Mutagenesis vol. 19 no. 3 pp. 169–185, 2004 DOI: 10.1093/mutage/geh025

REVIEW

Endogenous DNA damage in humans: a review of quantitative data

Rinne De Bont and Nik van Larebeke[1]

Study Centre for Carcinogenesis and Primary Prevention of Cancer, Department of Radiotherapy, Nuclear Medicine and Experimental Cancerology, Ghent University, Universitair ziekenhuis 4K3, De Pintelaan 185, B9000 Gent, Belgium

DNA damage plays a major role in mutagenesis, carcinogenesis and ageing. The vast majority of mutations in human tissues are certainly of endogenous origin. A thorough knowledge of the types and prevalence of endogenous DNA damage is thus essential for an understanding of the interactions of endogenous processes with exogenous agents and the influence of damage of endogenous origin on the induction of cancer and other diseases. In particular, this seems important in risk evaluation concerning exogenous agents that also occur endogenously or that, although chemically different from endogenous ones, generate the same DNA adducts. This knowledge may also be crucial to the development of rational chemopreventive strategies. A list of endogenous DNA-damaging agents, processes and DNA adduct levels is presented. For the sake of comparison, DNA adduct levels are expressed in a standardized way, including the number of adducts per 10^6 nt. This list comprises numerous reactive oxygen species and products generated as a consequence (e.g. lipid peroxides), endogenous reactive chemicals (e.g. aldehydes and *S*-adenosylmethionine), and chemical DNA instability (e.g. depurination). The respective roles of endogenous versus exogenous DNA damage in carcinogenesis are discussed.

Introduction

DNA damage plays a major role in mutagenesis, carcinogenesis and ageing. The chemical events that lead to DNA damage include hydrolysis, exposure to reactive oxygen substances (ROS) and other reactive metabolites. These reactions are triggered by exposure to exogenous chemicals or they can result from metabolic, endogenous processes. The concentrations and mutagenic potentials of known carcinogens to which we are exposed in our environment are insufficient to explain the high incidence of sporadic cancer that is actually seen in our population (Epe, 2002). Innate factors can also not suffice to explain this high incidence. Epidemiology shows that, in developed societies, exogenous factors are a necessary condition in about 75–80% of cancer cases (Doll and Peto, 1981; Trichopoulos *et al.*, 1994). So, mutations due to DNA damage, caused by unidentified exogenous agents, and to an increase in endogenous damage modulated by exogenous factors must play a role in most cases of cancer, in addition to changes in gene expression due to exogenous conditions. A thorough knowledge of the types and prevalence of endogenous DNA damage can be considered essential for an understanding of the interaction of exogenous agents and influences with endogenous processes in the induction of cancer and other diseases. In particular, this is important for risk analysis concerning low dose environmental factors. Endogenous DNA damage occurs at a high frequency compared with exogenous damage and the types of damage produced by normal cellular processes are identical or very similar to those caused by some environmental agents (Jackson and Loeb, 2001). The study of endogenous damage is also of importance to chemoprevention. It is evident that if an approach could be developed leading to a decrease in endogenous DNA damage and endogenous mutations, the incidence of cancer and other diseases might be substantially reduced, even without a reduction in exogenous mutations.

Here we present a review of quantitative data on the occurrence of endogenous DNA adducts in humans.

Oxidative DNA damage

In living cells ROS are formed continuously as a consequence of metabolic and other biochemical reactions as well as external factors. These ROS include superoxide ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^\bullet$) and singlet oxygen ($^1O_2$) and they can oxidize DNA, which can lead to several types of DNA damage, including oxidized bases and single- and double-strand breaks. DNA damage produced by ROS is the most frequently occurring damage.

Oxidatively modified DNA is, despite extensive DNA repair, abundant in many human tissues, especially in tumours (Iida *et al.*, 2001; Li *et al.*, 2002). Many defence mechanisms within the organism have evolved to limit the levels of reactive oxidants and the damage they induce (Slupphaug *et al.*, 2003). Oxidative stress occurs when the production of ROS exceeds the body's natural antioxidant defence mechanisms, causing damage to macromolecules such as DNA, proteins and lipids. ROS also inactivate antioxidant enzymes (Kono and Fidovich, 1982; Tabatabaie and Floyd, 1994). So, as pointed out by Epe (2002), any change in the endogenous generation of ROS or cellular antioxidants or in the efficiency of DNA repair should cause a corresponding modulation of the steady-state levels of oxidative DNA modifications, which in turn should modulate the mutation rate and ultimately the cancer incidence. Epidemiological evidence from different studies points to reduced risks for cancer, particularly in the upper gastrointestinal tract and airways, associated with a diet rich in antioxidants and/or a high content of antioxidants in plasma samples (Loft and Poulsen, 1996).

Data suggest that the rate of damage decreases with age, possibly along with the decreasing rate of metabolism, whereas the steady-state levels increase due to failing repair (Loft and Poulsen, 1996).

Apart from exogenous substances, the production of ROS occurs through a variety of endogenous processes. During mitochondrial respiration 1–5% of the oxygen undergoes

[1]To whom correspondence should be addressed. Tel: +32 9 240 66 12; Email: nicolas.vanlarebeke@ugent.be

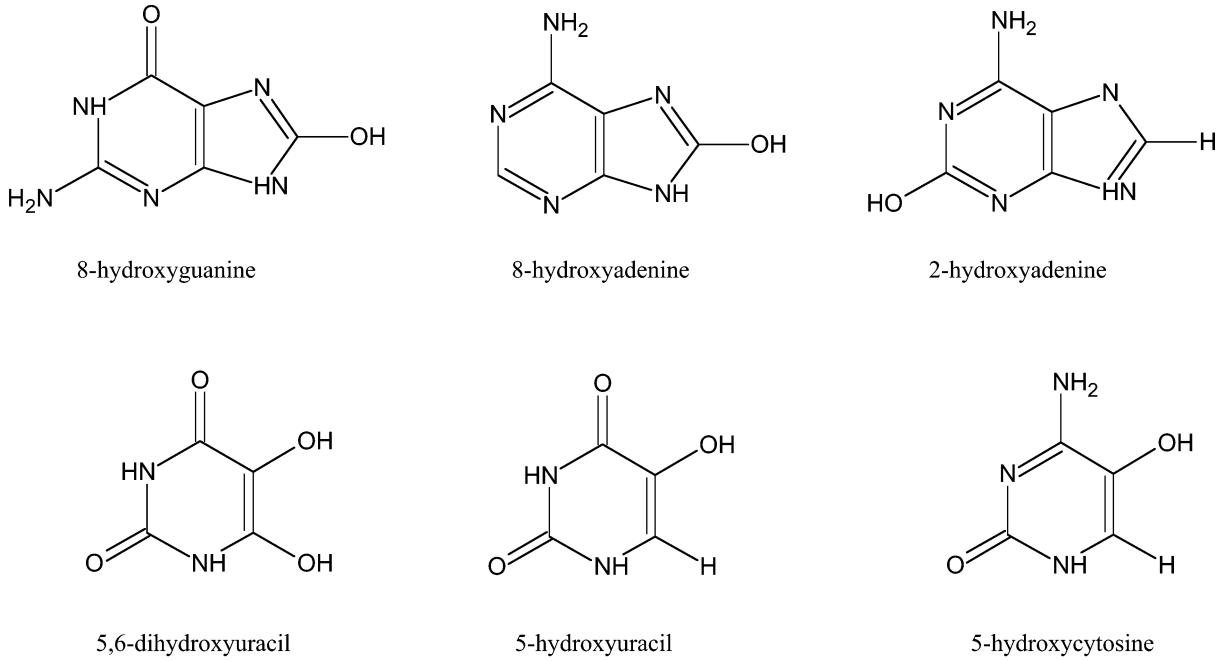


Fig. 1. Some oxidative DNA adducts.

single electron transfer, generating the superoxide anion radical (superoxide, $O_2^{-\bullet}$) (Chance *et al.*, 1979). This molecule shows limited reactivity but is converted to hydrogen peroxide by superoxide dismutase. Reduction of hydrogen peroxide to water is secured by catalase and glutathione peroxidase. However, in the presence of transition metals, such as iron and copper, hydrogen peroxide is reduced to hydroxyl radicals ($HO^{\bullet}$) (Loft and Poulsen, 1996). The reactivity of $HO^{\bullet}$ is so great that it does not diffuse more than one or two molecular diameters before reacting with a cellular component (Pryor, 1986). It must be generated immediately adjacent to DNA to be able to oxidize it. It is likely that H_2O_2 serves as a diffusible, latent form of $HO^{\bullet}$ that reacts with a metal ion in the vicinity of a DNA molecule to generate $HO^{\bullet}$ (Marnett, 2000). This is called the Fenton reaction:

$$H_2O_2 + Fe^{2+} \rightarrow {}^{\bullet}OH + OH^- + Fe^{3+}$$

Chronic infections that elicit an inflammatory response are potent generators of ROS. Neutrophils produce oxygen bursts of superoxide radical and hydrogen peroxide, which can subsequently interact via the Haber–Weiss reaction to form the potent hydroxyl radical (Jackson and Loeb, 2001):

$$O_2^{-\bullet} + H_2O_2 \rightarrow O_2 + OH^{\bullet} + OH^-$$

Shen *et al.* (2000) exposed DNA to activated neutrophils or eosinophils and this resulted in 8-oxo-deoxyguanosine (8-oxo-dG) formation through a pathway that was blocked by antioxidant agents and enzymes. Another DNA oxidant is peroxynitrite (ONO_2^-). It is the product of nitric oxide, a vascular relaxant and neurotransmitter, and superoxide and although it generates small quantities of $HO^{\bullet}$, its protonated form (peroxynitrous acid, ONO_2H) is an extremely reactive oxidant capable of oxidizing DNA independent of its ability to generate $HO^{\bullet}$ (Richeson *et al.*, 1998). Nitric oxide and superoxide are produced simultaneously in macrophages, so it can be assumed that elevated levels of ONO_2^- would be produced in activated inflammatory cells. Ambs *et al.* (1999) demonstrated an association between the occurrence of G→T transversions in the *p53* gene of human colorectal cancers and the level of expression of the inducible form of nitric oxide synthase in gastric precancerous and cancerous lesions. Many studies found a relationship between increased levels of oxidized bases and cancer or inflammatory diseases like hepatitis, cirrhosis or *Helicobacter pylori* infection (Marnett, 2000). Exposure of DNA to ONO_2^- can form strand breaks and base oxidation products and can cause, in some systems, deamination of G and A, but the major product of reaction between DNA and ONO_2^- is 8-nitro-deoxyguanosine (Burcham, 1999). This adduct has a tendency to depurinate. It has also been shown that nitrogen oxide inhibits various DNA repair enzymes, such as FAPY glycosylase, which removes 8-oxo-guanine (8-oxo-G). This suggests that there may exist a synergism between the ability of nitrogen oxide to stimulate DNA damage by the generation of peroxynitrite and the ability to inhibit the repair of this damage (Laval *et al.*, 1997; Jaiswal *et al.*, 2001).

