (1983). Organ species and compound specificity in the metabolic activation of primary aromatic amines. J. Natl. Cancer Inst., 70, 1077-1080.
- Prough, R.A. and Moloney, S.J. (1985). Hydrazines, in Bioactivation of foreign compounds. M.W. Anders (ed.), Academic-Press, Inc., Orlando, pp. 433-449.
- Purchase, I.F.H., Kalinowski, A.E., Ishmael, J.E., Wilson, J., Gore, C.W. and Chart, I.S. (1981). Lifetime carcinogenicity study of 1- and 2-naphthylamine in dogs. Br. J. Cancer, 44, 892-901.
- Radomski, J.L. (1979). The primary aromatic amines, their biological properties and structure-activity relationships. Ann. Rev. Pharmacol. Toxicol., 19, 129-157.
- Rahn, R.O., Chang, S.S., Holland, J.M. and Shugart, L.R. (1982). A fluorimetric HPLC assay for quantitating the binding of benzo[a]pyrene metabolites to DNA. Biochem. Biophys. Res. Commun., 109, 262-268.
- Randerath, K., Reddy, M.V. and Gupta, R.C. (1981). ^{32}P-labelling test for DNA damage. Proc. Natl. Acad. Sci. USA, 78, 6126-6129.
- Randerath, E., Agraval, H.P., Weaver, J.A., Bordelon, C. and Randerath , K. (1985). ^{32}P-postlabelling analysis of DNA adducts persisting up to 42 weeks in the skin, epidermis and

dermis of mice treated topically with 7, 12-dimethylbenz[a]anthracene. Carcinogenesis, 6, 1117-1126.

- Randerath, K., Reddy, M.V., Disher, R.M. (1986): Age- and tissue-related PS DNA modifications in untreated rats: detection by ^{32}P-postlabeling assay and possible significance for spontaneous tumor induction and aging. Carcinogenesis, 7, 1615 - 1617.
- Reddy, M.V. and Randerath, K. (1986). Nuclease P1-mediated enhancement of sensitivity of ^{32}P-postlabelling test for structurally diverse DNA adducts. Carcinogenesis, 7, 1543-1551.
- Reddy, M.V., Gupta, R.C., Randerath, E. and Randerath, K. (1984). ^{32}P-postlabelling test for covalent DNA binding of chemicals in vivo: Application to a variety of aromatic carcinogens and methylating agents. Carcinogenesis, 5, 231-243.
- Reed, E., Ozols, R.F., Fasy, T., Yuspa, S.H. and Poirier, M.C. (1986). Biomonitoring of cisplatin-DNA adducts in cancer patients receiving cisplatin chemotherapy. In: Genetic Toxicology of Environmental Chemicals, Part B: Genetic Effects and Applied Mutagenesis Eds: Ramel, C., Lambert, B. and Magnusson, J.; Alan R. Liss, Inc. New York, p. 247.
- Reed, E., Ozols, R.F., Tarone, R., Yuspa, S.H., and Poirier, M.C. (1987). Platinum-DNA adducts in leukocyte DNA correlate with disease response in ovarian cancer patients receiving platinum-based chemotherapy. Proc. natl. Acad. Sci. USA, 84, 5024.
- Richardson, F.C.and Swenberg, J.A. (1987): Evaluating the utility of molecular dosimetry and cell replication in carcinogenic risk assessment. CIIT Activities, 7, 1 - 6.
- Roggers, K.J. and Pegg, A.E. (1977). Formation of O^6-methylguanine by alkylation of rat liver, colon and kidney DNA following administration of 1,2-dimethylhydrazine. Cancer Res., 37, 4082-4087.
- Ross, W.C.J. (1962). "Biological Alkylating Agents". London : Buttterworth.
- Rüdiger, H.W. and Lehnert G. (1988), Toxikogenetik: Grundlagen, Methoden und Bedeutung für die Arbeitsmedizin. ASP Sonderheft, 11, 3-16.
- Saffhill, R., Margison, G,P., O'Connor, P.J. (1985): Mechanisms of carcinogenesis induced by alkylating agents. Biochem. Biophys. Acta, 823, 111 - 145.
- Saffhill, R., Strickland, P.T. and Boyle, J.M. (1982). Sensitive radioimmunoassays for O^6-n-butyldeoxyguanosine, O^2-n-butylthymidine and O^4-n-butylthymidine. Carcinogenesis, 3, 547-552.
- Santella, R.M. (1988) Application of new techniques for the detection of carcinogen adducts to human population monitoring. Mutat. Res. 205, 271-282.
- Santella, R.M., Dharmaraja, N., Gasparro, F.P. and Edelson, R.L. (1985). Monoclonal antibodies to DNA modified by 8-methoxypsoralen and ultraviolet A light. Nucleic Acids Res., 13, 2533-2544.
- Santella, R.M., Lin, C.D and Dharmaraja, N. (1986). Monoclonal antibodies to a benzo[a]pyrene diolepoxide modified protein. Carcinogenesis, 7, 441-444.
- Santella, R.M., Weston, A., Perera, F.P., Trivers, G.T., Harris, C.C., Young, T.L., Nguyen, D., Lee, B.M. and Poirier, M.C. (1988). Interlaboratory comparison of anti-sera and immunoassays for benzo(a)pyrene-diol-epoxide-I-modified DNA. Carcinogens, 9, 1265-1269.
- Scherer E., Van der Laken, C.J., Gwinner, L.M., Laid, R.J., and Emmelot, P. (1981) Modification of deoxyguanosine by chloroethylene oxide. Carcinogenesis, 2, 671-677.
- Segerbäck, D. (1983): Alkylation of DNA and haemoglobin in the mouse following exposure to ethene and ethene oxide. Chem.-Biol. Interactions, 45, 139 - 151.
- Segerbäck, D. (1985): In vivo dosimetry of some alkylating agents as a basis for risk estimation. PhD Thesis, University of Stockholm, Stockholm.
- Segerbäck, D., Calleman, C.J., Ehrenberg, L., Lofroth, G. and Osterman-Golkar, S. (1978): Evaluation of genetic risks of alkylating agents. IV. quantitative determination of alkylated amino acids in haemoglobin as a measure of the dose after treatment of mice with methyl methanesulfonate. Mutat. Res., 49, 71 - 82.
- Shimada, T. and Sato, R. (1978). Covalent binding in vitro of polychlorinated biphenyls to microsomal macromolecules. Biochem. Pharmacol., 27, 585-593.
- Shugart, L. (1985): Quantitating exposure to chemical carcinogens: in vivo alkylation of haemoglobin by benzo(a)pyrene. Toxicology,34, 211 - 220.