Figure 1 shows some oxidative DNA adducts. Among the oxidatively modified bases, 8-oxo-dG is apparently the most abundant but certainly the most investigated. Steady-state levels of this adduct determined by the various techniques in human cells ranged over several orders of magnitude: between 0.07 and 145.25 adducts/10^6 nt (Higuchi and Lin, 1995; Spencer *et al.*, 1996). It base pairs preferentially with adenine rather than cytosine and thus generates GC→TA transversion mutations after replication. Oxidative DNA damage is predominantly repaired by base excision repair (BER) enzymes. The rate of repair of purine lesions is slower than that of pyrimidine lesions (Jaruga and Dizdaroglu, 1996). Most of the damage is removed before the cell reaches replication, the stage at which damage can be fixed as a mutation. It is evident that variations in repair capability would affect the amount of oxidative DNA damage, and indeed some rare human diseases

characterized by aberrant DNA repair (xeroderma pigmentosum, ataxia telangiectasia, Bloom's syndrome and Fanconi's anaemia) are also characterized by an elevated amount of 8-oxo-dG (Degan *et al.*, 1995; Evans *et al.*, 2000). NO, an inflammatory mediator, directly inhibits a key BER enzyme (hOgg1) responsible for BER of 8-oxo-G. NO-mediated inhibition of DNA repair could thus contribute to the mutagenic environment of chronic inflammation (Jaiswal *et al.*, 2001).

Other abundant oxidatively modified bases are the formamidopyrimidine adducts of adenine and guanine: 4,6-diamino-5-formamidopyrimidine (FapyAdenine) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGuanine) (Burgdorf and Carell, 2002). The latter is especially formed in the absence of oxygen by ionizing radiation and other ROS-producing agents. This lesion is efficiently repaired by a special formamidopyrimidine glycosylase but it can lead to GC→CG transversions (Ono *et al.*, 1995). Other modified adenine bases include 8-oxo-adenine (7,8-dihydro-8-oxoadenine) and 2-hydroxy-2′-deoxyadenosine. Oxidation of 2′-deoxycytidine can lead to the formation of 5-hydroxy-2′-deoxycytosine, 5-hydroxy-2′-deoxyuridine and 5,6-dihydroxy-5,6-dihydro-2′-deoxyuridine (Wagner *et al.*, 1992).

Other oxidized bases are 5-hydroxymethylthymidine, 5-hydroxymethyluracil, uracil glycol, cytosine glycol and thymine glycol. 5-Hydroxyuracil and uracil glycol are products of the oxidative deamination of deoxycytosine (dC). They are detected in comparable amounts to 8-oxo-dG in human DNA (Burcham, 1999). Oxygen radicals react with 5-methylcytosine to oxidize the 5,6-double bond; the intermediate product, 5-methylcytosine glycol, then deaminates to form thymine glycol. Thymine glycol base pairs with A and results in a C→T transition (Marnett and Plastaras, 2001). It is a weak mutagen (Basu *et al.*, 1989) and it blocks transcription and replication (Dianov *et al.*, 2000). This lesion is primarily repaired by the BER pathway (Dianov *et al.*, 2000). Of the pyrimidine-derived lesions, 5-hydroxy-2′-deoxycytosine (5-OH-Cyt) and 5-hydroxyuracil (5-OH-Ura) have been shown to be potentially premutagenic lesions leading to GC→AT transitions and GC→CG transversions (Purmal *et al.*, 1994). 5-OH-Cyt appears to be more mutagenic than any other product of oxidative DNA damage (Feig *et al.*, 1994). 5-Hydroxymethyluracil is generated through oxidation of the methyl group of thymine (Rogstad *et al.*, 2002). This methyl group plays a major role in various DNA–protein interactions. This adduct thus interferes with the binding of transcription factors to DNA (Rogstad *et al.*, 2002). This is a non-mutagenic lesion but it is removed by higher organisms. This removal can trigger apoptosis as a consequence of chromosomal breakage and it can also result in deletion mutations (Rogstad *et al.*, 2002).

Table I summarizes measurements of oxidative DNA adducts in human tissues and Table VI shows the types of mutations that can be induced by different oxidative DNA lesions.

Lipid peroxidation

The polyunsaturated fatty acid residues of phospholipids are extremely sensitive to oxidation. Lipid hydroperoxides are the initial products of unsaturated fatty acid oxidation, but they are relatively short lived. They are either reduced by glutathione peroxidases to unreactive fatty acid alcohols or they react with metals to produce epoxides, aldehydes, etc. The major aldehyde products of lipid peroxidation are crotonaldehyde, acrolein, 4-hydroxynonenal (HNE) and malondialdehyde (MDA). These reactive substances damage DNA by the formation of exocyclic adducts. They block the Watson–Crick base pairing region and most of them are anticipated to be highly mutagenic. MDA appears to be the most mutagenic product of lipid peroxidation, whereas HNE is the most toxic (Esterbauer *et al.*, 1990).

The levels of DNA adducts in rodent and human tissues and leucocytes were found to be highly variable and to be affected by lifestyle, the dietary intake of antioxidants and the type and amount of fatty acids and persistent chronic infections or inflammation, in which nitric oxide is often overproduced (Bartsch, 1999). Consumption of a diet high in polyunsaturated fatty acids (PUFAs) has been shown to cause an increase in propano-, etheno- and malondialdehyde-derived adducts in human leucocyte DNA in women but not in men (Fang *et al.*, 1996; Nair *et al.*, 1997). It could be suggested that etheno and propano adducts are involved in carcinogenesis because they have been detected in target tissues of rodents treated with carcinogens such as vinyl chloride, ethyl carbamate and *N*-nitrosopyrrolidine (Chung *et al.*, 1996). Table II summarizes measurements of lipid peroxidation induced DNA adducts in human tissues.

Propano adducts

These adducts appear to be derived by reaction of DNA with acrolein, crotonaldehyde and HNE, all generated by lipid peroxidation. Crotonaldehyde and acrolein are also widespread in the environment: they are generated by burning fats and by cigarette smoking. Crotonaldehyde can also react with H_2O to form 3-hydroxybutanal, which reacts with DNA to produce the Schiff base N^2-(3-hydroxybut-1-ylidene)dG as well as several diastereomers of N^2-paraldol-dG (Hecht *et al.*, 2001) (Figure 2). HNE is weakly mutagenic but appears to be the major toxic product of lipid peroxidation (Esterbauer *et al.*, 1991). It modulates the expression of genes that are involved in cell cycle control and apoptosis.

Pan and Chung (2002) showed that the rate of acrolein adduct formation is ~5- to 10-fold that of crotonaldehyde adducts, depending on the type of PUFA, and the rate of formation of HNE adducts from arachidonic acid is also considerably slower than that of acrolein adducts. The reactivity of enals toward dG decreases with the increasing chain length acrolein > crotonaldehyde > HNE (Pan and Chung, 2002). The numbers of propano adducts measured in humans ranged between 0.0006 and 0.40 adducts/10^6 nt (Nath and Chung, 1994; Chung, 2000).

Some rodent studies suggest that a higher level of 1,N^2-propano-dG adducts occurs in tissues with a high fat content, such as brain, liver and colon (Nath *et al.*, 1996). Studies in humans showed that intake of dietary fats increases the levels of these adducts in lymphocyte DNA of women (Fang *et al.*, 1996; Nair *et al.*, 1997).

Etheno adducts

The former aldehydes (acrolein, croton aldehyde and HNE) can be converted to epoxyaldehydes by different oxidative processes (Chung *et al.*, 1996). The resulting epoxyaldehydes could modify DNA bases yielding etheno adducts. Epoxyaldehydes are more reactive towards DNA than the parent enals, especially the long-chain enals (Chung *et al.*, 1996). The reaction of epoxyaldehydes of acrolein (glycidaldehyde),

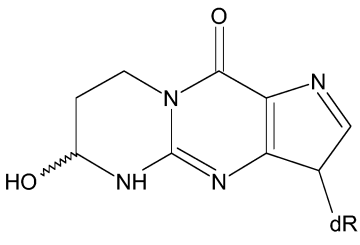
Table I. Oxidative DNA damage

Adduct	Method	Tissue	Quantity		Reference
				Adducts/10^6 nt	
5-Hydroxy-2′-deoxycytidine	HPLC/EC	Leukocytes	3.1 ± 1.6 fmol/μg DNA	1.02	Wagner *et al.* (1992)
	HPLC/EC[a]	Lymphocytes	2.9 ± 1.4 adducts/10^6 dG	0.58	Lenton *et al.* (1999)
	GC/MS/SIM	Bronchial epithelium	0.161 ± 0.005 nmol/mg DNA	52.79	Spencer *et al.* (1996)
5-Hydroxy-2′-deoxyuridine	HPLC/EC	Leucocytes	2.1 ± 1.8 fmol/μg DNA	0.69	Wagner *et al.* (1992)
5,6-Dihydroxy-5,6-dihydro-2′-deoxyuridine	HPLC/EC	Leucocytes	6.2 ± 4.6 fmol/μg DNA	2.03	Wagner *et al.* (1992)
8-Oxo-7,8-dihydro-2′-deoxyguanosine	HPLC/EC	Human leucocytes	3.5 ± 2.1 fmol/μg DNA	1.15	Wagner *et al.* (1992)
	^{32}P/HPLC and HPLC/EC	Human leucocytes	8 adducts/10^5 dG	15.92	Wilson *et al.* (1993)
	HPLC/EC	Leucocytes before versus after smoking	3.3 ± 0.8 versus 5.1 ± 2.5 adducts/10^6 dG	0.66 versus 1.01	Kiyosawa *et al.* (1990)
	HPLC/EC	Leucocytes	3.9 ± 0.26 adducts/10^5 dG	7.76	Degan *et al.* (1995)
	HPLC/EC	PMN leukocytes	118 mol/10^6 mol dG	23.48	Bashir *et al.* (1993)
	GC/MS	Human peripheral lymphocytes	30 adducts/10^5 dG	59.70	Podmore *et al.* (1998)
	HPLC/EC	Lymphocytes	68 mol adducts/10^6 mol dG	13.52	Bashir *et al.* (1993)
	HPLC/EC[a]	Lymphocytes	4.5 ± 1.8 adducts/10^6 dG	0.90	Lenton *et al.* (1999)
	HPLC/EC[a]	Lymphocytes	1.19–2.17 adducts/10^6 dG	0.24–0.43	Bianchini *et al.* (2001)
	HPLC/EC	HeLa cells (mtDNA)	0.35 adducts /10^6 dG	0.07	Higuchi and Lin (1995)
	HPLC/EC	Brain (mtDNA)	15.78–27.34 adducts/10^5 dG	31.40–54.41	Mecocci *et al.* (1993)
	HPLC/EC	Brain (nDNA)	1.25–2.70 adducts/10^5 dG	2.49–5.37	Mecocci *et al.* (1993)
	HPLC/EC	Brain (mtDNA)	15.3–35.7 adducts/10^5 dG	30.45–71.04	Mecocci *et al.* (1994)
	HPLC/EC	Brain (nDNA)	1.20–2.60 adducts/10^5 dG	2.39–5.17	Mecocci *et al.* (1994)
	GC/MS/SIM	Bronchial epithelium	0.443 ± 0.095 nmol/mg DNA	145.25	Spencer *et al.* (1996)
	Fpg/Southern blot	mtDNA	5 adducts/10^6 bases	5.00	Anson *et al.* (2000)
	HPLC/EC	Sperm cells	13 fmol/μg DNA	4.26	Fraga *et al.* (1991)
	HPLC/EC	Pancreas	19 ± 6.1 add adducts/10^8 nt	0.19	Kadlubar *et al.* (1998)
	HPLC/EC	Peripheral blood mononuclear cells from controls	11.1 ± 6.4 add./10^5 dG	22.09	Evans *et al.* (2000)

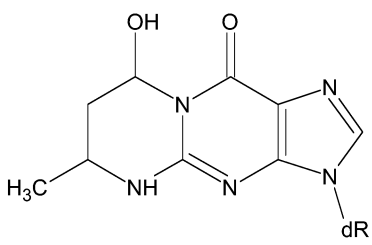
Molecular weight of DNA 327.6 g/mol; human genome = 3.2 × 10^9 bp; 19.9% guanine, 30.2% adenine, 19.6% cytosine, 30.3% thymine (Snustad *et al*., 1997).
HPLC, high performance liquid chromatography; EC, electrochemical detection; GC/MS, gas chromatography/mass spectrometry; SIM, selective ion monitoring; Fpg, formamidopyrimidine glycosylase; ^{32}P, ^{32}P-post-labelling.
[a]Special care was taken to avoid artefactual oxidation.