- Shugart, L. and Kao, J. (1985). Examination of adduct formation in vivo in the mouse between benzo[a]pyrene and DNA of skin and haemoglobin of red blood cells. Environ. Health Perspect.,62, 223-226.
- Shuker, D.E.G. (1988). Detrermination of N7-methylguanine by immunoassay. Methods for detecting DNA damaging agents in humans: application in cancer epidemiology and prevention. IARC Scientific Publications, 89, 296-300.
- Shut, H.A.J., Daniel, F.B., Schenck, K.M., Loeb, T.R., Stoner, G.D. (1984). Metabolism and DNA adduct formation of 2-AAF by bladder explants from human, dog, monkey, hamster and rat. Carcinogenesis, 5, 1287-1292.
- Sies, H. (ed) (1985). Oxidative Stress, Academic Press, New York.
- Sims, P., Grover, P.L., Swailsand, A., Pal, K. and Hewer, A. (1974). Metabolic activation of benzo(a)pyrene proceeds by a diol expoxide. Nature, Lond., 252, 326-327.
- Singer, B.,and Grunberger, D. (1983): Molecular Biology of Mutagens and Carcinogens. Plenum Press, New York.
- Singer, B., Bartsch, H. (eds.). The role of cyclic nucleic acid adducts in carcinogenesis and mutagenesis. IARC Scientific Publications No.70, IARC, Lyon, 1986.
- Sirover, M.A. and Loeb, L.A. (1976). Infidelity of DNA synthesis in vitro : screening for potential metal mutagens or carcinogens. Science 194, 1434-1436.
- v Sittert, N.J., De Jong, G., Clare, M.G., Davies, R., Dean, B.J., Wren, L.J. and Wright, A.S. (1985). Cytogenetic, immunological, and haematological effects in workers in an ethylene oxide manufactoring plant. Brit. J. Ind. Med., 42, 19 - 26.
- Skipper, P.L., Green, L.C., Bryant, M.S. and Tannenbaum, S.R. (1984): Monitoring exposure to 4-aminobiphenyl via blood protein adducts IARC Scientific Publications, No. 59, pp. 143 - 150.
- Skipper, P.L., Bryant, M.S., Tannenbaum, S.R. and Groopman, J.D. (1986). Analytical methods for assessing exposure to 4-aminobiphenyl based on protein adduct formation. J. Occup. Med., 28, 643-646.
- Snyder, R., Jowa, L., Witz, G. and Rushmore, T. (1987). Formation of reactive metabolites from benzene. Arch. Toxicol. 60, 61-64.
- Sterzel, W., Bedford, P. and Eisenbrand, G. (1985). Measurement of DNA single strand breaks induced in rat liver by N-nitrosodiethanolamine with a continuous flow analyser. J. Cancer Res., 109, A 20.
- Stillwell, W.G., Bryant, M.S. and Wishnok, J.S. (1987). GC-MS analysis of biologically important aromatic amines. Application to human dosimetry. Biomed. Environ. Mass Spectrom, 14, 221 - 227.
- Stowers, S.J. and Anderson, M.W. (1984): Ubiquitous binding of benzo(a)pyrene metabolites to DNA and protein in tissues of the mouse and rabbit. Chem.-Biol. Interaction, 51, 151-166.
- Stowers, S.J. and Marshall, W.A. (1985). Formation and persistance of benzo(a)pyrene metabolite-DNA adducts. Environ. Health Perspect., 62, 31-39.
- Stowers, S.J., Maronpot, R.R., Reynolds, St.H. and Anderson, M.W. (1987). The role of oncogenes in chemical carcinogenesis. Environ. Health Perspect., 75, 81-86.
- Straub, K.M., Meehan, T., Burlingame, A.L. and Calvin, M. (1977). Indentification of the major adducts formed by reaction of benzo(a)pyrene diol epoxide with DNA in vitro. Proc. Natl. Acad. Sci., (US), 74, 5285-5289.
- Strickland, P.T. and Boyle, J.M. (1981). Characterisation of two monoclonal antibodies specific for dimerised and non-dimerised adjacent thymidines in single stranded DNA. Photochem. Photobiol., 34, 595-601.
- Strickland, P.T. and Boyle, Y.M. (1984). Immunoassay of carcinogen-modified DNA, in W.E. Cohn (Ed.), Progress in nucleic acid research and molecular biology, Academic Press, New York, pp. 1-58.
- Stumpf, R., Margison, G.P., Montesano, R., and Pegg, A.E. (1979): Formation and loss of alkylated purines from DNA of hamster liver after administration of dimethylnitrosamine. Cancer Res., 39, 50 - 54.
- Sunderman, F.W. (1988). Mechanistic aspects of nickel carcinogenicity. In: Proceedings of the Eurotox. 88, Munich, in press.