Table II. Epoxyaldehydes and their etheno adducts

Aldehyde	Epoxyaldehyde	Adduct	Reference
Acrolein	Glycidaldehyde	1,N^2-ethenoguanine	Golding *et al.* (1996)
Crotonaldehyde	2,3-Epoxybutanal	1,N^2-ethenoguanosine	Nair and Offerman (1985)
		1,N^6-ethenoadenosine	
		3,N^4-ethenocytidine	
HNE	2,3-Epoxy-4-hydroxynonanal	1,N^2-ethenodeoxyguanosine	Sodum and Chung (1988)
		1,N^6-ethenoadenine	Sodum and Chung (1991)
		3,N^4-ethenocytidine	Nair *et al.* (1999)
		N^2,3-ethenodeoxyguanosine	



Acrolein-dG



Crotondialdehyde-dG

Fig. 2. Propano adducts.

Table III. Lipid peroxidation derived DNA adducts

Adduct	Method	Tissue	Quantity		Reference
				Adducts/10^6 nt	
M1G	^{32}P/reversed phase HPLC	White blood cells	2.6 ± 1.2 adducts/10^7 nt	0.26	Vaca *et al.* (1995)
	Imafin/GC/EC NCI/MS	Leucocytes	6.2 ± 1.2 adducts/10^8 bases	0.062	Rouzer *et al.* (1997)
	^{32}P/HPLC	Lung	1.00 ± 0.40 adducts/10^7 nt	0.1	Yi *et al.* (1998)
	GC/EC NCI/MS	Pancreas	32 ± 23 adducts/10^8 nt	0.32	Kadlubar *et al.* (1998)
	^{32}P/HPLC	Pancreas	3.58 ± 1.46 adducts/10^7 nt	0.36	Yi *et al.* (1998)
	^{32}P/HPLC	Liver	1.40 adducts/10^7 nt	0.14	Yi *et al.* (1998)
	GC/EC NCI/MS	Hepatic DNA	9 adducts/10^7 bases or 5400 adducts/cell	0.90	Chaudhary *et al.* (1994)
	^{32}P/reversed phase HPLC	Breast tissue	3.0 ± 1.3 adducts/10^7 nt	0.30	Vaca *et al.* (1995)
EdA, EdC	Imafin/32P/TLC	Liver	0–27 adducts/10^9 parent bases	0–0.027	Nair *et al.* (1995)
εdA	GC/NCI/MS/SIM	Placental DNA	2.32 ± 0.02 adducts/10^6 adenine	0.70	Chen *et al.* (1999)
	LC/ESI/MS/MS	Placental DNA	2.48 ± 0.25 εA/10^6 adenine	0.75	Chen *et al.* (1999)
	HPLC/fluorescence assay	Placental DNA	2.77 ± 0.24 εA/10^6 adenine	0.84	Chen *et al.* (1999)
	LC/MS	Placental DNA	1.1 adducts/10^8 normal bases	0.011	Doerge *et al.* (2000)
	Imafin/^{32}P/TLC	Pancreas	1.8 ± 1.3 adducts/10^8 nt	0.018	Kadlubar *et al.* (1998)
εdC	Imafin/^{32}P/TLC	Pancreas	1.2 ± 0.92 adducts/10^8 nt	0.012	Kadlubar *et al.* (1998)
N^2,3-ethenoguanine	LC/ESI/MS	Liver	0.06 ± 0.01 pmol/mg DNA	0.020	Yen *et al.* (1996)
HNE-dG	HPLC/^{32}P/TLC/HPLC[a]	Liver, colon	3–9 nmol/mol guanine	0.0006–0.0018	Chung (2000)
Acrolein-dG (AdG)	^{32}P/HPLC	Gingival tissue non-smokers	0.46 ± 0.26 μmol/mol guanine	0.092	Nath *et al.* (1998)
	^{32}P/HPLC	Gingival tissue smokers	1.36 ± 0.90 μmol/mol guanine	0.27	Nath *et al.* (1998)
	HPLC/^{32}P/TLC/HPLC[a]	Liver	0.3–2.0 adducts/10^6 guanine	0.06–0.40	Nath and Chung (1994)
Crotonaldehyde-dG1	^{32}P/HPLC	Gingival tissue non-smokers	0.06 ± 0.07 μmol/mol guanine	0.012	Nath *et al.* (1998)
	^{32}P/HPLC	Gingival tissue smokers	0.53 ± 0.44 μmol/mol guanine	0.11	Nath *et al.* (1998)
Crotonaldehyde-dG2	^{32}P/HPLC	Gingival tissue non-smokers	0.31 ± 0.40 μmol/mol guanine	0.062	Nath *et al.* (1998)
	^{32}P/HPLC	Gingival tissue smokers	1.72 ± 1.26 μmol/mol guanine	0.34	Nath *et al.* (1998)

Molecular weight of DNA 327.6 g/mol; human genome = 3.2 × 10^9 bp; 19.9% guanine, 30.2% adenine, 19.6% cytosine, 30.3% thymine (Snustad *et al*., 1997). HPLC, high performance liquid chromatography; EC, electrochemical detection; GC/MS, gas chromatography/mass spectrometry; SIM, selective ion monitoring; Fpg, formamidopyrimidine glycosylase; ^{32}P, ^{32}P-post-labelling. Imafin, immunoaffinity purification; NCI, negative chemical ionization; ESI, electrospray ionization; LC, liquid chromatography; TLC, thin layer chromatography.
[a] ^{32}P post-labelling followed by TLC and several HPLC steps.

crotonaldehyde (2,3-epoxybutanal) and HNE (2,3-epoxy-4-hydroxynonanal) give rise to four etheno adducts: 3,N^4-ethenodeoxycytidine (εdC), N^2-3-ethenodeoxyguanosine (N^2-3-εdG), 1,N^2-ethenodeoxyguanosine (1,N^2-εdG) and 1,N^6-ethenodeoxyadenosine (εdA) (Table III and Figure 3). These adducts are also formed from the carcinogens vinyl chloride and urethane (Bartsch and Nair, 2000). N^2,3-εdG levels in liver DNA were similar to levels of propano adducts, ~2 orders of magnitude higher than εdA and εdC (Swenberg *et al.*, 1995). Background ε adduct levels in tissues from humans and rodents were found to be highly variable, which in part may be associated with differences in dietary intake of antioxidants and/or ω-6 PUFAs (Nair *et al.*, 1999).

The ε bases mainly produce base pair substitution mutations. εdA is a highly mutagenic DNA lesion (Palejwala *et al.*, 1993) and can lead to AT→GC transitions, to AT→TA and AT→CG transversions (Pandya and Moriya, 1996; Basu *et al.*, 1999). εdC can lead to CG→AT transversions and CG→TA transitions (Palejwala *et al.*, 1993; Moriya *et al.*, 1994), N^2,3-εdG can lead to GC→AT transitions (Cheng *et al.*, 1991) and 1,N^2-εdG to GC→TA and GC→CG transversions (Langouët *et al.*, 1997). Pandya and Moriya (1996) indicated that the intrinsic mutagenic potency of εdA is comparable to that of εdC in mammalian cells. *In vitro* studies found that 1,N^2-ethenoguanine tends to strongly block replication and generate deletions, rearrangements, double mutants and base pair substitutions (Langouët *et al.*, 1997; Akasaka and Guengerich, 1999). Table VI shows the types of mutations induced by etheno adducts.

Bartsch and Nair (2000) showed that the level of these DNA lesions was increased in conditions resulting in oxidative stress and known to be associated with an increased risk of cancer. Oxygen/nitrogen intermediates generated during inflammatory processes led to the formation of ε adducts, likely through peroxynitrite-mediated lipid peroxidation and/or increased oxidative arachidonic acid metabolism. A high ω-6 PUFA diet increased ε-DNA adducts in white blood cells, particularly in female subjects. Nair *et al.* (1998) showed that NO overproduction *in vivo* is associated with the formation of etheno adducts.

Etheno bases are repaired efficiently by mammalian DNA glycosylases *in vitro* (Bartsch, 1999). εdC is removed by a human mismatch-specific thymine-DNA glycosylase (Hang *et al.*, 1998; Saparbaev and Laval, 1998). 1,N^2-ethenoguanine is a primary substrate of human alkylpurine-DNA-*N*-glycosylase (Saparbaev *et al.*, 2002). It has been shown that the etheno adducts of adenine and cytosine appear to be repaired much more efficiently than the guanine adduct (Dosanjh *et al.*, 1994).

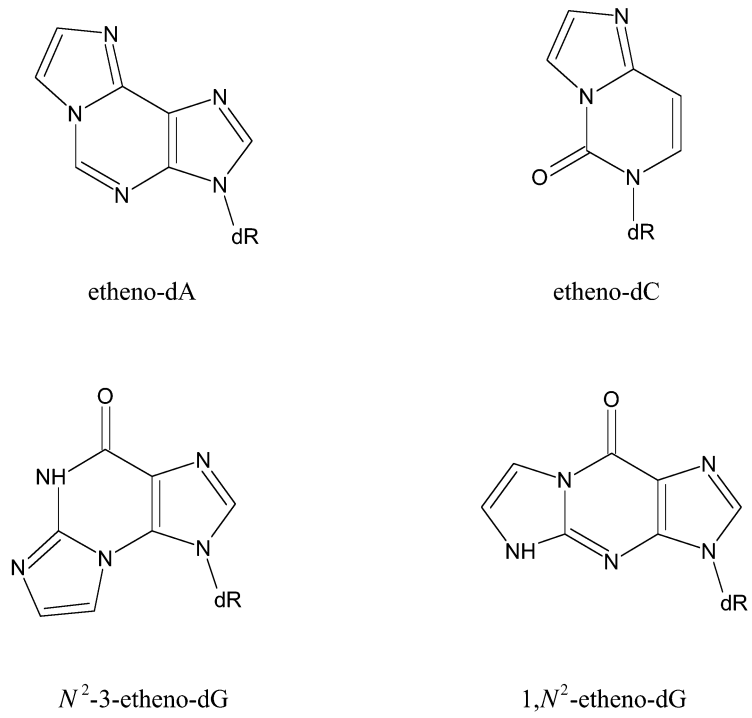


Fig. 3. Etheno adducts.