- Svensson, K. and Osterman-Golkar, S. (1987). In vivo 2-oxoethyl adducts in hemoglobin and their possible origin. In: Short-term bioassays in the analysis of complex environmental mixtures V. Ed: S.I. Milton. Plennen Press. New York and London.
- Swain, C.G. and Scott, C.B.(1953). Quantitative correlation of relative rates. Comparison of hydroxide ion with other nucleophilic reagents towards alkyl halides, esters, epoxides and acyl halides. J. Chem. Soc. 75, 141-147.
- Swenberg, J.A., Richardson, F.C., Boucheron, J.A., and Dyroff, M.C. (1985). Relationships between DNA adduct formation and carcinogenesis. Environ. Health. Perspect., 62, 177-183.
- Swenberg, J.A., Richardson, F.C., Tyeryar, L., Deal, F. and Boucheron, J. (1987). The molecular dosimetry of DNA adducts formed by continuous exposure of rats to alkylating heptacarcinogens. Progress exp. Tumor Res., 31, 42-51.
- Talaska, G., Au, W.W., Ward, J.B., Randerath, K., and Legator, M.S. (1987): The correlation between DNA-adducts and chromosomal abberations in the target organ of benzidine exposed, partially-hepatectomized mice. Carcinogenesis 8, 1899 - 1905.
- Tates,A.D., Bernine, L.F., Natarajan, A.T., Ploem, J.S., Cole, J., Green, M.H.L. and Arlett, C.F. (1989) Detection of somatic mutants in man: HPRT mutations in Lymphocytes and Haemoglobin and Mutations in Erythrocytes. Mutat. Res. (in press)
- Tilby, M.J., Styles, J.M., and Dean C.J. (1987). Immunological detection of DNA damage caused by melphalan using monoclonal antibodies. Cancer Res., 47, 1542-1546.
- Tong, W.P., Kirk, M.C. and Ludlum, D.B. (1982). Formation of the cross-linked. 1-(N-3-deoxycytidyl), 2-(N^1-deoxyguanosinyl)-ethane in DNA treated with N,N'-bis (2-chloroethyl)-N-nitrosourea. Cancer Res., 42, 3102-3105.
- Törnqvist, M.; Mowrer, J.; Jensen, S. and Ehrenberg, L. (1986a). Monitoring of environmental cancer initiators through hemoglobin adducts by a modified Edman degradation method. Anal. Biochem., 154, 255 - 266.
- Törnqvist, M., Osterman-Golkar, S., Kantiainen, A., Jensen, S., Farmer, P.B. and Ehrenberg, L. (1986 b). Tissue dose of ethylene oxide in cigarette smokers determined from adduct levels in hemoglobin. Carcinogenesis, 7, 1519.
- Tunek, A., Platt, K.L., Bentley, P., and Oesch, F. (1978) Microsomal metabolism of benzene to species irreversibly binding to microsomal protein and effects of modifications to this metabolism. Mol. Pharmacol. 14, 920 - 929.
- Umbenhauer, D., Wild, C.P., Montesano, R., Saffhill, R., Boyle, J.M., Huh, N., Kirsten, U., Thomale, J., Rajewsky, M.F., Lu, S.H. (1985) O-(6)-methyl-deoxyguanosine in oesophageal DNA among individuals at high risk of oesophageal cancer. Int. J. Cancer, 36, 661.
- Vahakangas, K., Haugen, A. and Harris, C.C. (1985a). An applied synchronous fluorescence spectrophotometric assay to study benzo(a)pyrene-diolepoxide-DNA adducts. Carcinogenesis, 6, 1109-1116.
- Vahakangas, K., Trivers, G., Rowe, M. and Harris, C.C.(1985b) Benzo[a]pyrene diolepexide DNA adducts detected by synchronous fluorescence spectrophotometry. Environ. Health Perspect. 62; 101-104.
- Varmus, H.E. (1984). The molecular genetics of cellular oncogenes. Ann. Rev. Genet., 18, 553-612.
- Vogel, E.W., Blijleven, W.G.H., Kortselius, M.J.H. and Zijlstra, J.A. (1982) A search for some common characteristics of the effects of chemical mutagens in Drosophila. Mutat. Res., 92, 69-87.
- Vogel, E.W., Nivard, M.J.M., Raaymakers-Jansen Verplanke, C.A., van Zealand, A.A. and Zijlstra, J.A. (1986) Alkylation induced mutagenesis in higher eukaryotic systems: Significance of DNA modifications and DNA repair with regard to genetic endpoints. In Genetic Toxicolgy of Environmental Chemicals, Part A: Basic principles and mechanisms of action. Eds Ramel, C., Lambert, B. and Magnusson, J; Liss, New York, pp 219-228.
- Watanabe, P.G., Zempel, J.A., Pegg, D.G., and Gehring, P.J. (1978): Hepatic macromolecular binding following exposure to vinyl chloride. Toxicol. Appl. Pharmacol., 44, 571 - 579.

- Watson, W.P., Crane, A.E., Davies, R., Smith, R.J. and Wright, A.S. (1987b). A post-labelling assay for N-7-(2-oxoethyl)guanine, the principal vinyl chloride-DNA adduct Arch. Toxicol. Suppl. 11, 89-92.
- Watson, W.P. (1987). Post-radiolabelling for detecting DNA damage. Mutagenesis, 2, 319-331.
- Weber, W.W. and Hein, D.W.(1985). N-acetylation pharmacogenetics. Pharmacological Reviews, 37, 25-79.
- West, G.J., West, I.W.-L. and Ward, J. (1982). Radioimmunoassay of a thymidine glycol. Rad. Res., 90, 595-608.
- Whiting, R.F., Stich, H.F. and Korapatinek, D.J. (1979). DNA damage and DNA repair in cultured human cells exposed to chromate. Chem.-Biol. Interactions, 26, 267-280.
- Wiegand, H.J., Ottenwälder, H. and Bolt, H.M. (1984a). Disposition of intratracheally instilled Cr(III) and Cr(VI) in rabbits. Toxicol Lett., 22, 273-276.
- Wiegand, H.J., Ottenwaelder, H., Bolt, H.M.(1984b). The reduction of chromium (VI) to chromium (III) by glutathione : an intracellular redox pathway in the metabolism of the carcinogen chromate. Toxicology, 33, 341-348.
- Wiegand, H.J., Ottenwälder, H. and Bolt, H.M. (1985). Die Chrombestimmung in Humanerythozyten, Arbeitsmed., Sozialmed., Präventmed., 20, 1-4.
- Wild, C.P., Smart, G., Saffhill, R. and Boyle, J.M. (1983). Radioimmunoassay of O^6-methyldeoxyguanosine in DNA of cells alkylated in vitro and in vivo. Carcinogenesis, 4, 1605-1609.
- Wiley, R.A., Hanzlik, R.P. and Gillesse, T.(1979). Effect of substituents on in vitro metabolism and covalent binding of substituted bromobenzenes. Toxicol. Appl. Pharmacol., 49, 249-255.
- Willems, M.I., de Raat, W.K., Baan, R.A., Wilmer, J.W.G.M., Lansbergen, M.J. and Lohman, P.H.M. (1987). Monitoring the exposure of rats to 2-AAF by the estimation of mutagenic activity in excreta, sister-chromatid exchanges in peripheral blood cells and DNA adducts in peripheral blood, liver and spleen. Mutat. Res., 176, 211-223.
- Wogan, G.N., Croy, R.G., Essigmann, J.M., Groopman, J., Thilly, W.G., Skopek, T.R., Liber, H.L. (1979). "Mechanisms of action of aflatoxin B and sterigmatocystin : relationship of macromolecular binding to carcinogenicity and mutagenicity", in : Environmental carcinogenesis, eds. P. Emmelot, E. Kriek, Elsevier: North Holland , Amsterdam, Biomedical Press, 97-121.
- Wong, A., Basrur, P. and Safe, S. (1979). The metabolically mediated DNA damage and subsequent DNA repair by 4-chlorobiphenyl in Chinese hamster ovary cells. Res. Commun. Chem. Pathol. Pharmacol., 24, 543-550.
- Wraith, M.J., Watson, W.P., Eadsforth, C.V., van Sittert, N.J., Törnquist, M. and Wright, A.S. (1988). Methods for detecting DNA-damaging agents in humans: Applications in Cancer Epidemiology and Prevention. IARC Scientific Publications, 89, 271-274.
- Wright, A.S. (1983). Molecular dosimetry techniques in human risk assessment: An industral perspective. In: Proceedings of the Third International Congress on Toxicology, San Diego, USA.
- Yuasa, Y., Srivastava, S.K., Dunn, C.Y., Rhim, J.A., Reddy, E.P. and Aaronson, S.A. (1983). Acquisition of transforming properties by alternative point mutations with c-bas/has human proto-oncogene. Nature, 303, 775-779.
- v Zeeland, A.A., Mohn, G.R., Neuhäuser-Klaus, A. and Ehling, U.H. (1985). Quantitative comparision of genetic effects of ethylating agents on the basis of DNA adduct formation. Use of O^6-ethylguanine as a molecular dosimeter for extrapolation from cells in culture to the mouse. Environ. Health Perspect., 62, 163-169.