MDA-induced damage

MDA is a toxic and mutagenic metabolite produced by lipid peroxidation and in the biosynthesis of prostaglandin. It is highly mutagenic in bacterial and mammalian cells and carcinogenic in rats (Basu and Marnett, 1983; Spalding, 1988). MDA reacts with DNA to form adducts of dG [pyrimido[1,2-α]purin-10(3*H*)-one (M1G)] (Figure 4), dA [N^6-(3-oxopropenyl)deoxyadenosine (M1A)] and dC [N^4-(3-oxopropenyl)deoxycytidine (M1C)]. The deoxyguanosine adduct (M1G) has been detected in liver, white blood cells, colon, pancreas and breast from healthy human beings (Marnett, 2002) and levels ranged between 0.062 and 0.9 adducts/10^6 nt (Chaudhary *et al.*, 1994; Rouzer *et al.*, 1997). Comparison of the yields of the various adducts produced in the reaction of MDA with DNA *in vitro* indicates that M1G is produced at roughly five times the amount of M1A while M1C is formed in trace amounts (Marnett, 2002). There is also considerable tissue-to-tissue variation observed in levels of M1G. M1G levels appear to increase with age and the unsaturated fatty acid content of the diet (Marnett, 1999).

Genotoxic substances derived from DNA oxidation: base propenals

Oxidation of DNA and other macromolecules leads to electrophilic mutagenic substances, such as base propenals (Dedon *et al.*, 1998). Direct oxidation of DNA by agents that abstract the 4′-hydrogen atom of the sugar backbone initiates a cascade of reactions that lead to the formation of these base propenals. Kadlubar *et al.* (1998) found a correlation between M1G and 8-oxo-dG, which seems consistent with the hypothesis that M1G is formed primarily by reaction of DNA with a base propenal (Dedon *et al.*, 1998). According to Dedon *et al.* (1998), several lines of evidence suggest that base propenals derived from DNA are a more important source of M1G than lipid peroxide-derived MDA. Indeed, these base propenals arise in immediate proximity to DNA. Plastaras *et al.* (2000) showed that base propenals are 30–150 times more potent than MDA in M1G formation and are 30–60 times more mutagenic than MDA. The order of reactivity is as follows: adenine propenal > cytosine propenal > thymine propenal > MDA (Plastaras *et al.*, 2000). The most common sequence changes induced by MDA were base pair substitutions (see Table VI). Of these, 43% were transversions, most of which were G→T, and 57% were transitions and consisted exclusively of C→T and A→G (Marnett, 1999). These are mutations frequently detected in oncogenes or tumour suppressor genes from human tumours (Marnett, 2002).

Endogenous oestrogens

Several oestrogen metabolites can cause DNA damage directly or indirectly, through redox cycling processes that generate reactive radical species (Yager and Liehr, 1996). In oestrogen-induced carcinogenesis, oestrogen metabolites, particularly catechol oestrogens (CEs), are involved in the initiation process through oxidative DNA damage and oestrogens themselves enhance cell proliferation, leading to tumour promotion (Hiraku *et al.*, 2001). Oestrogen-induced direct or indirect DNA damage *in vitro* or in rodents include single-strand breaks, 8-hydroxylation of guanine bases, bulky DNA adducts (unknown structure), estradiol-induced MDA adducts and oestrogen–DNA adducts (Liehr, 2000).

Oxidation of estradiol (E2) by specific cytochrome P-450 isoforms includes formation of 2,3- and 3,4-catechols. The catechols are inactivated by *O*-methylation mediated by catechol *O*-methyltransferase as well as by glucuronidation and sulphation (Martucci and Fishman, 1993). The metabolic clearance of 4-hydroxy-E2 is slower than that of 2-hydroxy-E2 (Roy *et al.*, 1990).

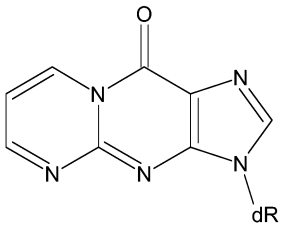


Fig. 4. M1G.

If this inactivation is incomplete, CEs may be oxidized to semiquinones (CE-SQ) and quinones (CE-Q) by redox cycling. This process consists of organic hydroperoxide-dependent oxidation of the CE to the quinone and NADPH-dependent cytochrome P450 reductase-catalysed reduction of the quinone intermediate back to the CE. The semiquinone free radical is an intermediate in each of these metabolic conversions. It may react with molecular oxygen and form quinone and superoxide radicals. Alternatively, non-enzymatic redox couples between copper ions and CEs also generate reactive oxygen radicals. Thus, metal ion-catalysed or enzyme-mediated redox cycling results in the continuous formation of free radicals (Liehr, 2000).

Direct damage

CE-Q is inactivated by conjugation with glutathione or by reduction to CE. If these two inactivating processes are incomplete, CE-2,3-Q reacts with DNA to form stable adducts [N^2-(2-hydroxyestron-6-yl)deoxyguanosine (2-OHE1-6-N^2dG) and N^6-(2-hydroxyestron-6-yl)deoxyadenosine (2-OHE1-6-N^6dA)] that remain in DNA unless repaired (Dwivedy *et al.*, 1992; Stack *et al.*, 1996). The other quinone, estradiol-3,4-quinone and estrone-3,4-quinone (CE-3,4-Q) can react with DNA to form depurinating adducts. These adducts are lost from DNA by cleavage of the glycosidic bond, leaving apurinic sites. CE-Q leads to the formation of following adducts (Li *et al.*, 1998; Stack *et al.*, 1996): 7-[4-hydroxyestron-1(α,β)-yl]guanine [4-OHE1-1(α,β)-N7Gua], 7-[4-hydroxyestradiol-1(α,β)-yl]guanine [4-OHE2-1(α,β)-N7Gua] (Figure 5), 3-[4-hydroxyestron-1(α,β)-yl]adenine [4-OHE1-1(α,β)-N3Ade], 3-[4-hydroxyestradiol-1(α,β)-yl]adenine [4-OHE2-1(α,β)-N3Ade], 2-OHE1-6-N^2dG and 2-OHE1-6-N^6dA. Exposure of DNA *in vitro* to CE-3,4-Q leads to the depurinating adduct 7-[4-hydroxyestron-1(α,β)-yl]guanine or 7-[4-hydroxyestradiol-1(α,β)-yl]guanine [4-OHE1(E2)-1(α,β)-N7Gua] (Cavalieri *et al.*, 1997). The 4-CE that form predominantly depurinating adducts are carcinogenic, whereas the non-carcinogenic 2-CE exclusively form stable DNA adducts (Cavalieri *et al.*, 2000).

Indirect damage

Redox cycling generated by enzymatic reduction of CE-Q to CE-SQ and subsequent autoxidation back to CE-Q by oxygen forms superoxide radicals and hydroxy radicals (Figure 6).

Secondarily, oestrogens can cause macrophage proliferation and activation (Cavalieri *et al.*, 2000). On stimulation, macrophages produce oxidants such as $O_2^{-\bullet}$ and H_2O_2. Furthermore, they produce nitric oxide, which can interact with $O_2^{-\bullet}$ to form peroxynitrite, a very potent oxidant (Cavalieri *et al.*, 2000). Yoshie and Ohshima (1998) have demonstrated that DNA strand breakage is induced synergistically in the presence of both a NO-releasing compound [2-(*N*,*N*-diethylamino)-diazenolate-2-oxide.diethylammonium salt] and a CE.

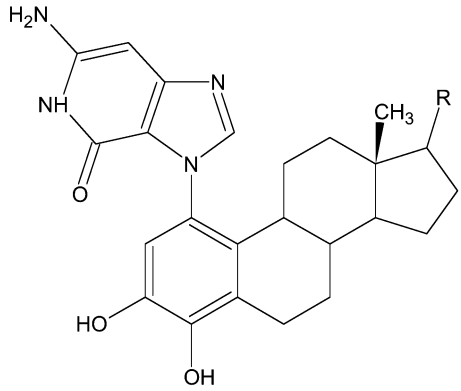


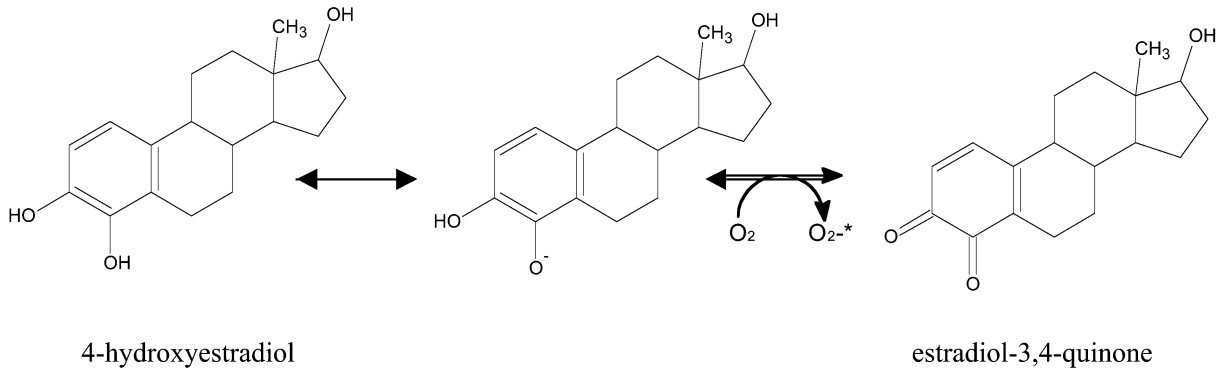
Fig. 5. 4-OHE1(E2)-1-(α,β)-N7Gua (E1, R is =O; E2, R is –OH).

Oestrogens can also affect the function of polymorphonuclear leucocytes (PMNs) resulting in the release of oxidants, including hypochlorite/hypochlorous acid ($HOCl/OCl^-$) (Jansson, 1991; Frenkel, 1992). 2-Hydroxylated oestrogens, however, act as powerful inhibitors of PMN activity, possibly one of the protective properties of the 2-hydroxylated CE.

Endogenous alkylating agents

Besides oxygen, living cells contain several other small reactive molecules that might cause DNA damage. The most important of these is *S*-adenosylmethionine (SAM). It is a reactive methyl group donor contributing to physiological enzymatic DNA methylation, which plays a role in regulation of gene expression (Holliday and Ho, 1998). However, mutagenic adducts can also be formed (Stern *et al.*, 2000). Rydberg and Lindahl (1982) showed that purified DNA can be non-enzymatically methylated by SAM and, as this reaction probably occurs to the same extent *in vivo*, they expect that an intracellular concentration of 4×10^{-5} M SAM generates 4000 7-methylguanine, 600 3-methyladenine and 10–30 O^6-methylguanine residues per day in a mammalian cell. Other endogenous methylation agents are betaine and choline and simple alkylating agents. The latter may be formed endogenously from cellular precursors, but they may also originate from exogenous sources such as diet, tobacco smoke or environmental pollution. Some of these sources may be so common that most humans are exposed to small amounts and it is difficult to apportion the exogenous and endogenous contributions (Zhao *et al.*, 1999). Examples are *N*-nitroso compounds.