Table 1: Examples of Antibodies Produced for the detection of modified DNA Constituents

Acetoxy-N-2-acetylaminofluorene-2'-deoxyguanosine, -8	Baan et al, 1985a
Acetoxy-aminofluorene-2'-deoxyguanosine, -8	Poirier et al, 1979
Aflatoxin B_1-modified DNA	Haugen et al, 1981
Benzo[a]pyrene-modified DNA	Poirier et al, 1980
	Kriek et al, 1984
Butyl-2'-deoxyguanosine, $-O^6$	Saffhill et al, 1982
Butyl-2'-deoxythymidine, $-O^4$	ibid
Butyl-2'-deoxythymidine, $-O^2$	ibid
Diamminedichlorplatinum(II)-DNA, - cis	Poirier et al, 1985
	Fichtinger-Schepman et al, 1985.
Dihydroxy-5,6-dihydrothymidine, -5,6	West et al, 1982
	Leadon and Hanavalt, 1983
DNA, Melphalan-modified	Tilby et al, 1987
DNA, 8-Methoxypsoralen-modified	Santella et al, 1985
DNA, Ultraviolet-modified	Strickland and Boyle, 1981
Ethyl-2'-deoxyguanosine, $-O^6$	Muller and Raewsky, 1980
Ethyl-2'-deoxythymidine, $-O^4$	Adamkiewicz et al, 1982
(2-Hydroxyethyl)-2'-deoxyguanosine, $-O^6$	Ludeke and Kleihues, 1988
Methyl-2'-deoxyguanosine, $-O^6$	Wild et al, 1983
Methyl-2'-deoxythymidine, $-O^2$	Strickland and Boyle, 1984
Isopropyl-2'-deoxyguanosine, $-O^6$	Ludeke and Kleihues, 1988
Methyl-guanine, -N7	Shuker et al, 1988
Cyclic 1, N^2-propano-2'-deoxyguanosine	Foiles et al, 1986

Table 2: Methods for the detection of DNA adducts

Method	Sensitivity[1]	Cost	Time	Remark
Postlabelling	1-10	low	high	only aromatic or bulky adducts
Fluorimetry	1000	low	low	only adducts with strong fluorescence
SFS	300	high	low	only adducts with strong fluorescence
GC/MS	100	high	low	needs derivatisation
Immunoassay	30	low	high	only adducts with known structure

[1] The sensitivity is given in fmol adduct/mg DNA.

Table 3: Methods for the detection of haemoglobin adducts

Method	Sensitivity[1]	Cost	Time	Remark
Fluorimetry	0.01	low	low	only adducts with strong fluorescence
GC/FID	0.05	low	low	only aromatic amines
GC/ECD	0.01	low	low	aromatic amines after derivatisation
GC/MS	0.01	high	high	needs derivatisation
Immunoassay	0.01	low	high	only adducts with known structure, partial degradation of haemoglogin necessary
AAS	0.1	high	low	only metals

[1] The sensitivity is given in nmol adducts/g haemoglobin

Figure 1.