Table IV summarizes measurements of alkylated DNA adducts in human tissues. The most frequent effect of DNA methylation is the generation of 7-methylguanine and 3-methyladenine. 7-Methylguanine is relatively harmless, because this modification does not alter the coding specificity of the base. However, destabilization of the glycosyl bond due to N-7 substitution on guanine results in generation of mutagenic apurinic (AP) sites and imidazole ring opening of 7-methylguanine, which results in the stoppage of DNA replication (Barbarella *et al.*, 1991; Tudek *et al.*, 1992). 3-Methyladenine is a cytotoxic DNA lesion that blocks replication. All living cells have an efficient DNA glycosylase that removes 3-methyladenine from DNA, generating an AP site. Atamna *et al.* (2000) showed that the activity of this enzyme decreases with age. 7-Methylguanine is poorly repaired and



4-hydroxyestradiol estradiol-3,4-quinone

Fig. 6. Metabolic redox cycling of catechol oestrogens.

might be expected to accumulate to a measurable degree in the DNA of mammalian cells, although the chemical lability of the 7-methylguanine–deoxyribose bond ensures that a steady-state of base modification and loss would be achieved within a few days. Measurements in humans ranged between 0.028 and 7.2 7-methylguanine/10^6 nt (Shields *et al.*, 1990; Kato *et al.*, 1993) but stayed generally below 1 adduct/10^6 nt. 3-Methyladenine was found in human cells at 0.094 adducts/10^6 nt/day (Rydberg and Lindahl, 1982). SAM also generates the minor pyrimidine lesions 3-methylthymine and 3-methylcytosine. 3-Methylthymine blocks DNA replication *in vivo* (Huff and Topal, 1987) and 3-methylcytosine is a strong inhibitor of DNA synthesis and could be responsible for the mutagenesis observed after cell treatment with alkylating agents (Boiteux and Laval, 1982; Saffhill, 1984).

Some other alkylated and highly mutagenic DNA lesions of endogenous origin are O^6-methylguanine (Figure 7), O^4-methylthymine and O^4-ethylthymine. They can cause GC→AT and TA→CG transitions during DNA replication (Gerchman and Ludlum, 1973; Abbot and Saffhill, 1979; Saffhill, 1985; Singer *et al.*, 1986) (see Table VI). Compared with *N*-alkylation products, however, the detection of *O*-alkylated adducts in human tissues is difficult, because of their low yield and rapid rate of repair (Lindahl, 1982; Pegg, 1983). O^6-methylguanine is repaired by ubiquitous O^6-methylguanine-DNA methyltransferases which demethylate O^6-methylguanine in cellular DNA and transfer the methyl group to one of their active site cysteine residues (Pegg *et al.*, 1995). The mean value of the ratio of O^6-methylguanine to O^4-methylthymine is about six (Kang *et al.*, 1995). Measurements of O^6-methylguanine in humans ranged between 0.0014 and 0.22 adducts/10^6 nt (Shields *et al.*, 1990; Kang *et al.*, 1995) and measurements of O^4-methylthymine ranged between 0.003 and 0.42 adducts/10^6 nt (Kang *et al.*, 1995). They are both formed by *N*-nitroso compounds, potential human carcinogens. Humans are exposed to *N*-nitroso compounds (e.g. *N*-nitrosodimethylamine) through nitrite-treated meat and other nitrate- or nitrite-containing food, in the workplace, from cigarette smoke (Blömeke *et al.*, 1996) and through endogenous formation in the oral cavity, stomach, lungs and by bacteria and macrophages in infected or inflamed organs (Bartsch *et al.*, 1990).

Nitrosated bile salts are carboxymethylating agents which predominantly form N^7-carboxymethylguanine when they react with DNA. N^3-Carboxymethyladenine and O^6-carboxymethylguanine are formed as minor products (O'Driscoll *et al.*, 1999).

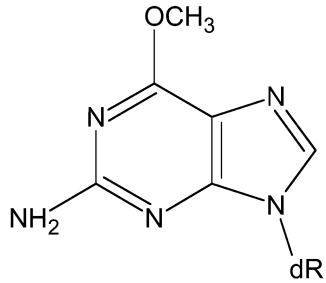


Fig. 7. O^6-methylguanine.

Cadet *et al.* (1999) described the formation of cyclic adducts of purine DNA bases after reaction with two aldehyde compounds, 4,5-dioxovaleric acid and 2,4-decadienal, which are involved in 5-aminolaevulinic acid accumulation and lipid peroxidation.

7-(2-Hydroxyethyl)-guanine adducts are formed by exogenous exposure to ethylene oxide, a known carcinogen, which besides being an industrial chemical is also a component of cigarette smoke. Ethene, which can be metabolically converted to ethylene oxide, is also formed endogenously from several possible sources, including lipid peroxidation of unsaturated fats, oxidation of free methionine, oxidation of hemin in haemoglobin and metabolism by intestinal bacteria (Törnqvist *et al.*, 1989). Measurements in humans ranged between 0.0095 and 1.90 7-(2-hydroxyethyl)-guanine/10^6 nt (Van Delft *et al.*, 1994; Bolt *et al.*, 1997) but lay mostly below 1 adduct/10^6 nt. Kumar and Hemminki (1996) revealed that the level of 7-(2-hydroxyethyl)-guanine was twice the level of 7-methylguanine adducts in total white blood cells, whereas in isolated lymphocytes it was at least four times more than the 7-methylguanine adduct.

In vivo many (but not all) methylated adducts are removed from DNA via the BER pathway, in which the modified bases are recognized by DNA glycosylases, leaving an abasic site (Ye *et al.*, 1998). The resulting abasic sites are potentially mutagenic if left unrepaired. Atamna *et al.* (2000) showed that human leucocytes isolated from old donors possess a reduced activity of glycosylases that remove methylated bases, suggesting a decline in the activity of BER. Zhao and Hemminki (2002), on the other hand, showed that at steady-state the levels of DNA alkylation products are independent of age, suggesting that the ratio between endogenous DNA damage, through

Table IV. Adducts as a consequence of DNA alkylation

Adduct	Method	Tissue	Quantity		Reference
				Adducts/10^6 nt	
7-Methylguanine	^{32}P/TLC/HPLC	White blood cells	2.9 adducts/10^7 nt	0.29	Zhao *et al.* (1999)
	^{32}P/TLC/HPLC	White blood cells smokers with lung cancer	3.3 ± 0.9 adducts/10^7 nt	0.33	Zhao *et al.* (1999)
	^{32}P/AEC	White blood cells smokers	6.9 adducts/10^7 nt	0.69	Mustonen and Hemminki (1992)
	^{32}P/AEC	White blood cells non-smokers	3.4 adducts/10^7 nt	0.34	Mustonen and Hemminki (1992)
	^{32}P/HPLC	White blood cells non-smokers	2.5 adducts/10^7 nt	0.25	Mustonen *et al.* (1991)
	^{32}P/HPLC	Lymphocytes (age ⩽ 50)	1.0 ± 0.9 adducts/10^7 nt	0.10	Zhao and Hemminki (2002)
	^{32}P/HPLC	Lymphocytes (age ⩾ 80)	0.8 ± 0.4 adducts/10^7 nt	0.080	Zhao and Hemminki (2002)
	HPLC/^{32}P	Lymphocytes	5.0–8.3 adducts/10^7 dGp	0.10–0.17	Kato *et al.* (1993)
	^{32}P/AEC	Lymphocytes non-smokers	2.3 adducts/10^7 nt	0.23	Mustonen *et al.* (1993)
	^{32}P/AEC	Lymphocytes smokers	11.5 adducts/10^7 nt	1.15	Mustonen *et al.* (1993)
	^{32}P/AEC	Lymphocytes smokers	23.6 adducts/10^7 nt	2.36	Mustonen and Hemminki (1992)
	^{32}P/AEC	Lymphocytes non-smokers	13.5 adducts/10^7 nt	1.35	Mustonen and Hemminki (1992)
	^{32}P/AEC	Granulocytes smokers	4.7 adducts/10^7 nt	0.47	Mustonen and Hemminki (1992)
	^{32}P/AEC	Granulocytes non-smokers	2.8 adducts/10^7 nt	0.28	Mustonen and Hemminki (1992)
	^{32}P/TLC/HPLC	Lung tissue	4.0 adducts/10^7 nt	0.40	Zhao *et al.* (1999)
	^{32}P/TLC/HPLC	Lung tissue smokers with lung cancer	6.3 ± 1.9 adducts/10^7 nt	0.63	Zhao *et al.* (1999)
	HPLC/^{32}P	Lung tissue	1.4–5.4 adducts/10^7 dGp	0.028–0.11	Kato *et al.* (1993)
	HPLC/^{32}P	Lung tissue smokers	14–72 adducts/10^7 nt	1.40–7.20	Shields *et al.* (1990)
	HPLC/^{32}P[a]	Lung tissue	2.5 ± 2.3 adducts/10^7 dGp	0.05	Blömeke *et al.* (1996)
	^{32}P/AEC	Bronchus smokers	17.3 adducts/10^7 nt	1.73	Mustonen *et al.* (1993)
	^{32}P/AEC	Bronchus non-smokers	4.7 adducts/10^7 nt	0.47	Mustonen *et al.* (1993)
	^{32}P/TLC/HPLC	Bronchus smokers with lung cancer	6.1±1.5 adducts/10^7 nt	0.61	Zhao *et al.* (1999)
	Imafin/HPLC/EC	Pancreas	2–7 pmol/μmol guanosine	0.40–1.39	Bianchini *et al.* (1993)
	Immunoslot blot	Colon mucosa	0.33–1.34 μmol/mol dG	0.066	Harrison *et al.* (2001)
	Immunoslot blot	Rectal mucosa	0.30 μmol/mol dG	0.060	Harrison *et al.* (2001)
O^6-methylguanine	PREPI[b]	Leucocytes	0.7–4.6 adducts/10^8 guanine	0.0014–0.0092	Kang *et al.* (1995)
	PREPI[b]	Liver	1.1–6.7 adducts/10^7 guanine	0.022–0.13	Kang *et al.* (1995)
	HPLC/^{32}P	Lung tissue smokers	1–11 adducts/10^7 dGp	0.020–0.22	Shields *et al.* (1990)
O^4-methylthymine	PREPI[b]	Liver	0.1–14 adducts/10^7 thymine	0.003–0.42	Kang *et al.* (1995)
O^4-ethylthymine	PREPI[b]	Liver	0.5–140 adducts/10^7 thymine	0.015–4.24	Kang *et al.* (1995)
7-(2-Hydroxyethyl)guanine	HPLC/fluorescence assay	Blood cells	2.1–5.8 pmol/mg DNA	0.69–1.90	Bolt *et al.* (1997)
	^{32}P/TLC/HPLC	White blood cells smokers with lung cancer	0.6±0.2 adducts/10^7 nt	0.06	Zhao *et al.* (1999)
	Immunoslot blot	White blood cells	0.65 adducts/10^7 nt	0.065	Van Delft *et al.* (1994)
	Immunoslot blot	WBC smokers	0.11 adducts/10^7 nt	0.011	Van Delft *et al.* (1994)
	Immunoslot blot	WBC non-smokers	0.095 adducts/10^7 nt	0.0095	Van Delft *et al.* (1994)
	^{32}P/HPLC	Lymphocytes (age ⩽50)	3.8 ± 3.4 adducts/10^7 nt	0.38	Zhao and Hemminki (2002)
	^{32}P/HPLC	Lymphocytes (age ⩾80)	3.0 ± 2.7 adducts/10^7 nt	0.30	Zhao and Hemminki (2002)
	^{32}P/TLC/HPLC	Lung samples smokers with lung cancer	0.8 ± 0.3 adducts/10^7 nt	0.08	Zhao *et al.* (1999)
	^{32}P/TLC/HPLC	Bronchus samples smokers with lung cancer	1.0 ± 0.8 adducts/10^7 nt	0.10	Zhao *et al.* (1999)
7-Alkylguanine	^{32}P/AEC	Leucocytes	13.1 adducts/10^7 nt	1.31	Szyfter *et al.* (1996)
	^{32}P/AEC	Larynx	22.7 adducts/10^7 nt	2.27	Szyfter *et al.* (1996)
7-Ethyl-dGp	HPLC/^{32}P	Lymphocytes	0.3–1.4 adducts/10^7 dGp	0.006–0.028	Kato *et al.* (1993)
	HPLC/^{32}P	Lung	0.6–3.1 adducts/10^7 dGp	0.012–0.062	Kato *et al.* (1993)
	HPLC/^{32}P[a]	Lung	1.6 ± 1.7 adducts/10^7 dGp	0.032	Blömeke *et al.* (1996)
7-Methylguanine and 7-(2-hydroxyethyl)-guanine	^{32}P/TLC/HPLC	White blood cells	0.7–1.5 adducts/10^7 nt	0.07–0.15	Kumar and Hemminki (1996)
	^{32}P/TLC/HPLC	Lymphocytes non-smokers	1.1–8.4 adducts/10^7 nt	0.11–0.84	Kumar and Hemminki (1996)
	^{32}P/TLC/HPLC	Lymphocytes smokers	5.6–12 adducts/10^7 nt	0.56–1.20	Kumar and Hemminki (1996)