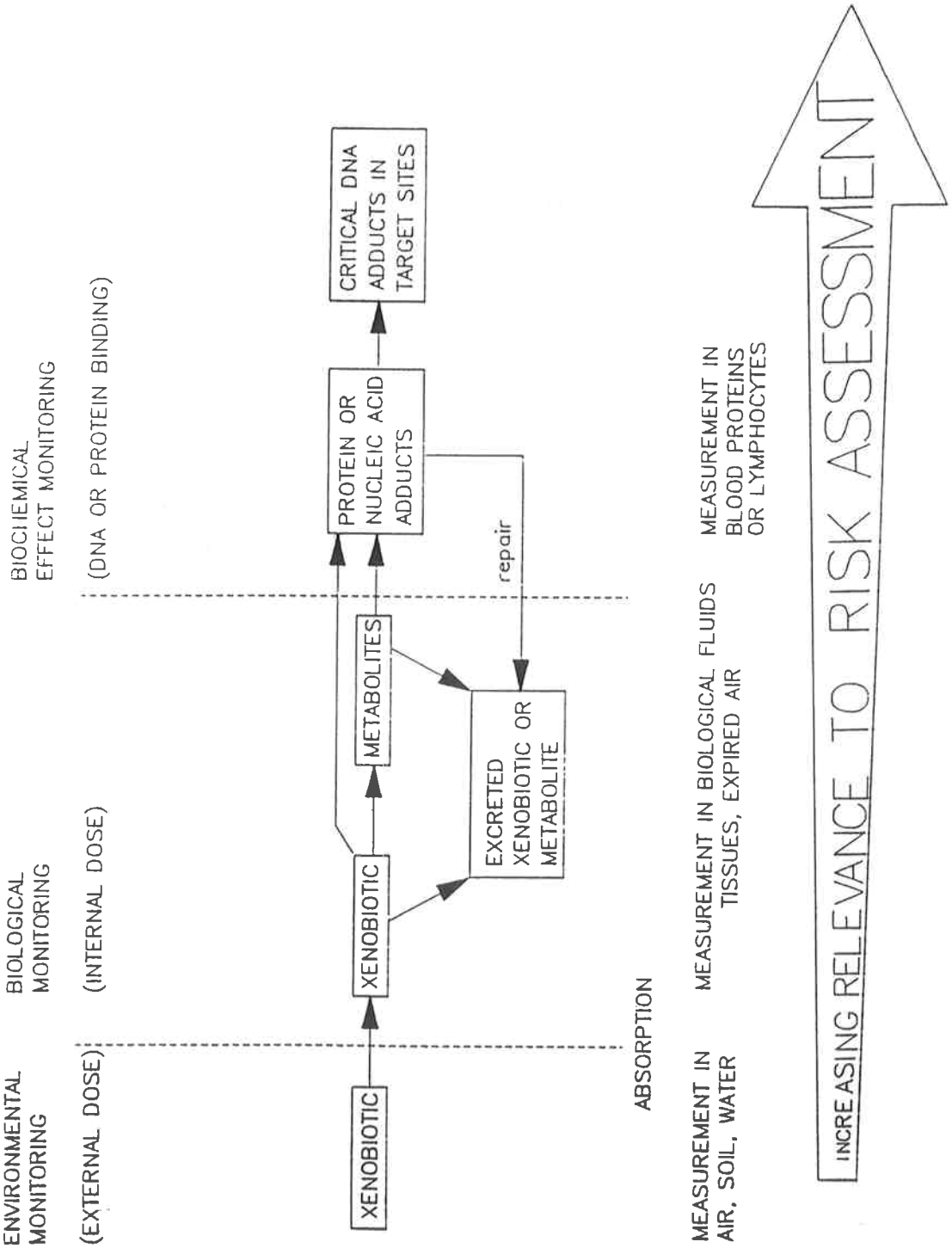

ENVIRONMENTAL MONITORING
(EXTERNAL DOSE)
BIOLOGICAL MONITORING
(INTERNAL DOSE)
BIOCHEMICAL EFFECT MONITORING
(DNA OR PROTEIN BINDING)
XENOBIOTIC
XENOBIOTIC
METABOLITES
PROTEIN OR NUCLEIC ACID ADDUCTS
CRITICAL DNA ADDUCTS IN TARGET SITES
repair
EXCRETED XENOBIOTIC OR METABOLITE
ABSORPTION
MEASUREMENT IN AIR, SOIL, WATER
MEASUREMENT IN BIOLOGICAL FLUIDS TISSUES, EXPIRED AIR
MEASUREMENT IN BLOOD PROTEINS OR LYMPHOCYTES
INCREASING RELEVANCE TO RISK ASSESSMENT

Figure 2.

Examples of organic carcinogens of different chemical classes with a known or strongly suspected electrophilic center (arrow) or chemically reactive derivative (on the right): 1. aziridine; 2. methyl methanesulphonate; 3. 1,2,3,4-diepoxybutane; 4.ß-propiolactone; 5. a sulphur mustard; 6. bis-chloromethylether; 7. dimethylcarbamoylchloride; 8. diazomethane; 9. carbon tetrachloride; 10. a polycyclic aromatic hydrocarbon (the reactive metabolite shown is known from benzo(a)pyrene; 11. benzidine; 12. 2-acetylaminofluorene; 13. 1,2-dimethylhydrazine; 14. N,N-dimethylnitrosamine; 15. aflatoxin B_1. (E = enzymatic activation required).
(Based on Lutz, W.K.(1979) with some additional examples).