Molecular weight of DNA 327.6 g/mol; human genome = 3.2 × 10^9 bp; 19.9% guanine, 30.2% adenine, 19.6% cytosine, 30.3% thymine (Snustad *et al*., 1997). HPLC, high performance liquid chromatography; EC, electrochemical detection; GC/MS, gas chromatography/mass spectrometry; SIM, selective ion monitoring; Fpg, formamidopyrimidine glycosylase; ^{32}P, ^{32}P-post-labelling. Imafin, immunoaffinity purification; NCI, negative chemical ionization; ESI, electrospray ionization; LC, liquid chromatography; TLC, thin layer chromatography; AEC, anion exchange chromatography.

[a]Adducts were isolated through two sequential HPLC steps.

[b] ^{32}P-post-labelling, followed by HPLC and immunoprecipitation.

Table V. Abasic sites

Adduct	Method	Tissue	Quantity		Reference
				AP sites/10^6 nt	
Abasic site	Radioactive labelling of purines	Cells	2000–10 000 lesions/cell generation	0.31–1.56 per day	Lindahl and Nyberg (1972)
	ASB[a]	Liver	8–9 lesions/10^6 nt, 50 000–200 000 lesions/cell	8–9	Nakamura and Swenberg (1999)
	ARP/ELISA	Cultured cells, leucocytes	<0.67 lesions/10^6 nt or 4000 lesions/genome	< 0.67	Atamna *et al.* (2000)

Molecular weight of DNA 327.6 g/mol; human genome = 3.2×10^9 bp; 19.9% guanine, 30.2% adenine, 19.6% cytosine, 30.3% thymine (Snustad *et al*., 1997).
ARP, biotin-containing aldehyde-reactive probe; ELISA, enzyme-linked immunosorbent assay.
[a]A combination of an ARP and slot blot techniques.

methylation or lipid peroxidation, and the repair of such damage may not be altered in lymphocytes of older individuals.

DNA hydrolysis

The glycosidic bond between bases and deoxyribose in DNA is labile under certain conditions, such as heating, alkylation of bases or the action of *N*-glycosylases (Lindahl, 1982). Cleavage of the glycosidic bond in DNA leads to an abasic site. AP sites are not only produced by spontaneous depurination but to a large extent also by ROS (Nakamura *et al.*, 2000). Abasic sites are among the most frequent endogenous lesions found in DNA, with an estimated 10 000 lesions/human cell/day (Lindahl, 1993). Nakamura and Swenberg (1999) found levels corresponding to 50 000–200 000 AP sites/genome in many human and rodent tissues. Table V summarizes measurements of AP sites in human tissues. In DNA purines are lost at a 20 times higher rate than pyrimidines (Lindahl and Karlström, 1973).

The number of endogenous AP sites varied widely between tissues but not within tissues (Nakamura and Swenberg, 1999). The brain is the most affected organ, followed by colon and heart, and then liver, lung and kidney (Nakamura and Swenberg, 1999). Atamna *et al.* (2000) showed that the number of AP sites in human leucocytes from old donors was about seven times that in young donors, apparently due to a decline in BER activity.

The major process in the repair of AP sites is the type II AP endonuclease-/β-polymerase-dependent pathway (Dianov *et al.*, 1992). AP sites are repaired rapidly and efficiently (Lindahl, 1993, 1996). Nakamura and Swenberg (1999) found high steady-state levels of AP sites in human liver (8–9 sites/10^6 nt). So the fraction of AP sites that escapes repair is likely to contribute to mutations, chromosome aberrations and transcription errors (Nakamura and Swenberg, 1999).

Typical AP sites induce base pair substitutions (primarily AP site→T) (Gentil *et al.*, 1990; Lawrence *et al.*, 1990) (see Table VI). The 5′-cleaved AP sites might also induce frameshift mutations, such as those detected in microsatellite sequences following treatment of plasmids with H_2O_2 (Jackson *et al.*, 1998). Abasic sites are mutagenic due to the preferential incorporation of adenine opposite abasic sites by DNA polymerases during replication (Jackson and Loeb, 2001).

Hydrolytic deamination

DNA bases are susceptible to hydrolytic deamination, although deamination is much less frequent in double-stranded compared to single-stranded DNA (Lindahl, 1993). Cytosine and its homologue 5-methylcytosine are the main targets (Lindahl, 1993). Between 100 and 500 cytosines/cell/day are deaminated to uracil (Lindahl, 1993). DNA contains a fifth distinct base, 5-methylcytosine, which base pairs with guanine. At CpG sequences it is involved in silencing gene expression (Li *et al.*, 1992). 5-Methylcytosine is a preferred target for spontaneous mutagenesis, primarily because of the fact that 5-methylcytosine can be deaminated three to four times more rapidly than cytosines (Lindahl, 1979) and secondarily because of the different rates of DNA repair. The deaminated form of cytosine is very rapidly excised by the abundant uracil-DNA glycosylase to generate a base-free site, which is efficiently corrected. The GT base pair, formed by deamination of 5-methylcytosine, is a substrate for mismatch correction systems, but these processes are slow (Lindahl, 1993). As a consequence, GC→AT transitions at sites of cytosine methylation account for about one-third of the single site mutations that cause inherited disease in humans (Cooper and Youssoufian, 1988). The same base change is frequent in mutated *p53* tumour suppressor genes found in many human cancers (Rideout *et al.*, 1990). Chen *et al.* (1998) found that cytosine methylation greatly enhances guanine alkylation at all CpG sites in the *p53* gene by many different carcinogens. These findings suggest that mutational hot-spots at methylated CpG sequences in the *p53* gene may in part be a consequence of preferential carcinogen binding at these sites.

In comparison with the deamination of cytosine to uracil, the deamination of DNA purines is a minor reaction. Adenine is converted to hypoxanthine at 2–3% of the rate of cytosine deamination (Karran and Lindahl, 1980). Hypoxanthine pairs with C rather than with T and can thus form a mutagenic lesion. Because of the low cellular level of hypoxanthine-DNA glycosylase, the repair reaction is less efficient than that of deaminated cytosine (Hill-Perkins *et al.*, 1986). The rate of deamination of guanine to xanthine is similar to adenine deamination (Lindahl, 1993). This lesion is less mutagenic because xanthine pairs with C. The xanthine–deoxyribose bond is also susceptible to spontaneous hydrolysis, which can generate an abasic site.

Table VI. Mutation patterns of endogenous DNA lesions

Lesion	Mutation	Reference
Apurinic sites	AT→TA	Lindahl (1996); Jackson and Loeb (2001)
	GC→TA	
	APsite→T	Gentil *et al.* (1990); Lawrence *et al.* (1990)
	Frameshift mutations	Jackson *et al.* (1998)
Cytosine deamination	CG→TA	Lindahl (1996)
5-Methylcytosine deamination	5MeCG→TA	Lindahl (1996); Jackson and Loeb (2001)
8-Oxo-G	GC→TA	Lindahl (1996); Wagner *et al.* (1992)
Thymine glycol	C→T transition	Marnett and Plastaras (2001)
O^6-methylguanine	GC→AT	Lindahl (1996)
O^4-methylguanine	TA→CG	Lindahl (1996)
FapyGua	GC→CG transversion	Ono *et al.* (1995)
5-OH-Cyt	CG→AT transition, GC→CG transversion	Purmal *et al.* (1994)
5-OH-Ura	CG→AT transition, GC→CG transversion	Purmal *et al.* (1994)
1,N^2-(1,3-propano)-2′-deoxyguanosine	G→T transversion	Moriya *et al.* (1994)
εdC	CG→AT transversion	Palejwala *et al.* (1993); Moriya *et al.* (1994); Pandya and Moriya (1996)
	CG→TA transition	Moriya et al. (1994); Pandya and Moriya (1996)
N^2,3-εdG	GC→AT transition	Cheng *et al.* (1991)
1,N^2-εdG	GC→TA transversion	Langouët *et al.* (1997)
	GC→CG transversion	
εdA	AT→GC transition	Basu *et al.* (1993); Pandya and Moriya (1996)
	AT→TA transversion	Pandya and Moriya (1996)
	AT→CG transversion	
M1G	G→T transversion	Marnett (1999)
	C→T transition	
	A→G transition	
Frameshift mutations		
	M1G→A	Fink *et al.* (1997)
	M1G→T	
	M1G→C	

DNA damage by carbonyl stress

Reactive carbonyl species (RCS) are potent mediators of cellular carbonyl stress originating from endogenous chemical processes such as lipid peroxidation and glycation (Roberts *et al.*, 2003). Glycation is a major source of RCS such as glyoxal and methylglyoxal.

Methylglyoxal is a biologically important carbonyl compound. It is a reactive aldehyde endogenously formed as a product of glucose metabolism, but is also found as a constituent of various beverages (coffee) and foodstuffs. It readily forms the cyclic 3-(2′-deoxy-β-D-erythro-pentafuranosyl)-6,7-dihydro-6,7-dihydroxy-6-methylimidazo[2,3-*b*]purine-9(8*H*)one adduct (MG-3′-dGMP) in human buccal epithelial cells. Vaca *et al.* (1998) measured 0.26 MG-3′-dGMP adducts/10^6 nt.

Decomposition products of lipid hydroperoxides include the reactive aldehyde glyoxal. It can also be formed by oxidative deoxyribose breakdown or autoxidation of sugars, such as glucose, and it plays a role in the pathophysiology of diabetes and ageing (Abordo *et al.*, 1999). It readily forms DNA adducts, generating potential carcinogenic adducts such as glyoxalated deoxycytidine (Mistry *et al.*, 2003) and it has been shown to be mutagenic in bacterial and mammalian cells (Murata-Kamiya *et al.*, 1997). Awada and Dedon (2001) reported that 2-phosphoglycolaldehyde, formed by oxidation of the 3′-position of deoxyribose, reacts with dG to form 1,N^2-glyoxal adducts. The formation of glyoxal-dG adducts occurs almost five orders of magnitude more slowly with 2-phosphoglycolaldehyde than with glyoxal.

Elevated tissue concentrations of RCS are observed in pathological conditions such as diabetes and in skin upon solar irradiation (Mizutari *et al.*, 1997; Beisswenger *et al.*, 1999). Acute treatment of cultured human keratinocytes with glyoxal resulted in pronounced DNA strand breaks, whereas methylglyoxal treatment resulted in cross-linked DNA (Roberts *et al.*, 2003).

Conclusion

One of the major endogenous sources of DNA damage is that produced by ROS and the most studied oxidative DNA adduct is 8-oxo-dG. Aldehydes derived from lipid peroxidation form another threat to DNA. Adducts formed are etheno-, propano- and malondialdehyde-derived DNA adducts. Several oestrogen metabolites can cause DNA damage directly or indirectly, through redox cycling processes that generate reactive radical species (Yager and Liehr, 1996). In addition to oxygen and lipid peroxidation products, living cells contain several other small reactive molecules that might cause DNA damage, like methylating agents. The main adducts formed are 7-methylguanine, 3-methyladenine, O^4-methylthymine, O^6-methylguanine and 7-(2-hydroxyethyl)guanine, of which O^6-methylguanine is the most mutagenic. Another, pathway for DNA damage is attack by water, causing hydrolysis of the glycosylic bond and resulting in the formation of mutagenic abasic sites in DNA. DNA bases are also susceptible to hydrolytic deamination. A last pathway for endogenous DNA damage is that caused by carbonyl stress.

The adduct level data concerning some of these processes show a wide range covering several orders of magnitude. In some cases, at least part of this wide range might be due to differences in analytical methods. Generally, for the quantification of oxidative DNA adducts, GC/MS gives high values, HPLC intermediate and enzyme-based methods low values (Collins *et al.*, 1997). We compared the levels of 8-oxo-dG measured in human lymphocytes by GC/MS (59.70 adducts/10^6 nt) with those measured by HPLC/EC (0.24–13.52 adducts/10^6 nt) (see Table I). GC/MS clearly gives higher values. Oxidation may occur during DNA isolation and hydrolysis before HPLC or GC-MS; derivatization procedures for GC-MS can lead to the oxidation of normal unaltered guanine resulting in a spuriously high yield of 8-oxo-G (Wagner *et al.*, 1992; Collins *et al.*, 1997). However, this artificial oxidation may be prevented by, for example, prepurifying the compounds of interest or by derivatization at low temperature or by addition of antioxidants to the silylating reagents (Cadet *et al.*, 1999). HPLC, on the other hand, may underestimate the adduct levels due to incomplete enzymatic digestion of the DNA in the standard procedure (Schuler *et al.*, 1997). Enzymatic methods to convert oxidative lesions to breaks might underestimate adducts as there may be some adducts that are inaccessible to the enzyme. On the other hand, an enzyme such as Fpg might recognize additional (unknown) base modifications, which might lead to an overestimation of the number of 8-oxo-G adducts (Collins *et al.*, 1997). However, Anson *et al.* (2000) found that the FAPY glycosylase (Fpg) assay detected ~90% of the 8-oxo-dG detected by HPLC/EC. For the quantification of etheno adducts, the ^{32}P-post-labelling assay is probably one of the most sensitive methods available. However, quantification by this method is compromised by relatively high variability due to multiple steps and lack of an internal standard (Chung, 2000). Quantification, including immunoaffinity chromatography for adduct enrichment, can be poorly reproducible due to batch-to-batch preparation of antibody and ageing of antibody.

A number of techniques have been developed to detect alkyl DNA adducts, including immunological assays, fluorescence techniques, mass spectrometry, ^{32}P-post-labelling and electrochemical detection. When ^{32}P/HPLC was compared with GC/MS in quantifying 7-(2-hydroxyethyl)guanine from *in vivo* samples, a good qualitative agreement between methods with a correlation coefficient of 0.97 was obtained (Eide *et al.*, 1999).

It should be taken into account that over the years, the different techniques have been adjusted to avoid artefacts. Bianchini *et al.* (2001) and Lenton *et al.* (1999), for example, took measurements to avoid artefactual DNA oxidation during HPLC/EC.

Endogenous DNA damage occurs at a high frequency compared with exogenous damage (Jackson and Loeb, 2001). The high prevalence of endogenous DNA damage corresponds with highly efficient repair of such damage, necessary for cell survival (Wood, 1996; Ochs *et al.*, 1999). Most endogenous DNA damage is repaired by BER, O^6-methylguanine DNA-methyltransferase (MGMT) and mismatch repair (Pegg *et al.*, 1995; Seeberg *et al.*, 1995; Prolla, 1998). The spontaneous dissociation of bases, a frequent form of endogenous DNA damage that can lead to AP sites, is efficiently repaired by BER (Frosina *et al.*, 1994). O^6-methylguanine is repaired very efficiently by the direct repair enzyme MGMT. In spite of efficient repair, most mutations arise endogenously. Of particular significance for the thesis that most mutations in normal cells are of endogenous origin is the great similarity between the mutational spectra of *HPRT* genes in the lymphocytes of normal individuals from different population groups (Burkhart-Schultz *et al.*, 1996; Podlutsky *et al.*, 1998). Also, the mean mutation frequency of a given gene locus (e.g. *HPRT*) in a given cell type for a given age group is approximately the same in diverse Western countries (Robinson *et al.*, 1994). The same applies for different subgroups of the population in the same country (Tates *et al.*, 1991). This suggests that the mutations are mainly due either to endogenous mechanisms of mutagenesis or to ubiquitous environmental influences.

Adduct levels were reported to vary according to gender, ethnicity and individual characteristics. Thus, for gender only women have been reported to have increased levels of DNA adducts arising from lipid peroxidation products in white blood cell DNA following consumption of PUFAs (Fang *et al.*, 1996; Nair *et al.*, 1997). This also holds true for exogenous adduct levels, as female smokers have been reported to have higher bulky DNA adduct levels in lung DNA than do male smokers (Rydberg *et al.*, 1994). Mean 8-oxo-dG levels ranged from 0.1 to 40 adducts/10^5 dG residues and lower levels were observed in the Japanese population, as compared with western populations (Povey, 2000). 8-Hydroxyguanine may be formed as a result of both endogenous and exogenous processes, so these differences may reflect environmental and/or genetic factors or variations in analytical and sampling techniques (Povey, 2000). Also, pronounced interindividual variations in the activity of enzymes that participate in the activation and inactivation pathways of carcinogen metabolism and in DNA repair pathways have been described (Autrup *et al.*, 1986; Perera, 1996). A similar variability is expected concerning the reactions of endogenous genotoxic agents with DNA and repair of the lesions. Interindividual differences in the levels of endogenous DNA damage and risk of spontaneous tumour formation could therefore be expected (Lutz, 1990).

Some endogenous genotoxic substances are formed by more than one pathway. When considering chemopreventive strategies it might be important to know which metabolic pathway predominates in generating a given substance. An example is the reactive aldehyde glyoxal. It is a decomposition product of lipid hydroperoxides, but it can also be formed by oxidative deoxyribose breakdown or autoxidation of sugars. In addition, there are specific adducts that can be generated by different genotoxic substances. The M1G adduct, for example, can be formed by MDA as well as by base propenals. That we now know that M1G is primarily formed by base propenals rather than by MDA (Dedon *et al.*, 1998) might be relevant to our thinking on chemoprevention.

Distinguishing between endogenous and exogenous DNA damage and determining the relative importance of both is a complex matter. Some exogenous mutagens also occur endogenously. Secondly, some exogenous substances, although chemically different from endogenous ones, generate the same DNA adducts. An example of adducts formed by exogenous mutagens that also occur endogenously are the exocyclic adducts formed by the reactive aldehydes acrolein and crotonaldehyde. These adducts are not only attributable to endogenous exposure, because acrolein and crotonaldehyde also occur exogenously as environmental contaminants. Methylglyoxal is another substance that is formed endogenously but can also be found in various foods and beverages. Vinyl chloride is an occupational and environmental pollutant that gives rise to 1,N^6-ethenoadenine and 3,N^4-ethenocytosine,

adducts that are also formed endogenously by the products of lipid peroxidation. Smoking increases the levels of DNA adducts that also arise spontaneously. The amount of 8-oxo-dG appeared higher in human leucocytes after smoking (1.01 versus 0.66 adducts/10^6 nt) (Kiyosawa *et al.*, 1990). Nath *et al.* (1998) measured higher acrolein-dG and crotonaldehyde-dG levels in gingival tissue of smokers than in that of non-smokers (0.27 versus 0.091 and 0.11 versus 0.012 adducts/10^6 nt, respectively). The methylated DNA adduct 7-methylguanine also occurred in higher amounts in different tissues of smokers (bronchus, lymphocytes, white blood cells and granulocytes) (Mustonen and Hemminki, 1992; Mustonen *et al.*, 1993). In assessing the risks associated with exposure to certain exogenous DNA-damaging agents the fact that they generate adducts of a type that is also generated by endogenous substances has to be taken into account. Indeed, exposure to such an agent can never take place in a 'very low dose' context as there is the background of endogenous exposure. An increase in the amount of this type of damage will probably induce a proportional biological effect.

With respect to the endogenous versus exogenous origin of mutations, it is worth noting that methylated CpG sequences, besides being involved in spontaneous mutagenesis processes, can also create preferential targets for environmental mutagens and carcinogens (Denissenko *et al.*, 1997, 1998; Shen *et al.*, 2000).

It seems likely that certain less frequent exogenous DNA lesions may have unexpectedly large biological effects due to their relatively inefficient removal by DNA repair. Exogenous DNA damage differs from the endogenous form in that it more often entails bulky DNA adducts that are removed preferentially by nucleotide excision repair (Sancar, 1995), a more complex and slower DNA repair process (Cox and Lane, 1995). This pathway also repairs dipyrimidine photoproducts, which are formed on DNA by UV light. Double-strand breaks often arise by the action of exogenous agents. Double-strand breaks are repaired in mammals by homologous recombination and to a large extent by error-prone non-homologous end-joining (Shinohara and Ogawa, 1995; Strathern *et al.*, 1995; Henle and Linn, 1997; Vamvakas *et al.*, 1997; Critchlow and Jackson, 1998).