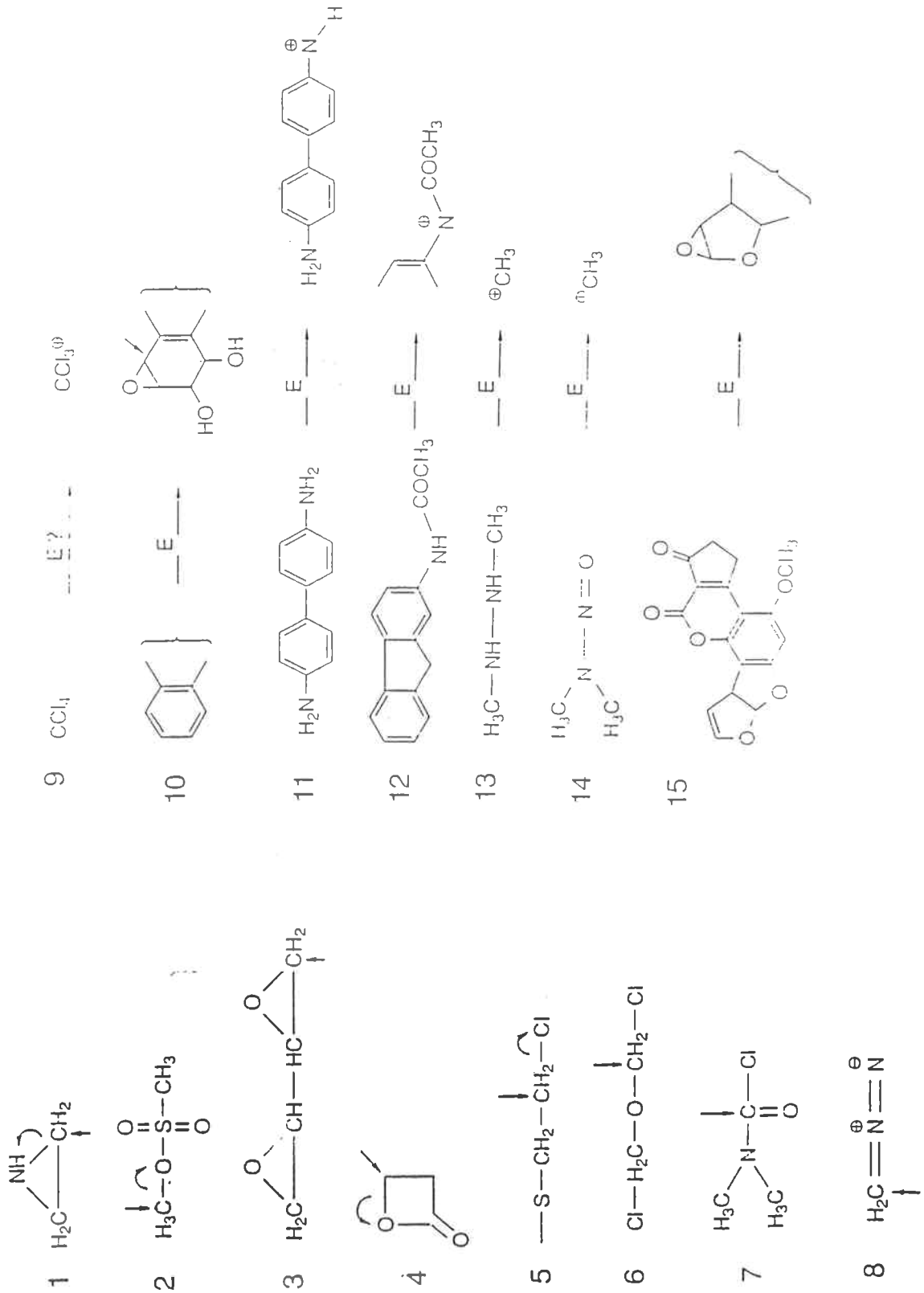
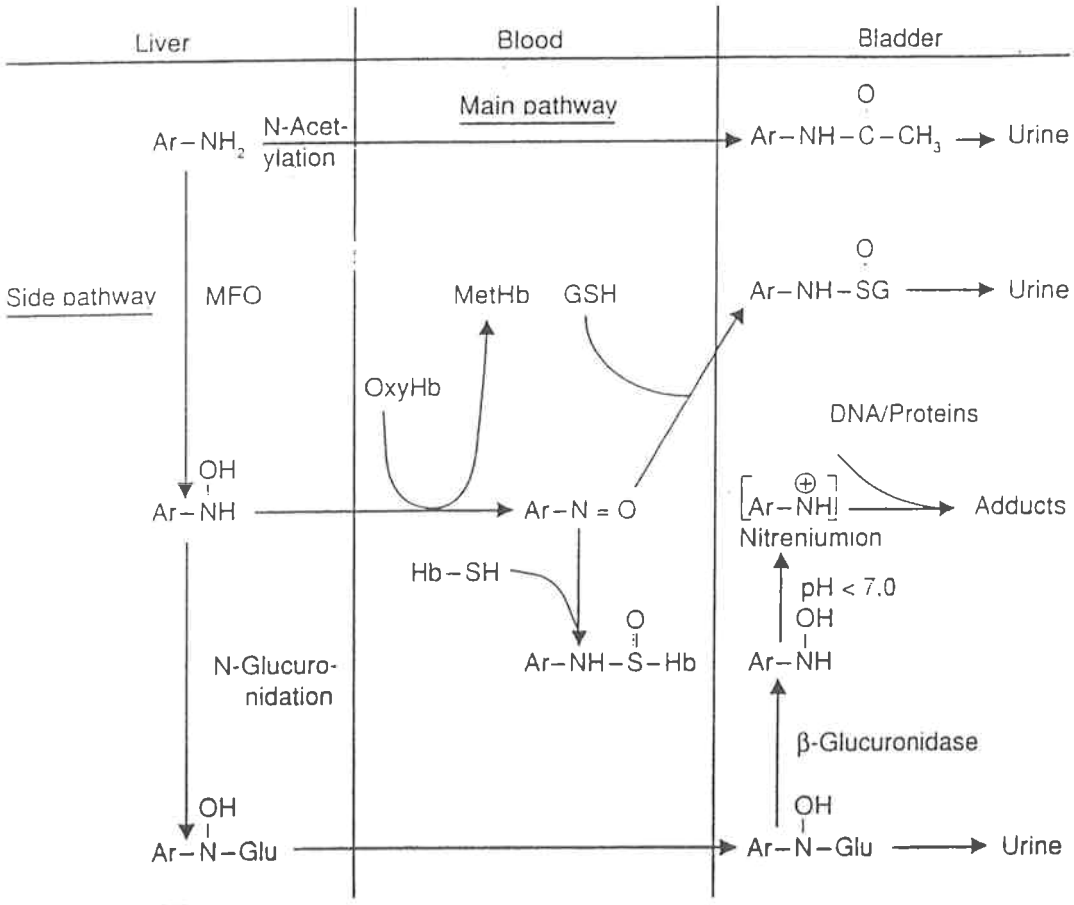


Figure 3.

Metabolism of primary aromatic amines ($Ar-NH_2$): Transportation between compartments and proposed relationship between the steps of detoxication (N-acetylation, N-glucuronidation, GSH-/Hb-conjugation) and steps of intoxication (deglucuronidation, protein- and DNA-adducts in bladder epithelial cells).

Glu = Glucuronide; Hb = Hemoglobin; GSH = Glutathione; MFO = Mixed-Function Oxidase



APPENDIX 1

GLOSSARY OF TERMS

Adduct is the addition product of a reaction between two molecules which involves covalent bonding. In the context of this document, an adduct is the product of a reaction between a chemical agent and macromolecule such as DNA or a protein.

Ambient or Environmental Monitoring(1) is the measurement and assessment of agents at the workplace to evaluate ambient exposure and health risk compared to an appropriate reference.

Biochemical Effect Monitoring is the measurement and assessment of early biochemical effects, eg formation of DNA or Haemoglobin adducts on which the relationship to health has not yet been established. It is a means of measuring tissue dose for which the biological consequences may not be known.

Biological Monitoring(1) is the measurement and assessment of workplace agents or their metabolites either in tissues, secreta, excreta or any combination of these to evaluate exposure and health risk compared to an appropriate reference.

Biological Effect Monitoring(1) is the measurement and assessment of early biological effects, of which the relationship to health impairment has not yet been established, in exposed workers to evaluate exposure and/or health risk compared to an appropriate reference.

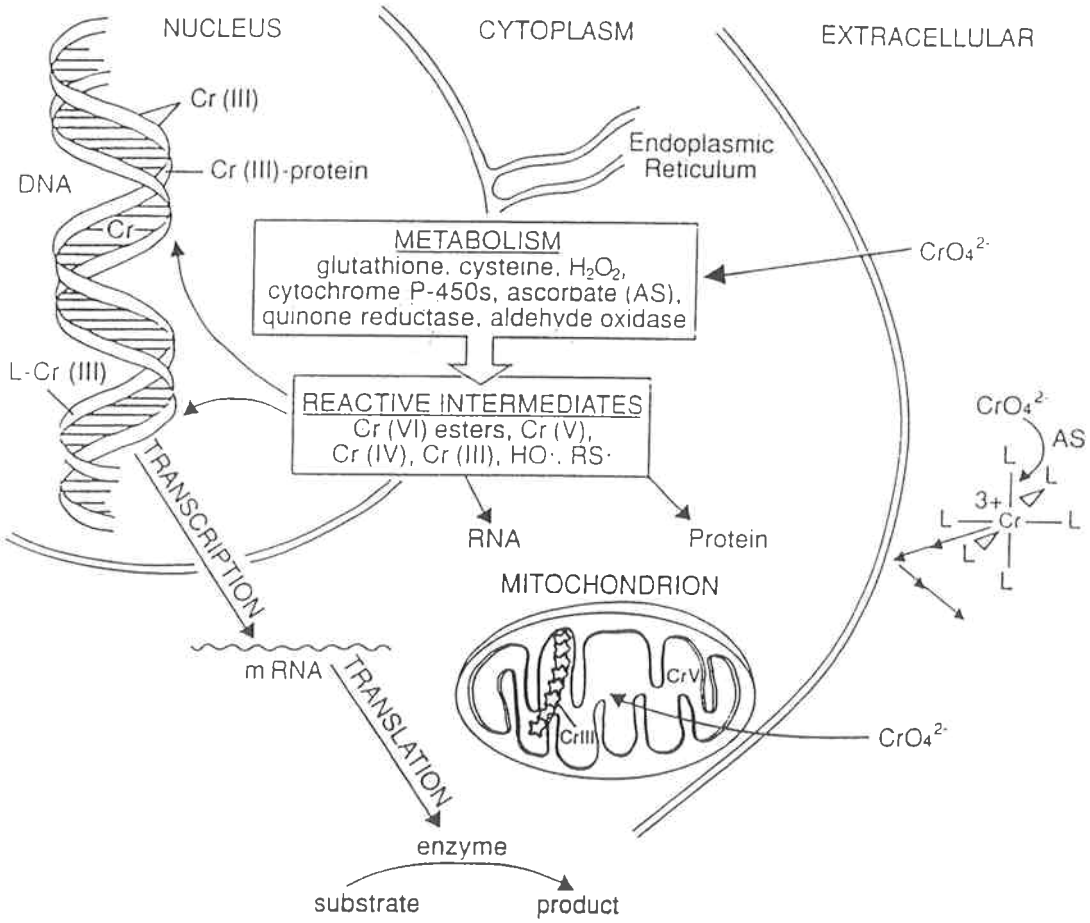
Covalent Bond is a conventional chemical bond between two atoms in which each provides on electron to a shared pair.

External Dose is the amount of a chemical agent in external contact with the organism and thus directly available for absorption into that organism.

Genotoxic Agent is a chemical capable of altering the integrity of DNA.

Figure 4.

The uptake-reduction model for Cr (VI) metabolism: Cr (VI) enters the cell as chromate, using normal cellular anion transport systems,react intracellularly with substrates like GSH, ascorbic acid, cysteine, etc. and enzymes to produce reactive intermediates such as Cr (VI) esters, hydroxyl and thiyl radicals, etc. and stable Cr (III) species.
The Cr (VI)-induced DNA damage in the form of Cr-DNA adducts, etc. disrupt the normal functioning of the DNA as a template in transcription and replication.
(Connett and Wetterhahn, 1983).



Health Surveillance(1) is the periodic medico-physiological examination of exposed workers with the objective of protecting them and preventing occupationally related diseases.

Integrated Dose is the dose received by the organism over a defined period of time.

Internal Dose is the total amount of a chemical agent taken up by the organism. This term includes the amount of a chemical recently absorbed, the amount already stored in the organism (body burden) and the amount of active chemical species bound to the site of action.

Molecular Dosimetry is a means of assessing the internal dose of a chemical agent by measurement of the extent and nature of covalent binding with biological macromolecular such as protein or DNA.

Molecular Lesion is an abnormal transformation of a macromolecule involving covalent bonding and which could ultimately result in adverse biochemical or toxicological consequences for the organism.

Monitoring(1)is a systematic, continuous or repetitive health-related activity, designed to lead if necessary to corrective action.

Target Dose(2) is the dose, expressed as a time integral of concentration, of the ultimate genotoxic agent (which may be systematically generated) which evades metabolic detoxification and penetrates to the biologically significant site in DNA.

Tissue Dose is the dose, expressed as a time intergral of concentration, of a genotoxic agent in any tissue.

Ultimate Mutagen/Carcinogen is an agent capable of direct interaction with DNA. In some cases this could be the chemical agent itself or alternatively it could be a metabolic product of that agent.

REFERENCES

1) - Zielhuis, R.L. and Henderson P.Th. (1986): Definitions of monitoring activities and their relevance for the practice of occupational health. Int. Arch. Occup. Environ. Health, 57, 249-257.

2) - Farmer P.B., Neumann H.G. and Henschler D. (1987) Estimation of exposure of man to substances reacting covalently with macromolecules. Arch. Toxicol., 60, 251-260.

APPENDIX 2

Abbreviations

CEC	Commission of the European Communities
ECETOC	European Chemical Industry Ecology and Toxicology Centre
EPA	Environmental Protection Agency (US)
IARC	International Agency for Research on Cancer
IPCS	International Programme on Chemical Safety
NIEHS	National Institute of Environmental Health Sciences (US)
WHO	World Health Organisation

BAT	Biologische Arbeitsstoff-Toleranz-Werte (Deutsche Forschungsgemeinschaft)
MAK	Maximale Arbeitsplatz-Konzentration (Deutsche Forschungs-gemeinschaft)
TLV	Threshold Limit Value (American Conference of Governmental Industrial Hygienists)
TWA	Time-weighted average

1,2DMH	1,2-dimethylhydrazine
2AAF	2-acetylaminofluorene
2-NA	2-napthylamine
3-MC	3-methylcholanthrene
4-ABP	4-aminobiphenyl
4NQO	4-nitroquinoline-1-oxide
7BrMBA	7-bromo-methylbenzanthracene
7-medGua	N-7-methylguanine
AAF	2-acetylaminofluorene
AABP	4-acetylaminobiphenyl
AB	aminoazobenzene
AFB	aflatoxin B_1
B(a)P	benzo(a)pyrene
BPDE	7ß,8-dihydroxy-(9,10)-epoxy-7,8,9,10-tetahydro-benzo(a)pyrene

BPL	ß-propiolactone
BZ	benzidine
CNU	bis-(chloroethyl)-nitrosourea
trans-DAS	*trans*-4-dimethylaminostilbene
DEHP	di-(2-ethylhexyl)phthalate
DEN	diethylnitrosamine (N-nitrosodiethylamine)
DMAB	N,N-dimethyl-4-aminozobenzene
DMBA	dimethylbenzanthracene
DMN	dimethyl nitrosamine (N-nitrosodimethylamine)
DMS	dimethylsulphate
cis DPP	*cis diamminedichloroplatinum*(II)
EMS	ethyl methanesulphonate
ENU	ethyl nitrosourea
EO	Ethylene Oxide
MAB	N-methyl-4-aminozobenzene
MAM	methylazoxymethanol
MCPP	15,16-dihydro-11-methyl-cyclopenta(a)phenanthren-17-one
MNNG	N-methyl-N-nitro-N-nitrosoguanidine
MNU	methyl nitrosourea (N-methyl-N-nitrosourea)
MMS	methyl methanesulphonate
N-OH-AAF	N-hydroxy-acetylaminofluorene
N-OH-1-NA	N-hydroxy-1-naphthylamine
N-OH-AAPB	N-hydroxy-2-acetoaminobiphenyl
PAH	polycyclic aromatic hydrocarbon
SAM	s-adenosylmethionine

A	adenine
Ac-CoA	acetyl coenzyme A
A-G	adenine-guanine
AP	apurinic/apyridimic site in DNA
ATP	adenosine-5^{1}-triphosphate
C	cytosine
DNA	deoxyribonucleic acid
EtOG	ethenöguanine
G	guanine
G-SH	glutathione

Hb	haemoglobin
Met-Hb	methaemoglobin
MFO	mixed function monooxygenase
N^2-G	N^2-guanine
NADH	reduced form of nicotinamide adenine dinucleotide
NAT	N-acetyl transferase
RNA	ribonucleic acid
T	thymine
3meA	3-methyladenine
7meG	7-methylguanine
O^6-alkG	O^6-alkylguanine
O^4-alkt	O^4-alkylthymine
O^4-EtT	O^4-ethylthymine
O^6-EtG	O^6-ethylguanine
O^6-meG	O^6-methylguanine
O^6-medGua	O^6-methyl-2′-deoxyguanosine
O^6-EtdGua	O^6-ethyl-2′-deoxyguanosine
O^4-EtdThy	O^4-ethyl-2′-deoxythymidine

AAS	atomic absorption spectrometry
CBI	covalent binding index
CHO	Chinese Hamster Ovary
EC	electron capture (detection)
ELISA	enzyme linked immunosorbent assay
GC-MS	gas chromatography-mass spectrometry
HBI	haemoglobin binding index
HPLC	high pressure liquid chromatography
ip	intra-peritoneal
LET	linear energy transfer
N-FID	nitrogen selective flame ionisation detection
NICI	negative ion chemical ionisation mass spectrometry
RIA	radioimmunoassay
SCE	sister chromatid exchange
SFS	synchronous fluorescence spectroscopy
TLC	thin-layer chromatography
USERIA	ultra sensitive enzyme radio immunoassay

APPENDIX 3

MEMBERS OF THE ECETOC TASK FORCE RESPONSIBLE FOR COMPILING THE REPORT

Dr. N.J. van Sittert (Chairman)	SHELL Internationale Petroleum Mij (den Haag)
Dr. M. Chamberlain	UNILEVER (Sharnbrook)
Dr. I. Fleig	BASF AG (Ludwigshafen)
Dr. J. Lewalter	BAYER AG (Leverkusen)
Prof. Dr. H.G. Neumann	Consultant (Würzburg)
Dr. P. Sagelsdorff	Ciba Geigy AG (Basel)
Dr. G. Steel	ICI PLC (Macclesfield)
Dr. H.J. Wiegand	Hüls AG (Marl)

APPENDIX 4

MEMBERS OF THE ECETOC SCIENTIFIC COMMITTEE

I.F.H. PURCHASE, (Chairman), Central Toxicology Laboratory	ICI, Plc, Alderley Park)
M. SHARRATT*, (Vice-Chairman), Group Toxicology Advisor	BP (Guildford)
B. BROECKER, Coordinator, Product-related Environmental Problems	HOECHST (Frankfurt)
H. DE HENAU Professional and Regulatory Services	PROCTER AND GAMBLE (Grimbergen)
H.O. ESSER*, Vice-Director Product Safety	CIBA-GEIGY (Basel)
P. GELBKE, Head, Department of Toxicology	BASF (Ludwigshafen)
P.A. GILBERT, Head, Environmental Relations	UNILEVER (Port Sunlight)
I.J. GRAHAM-BRYCE Head of Environmental Affairs Division	SHELL (The Hague)
Dr. J.R. JACKSON Director, Medicine and Health Science	MONSANTO (Brussels)
C. LUNDBERG, Chief Toxicologist	NOBEL INDUSTRIES (Karlskoga)
R. MILLISCHER, Chief Toxicologist	ATOCHEM (Paris La Défense)
H.G. NOESLER, Head, Coord. Centre For Environmental Protection and Consumer Safety	HENKEL (Düsseldorf)
S. PAGLIALUNGA, Consultant	MONTEDISON (MILAN)
W.F. TORDOIR*, Head of Occupational Health and Toxicology Division	SHELL (den Haag)
H. VERSCHUUREN, Head, Department of Toxicology	DOW CHEMICAL (Horgen)

* indicates Steward Responsibility.