As argued above, most mutations arise spontaneously. In tumour cells, the mutations which can be specifically ascribed to exogenous agents represent a minority of all mutations. Only in a limited number of instances was a significant correlation found between the mutational spectra of tumour cells and the type of exogenous carcinogenic agent, e.g. aflatoxin B_1 (Prieto-Alamo *et al.*, 1996; Lutz *et al.*, 1998). These were instances in which the relative risk of cancer increased significantly (typically by more than 10 times) following exposure to exogenous agents, such as skin cancer caused by UV light (Nakazawa *et al.*, 1994), lung cancer due to cigarette smoking and bladder cancer due to aromatic amines (Lutz *et al.*, 1998). It must be emphasized that the association between exposure to exogenous agents and relative cancer risk does not necessarily imply that these agents have initiated carcinogenesis. These exogenous agents might well act as a necessary condition, and thus be considered as a cause of cancer, by acting at a later stage, contributing to accumulation of mutations or to tumour promotion after initiation has taken place. Initiated cells can be assumed to be more susceptible to the mutagenic action of exogenous agents. (Deman and van Larebeke, 2001) which then contribute to the continuing accumulation of mutations leading to malignant transformation and further progression. This is corroborated by the following observations. (i) Mutational spectra in the *HPRT* gene of normal lymphocytes from different human populations are similar. This suggests that they are predominantly due to endogenous mechanisms of mutagenesis (Podlutsky *et al.*, 1998). (ii) Mutation spectra of the *p53* oncogene in cancer cells from different types of tumours (e.g. lung versus brain) show substantial differences, reflecting the fact that they have partly been caused by different exogenous agents (Lutz *et al.*, 1998). Deman and van Larebeke (2001) hypothesized that the most probable course of events in carcinogenesis is as follows. (i) Because of the low contribution of exogenous agents to the mutation rate of normal cells, initiation is expected to be chiefly due to endogenous causes. The exception might be a situation of acute excessive exposure to a carcinogenic agent. (ii) The mutation which brings about the enhancement of the mutation rate responsible for initiation is more often one which enhances the susceptibility to exogenous mutagenic agents than the susceptibility to endogenous causes of mutations. (iii) The same applies for subsequent mutations which further enhance the mutation rate in initiated cells.

References

Abbott,P.J. and Saffhill,R. (1979) DNA synthesis with methylated poly(dC-dG) templates. Evidence for a competitive nature to miscoding by O^6-methylguanine. *Biochim. Biophys. Acta*, **562**, 51–61.

Abordo,E.A., Minhas,H.S. and Thornalley,P.J. (1999) Accumulation of α-oxoaldehydes during oxidative stress: a role in cytotoxicity. *Biochem. Pharmacol.*, **58**, 641–648.

Akasaka,S. and Guengerich,F.P. (1999) Mutagenicity of site-specifically located 1,N^2-ethenoguanine in Chinese hamster ovary cell chromosomal DNA. *Chem. Res. Toxicol.*, **12**, 501–507.

Ambs,S., Bennett,W.P., Merriam,W.G., Ounfusika,M.O., Oser,S.M., Harrington,A.M., Shields,P.G., Felley-Bosco,E., Hussain,S.P. and Harris,C.C. (1999) Relationship between p53 mutations and inducible nitric oxide synthase expression in human colorectal cancer. *J. Natl Cancer Inst.*, **91**, 86–88.

Anson,R.M., Hudson,E. and Bohr,V.A. (2000) Mitochondrial endogenous oxidative damage has been overestimated. *FASEB J.*, **14**, 355–360.

Atamna,H., Cheung,I. and Ames,B.N. (2000) A method for detecting abasic sites in living cells: age-dependent changes in base excision repair. *Proc. Natl Acad. Sci. USA*, **97**, 686–691.

Autrup,H., Grafström,R., Vahakangas,K. and Harris,C.C. (1986) Inter-individual variations in carcinogen metabolism. *Arch. Toxicol.*, **9** (suppl.), 147–153.

Awada,M. and Dedon,P.C. (2001) Formation of the 1,N^2-glyoxal adduct of deoxyguanosine by phosphoglycolaldehyde, a product of 3′-deoxyribose oxidation in DNA. *Chem. Res. Toxicol.*, **14**, 1247–1253.

Barbarella,G., Tugnoli,V. and Zambianchi,M. (1991) Imidazole ring opening of 7-methylguanosine at physiologic pH. *Nucl. Nucl.*, **10**, 1759–1769.

Bartsch,H. (1999) Keynote address: exocyclic adducts as new risk markers for DNA damage in man. *IARC Sci. Publ.*, **150**, 1–16.

Bartsch,H. and Nair,J. (2000) Ultrasensitive and specific detection methods for exocyclic DNA adducts: markers for lipid peroxidation and oxidative stress. *Toxicology*, **153**, 105–114.

Bartsch,H., Ohshima,H., Shuker,D.E.G., Pignatelli,B. and Calmels,S. (1990) Exposure of humans to endogenous N-nitroso compounds: implications in cancer etiology. *Mutat. Res.*, **238**, 255–267.

Bashir,S., Harris,G., Denman,M.A., Blake,D.R. and Winyard,P.G. (1993) Oxidative DNA damage and cellular sensitivity to oxidative stress in human autoimmune diseases. *Ann. Rheum. Dis.*, **52**, 659–66.

Basu,A.K. and Marnett,L.J. (1983) Unequivocal demonstration that malondialdehyde is a mutagen. *Carcinogenesis*, **4**, 331–333.

Basu,A.K., Loechler,E.L., Leadon,S.A. and Essigmann,J.M. (1989) Genetic effects of thymine glycol: site-specific mutagenesis and molecular modeling studies. *Proc. Natl Acad. Sci. USA*, **86**, 7677–7681.

Basu,A.K., Wood,M.L., Niederhofer,L.J., Ramos,L.A. and Essigmann,J.M. (1993) Mutagenic and genotoxic effects of three vinyl chloride-induced DNA lesions: 1,N^6-ethenoadenine, 3,N^4-ethenocytosine and 4-amino-5-(imidazol-2-yl)imidazole. *Biochemistry*, **32**, 12793–12801.

Basu,A.K., McNulty,J.M. and McGregor,W.G. (1999) Solution conformation and mutagenic specificity of 1,N6-ethenoadenine. *IARC Sci. Publ.*, **150**, 325–333.

Beisswenger,P.J., Howell,S.K., Touchette,A.D., Lal,S. and Szwergold,B.S. (1999) Metformin reduces systemic methylglyoxal levels in type 2 diabetes. *Diabetes*, **48**, 198–202.

Bianchini,F., Montesano,R., Shuker,D.E.G., Cuzick,J. and Wild,C.P. (1993) Quantification of 7-methyldeoxyguanosine using immunoaffinity purification and HPLC with electrochemical detection. *Carcinogenesis*, **14**, 1677–1682.

Bianchini,F., Jaeckel,A., Vineis,P., Martinez-Garciá,C., Elmstahl,S., van Kappel,A.L., Boeing,H., Ohshima,H., Riboli,E. and Kaaks,R. (2001) Inverse correlation between alcohol consumption and lymphocyte levels of 8-hydroxydeoxyguanosine in humans. *Carcinogenesis*, **22**, 885–890.

Blömeke,B., Greenblatt,M.J., Doan,V.D., Bowman,E.D., Murphy,S.E., Chen,C.C., Kato,S. and Shields,P.G. (1996) Distribution of 7-alkyl-2′-deoxyguanosine adduct levels in human lung. *Carcinogenesis*, **17**, 741–748.

Boiteux,S. and Laval,J. (1982) Mutagenesis by alkylating agents: coding properties for DNA polymerase of poly(dC) template containing 3-methylcytosine. *Biochimie*, **64**, 637–641.

Bolt,H.M., Leutbecher,M. and Golka,K. (1997) A note on the physiological background of the ethylene oxide adduct 7-(2-hydroxyethyl)guanine in DNA from human blood. *Arch. Toxicol.*, **71**, 719–721.

Burcham,P.C. (1999) Internal hazards: baseline DNA damage by endogenous products of normal metabolism. *Mutat. Res.*, **443**, 11–36.

Burgdorf,L.T. and Carell,T. (2002) Synthesis, stability and conformation of the formamidopyrimidine G DNA lesion. *Chemistry*, **8**, 293–301.

Burkhart-Schultz,K.J., Thompson,C.L. and Jones,I.M. (1996) Spectrum of somatic mutations at the hypoxanthine phosphoribosyltransferase (hprt) gene of healthy people. *Carcinogenesis*, **17**, 1871–1883.

Cadet,J., Carvalho,V.M., Onuki,J., Douki,T., Medeiros,M.H.G. and Mascio,P.D. (1999) Purine DNA adducts of 4,5-dioxovaleric acid and 2,4-decadienal. *IARC Sci. Publ.*, **150**, 103–113.

Cavalieri,E.L., Stack,D.E., Devanesan,P.D. *et al.* (1997) Molecular origin of cancer: catechol estrogen-3,4-quinones as endogenous tumor initiators. *Proc. Natl Acad. Sci. USA*, **94**, 10937–10942.

Cavalieri,E., Frenkel,K., Liehr,J.G., Rogan,E. and Roy,D. (2000) Estrogens as endogenous genotoxic agents-DNA adducts and mutations. *J. Natl Cancer Inst. Monogr.*, **27**, 75–94.

Chance,B., Sies,H. and Boveris,A. (1979) Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.*, **59**, 527–605.

Chaudhary,A.K., Nokubo,M., Reddy,G.R., Yeola,S.N., Morrow,J.D., Blair,I.A. and Marnett,L.J. (1994) Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver. *Science*, **265**, 1580–1582.

Chen,H.J., Chiang,L.C., Tseng,M.C., Zhang,L.L., Ni,J. and Chung,F.L. (1999) Detection and quantification of 1,N^6-ethenoadenine in human placental DNA by mass spectrometry. *Chem. Res. Toxicol.*, **12**, 1119–1126.

Chen,J.X., Zheng,Y., West,M. and Tang,M.S. (1998) Carcinogens preferentially bind at methylated CpG in the p53 mutational hot spots. *Cancer Res.*, **58**, 2070–2075.

Cheng,K.C., Preston,B.D., Cahill,D.S., Dosanjh,M.K., Singer,B. and Loeb,L.A. (1991) The vinyl chloride DNA derivative N2,3-ethenoguanine produces G→A transitions in *Escherichia coli*. *Proc. Natl Acad. Sci. USA*, **88**, 9974–9978.

Chung,F.L., Chen H.J.C. and Nath,R.G. (1996) Lipid peroxidation as a potential endogenous source for the formation of exocyclic DNA adducts. *Carcinogenesis*, **17**, 2105–2111.

Chung,F.L., Nath,R.G., Ocando,J., Nishikawa,A. and Zhang,L. (2000) Deoxyguanosine adducts of *t*-4-hydroxy-2-nonenal are endogenous DNA lesions in rodents and humans: detection and potential sources. *Cancer Res.*, **60**, 1507–1511.

Collins,A., Cadet,J., Epe,B. and Gedik,C. (1997) Problems in the measurement of 8-oxoguanine in human DNA. Report of a workshop, DNA oxidation, held in Aberdeen, UK, 19–21 January, 1997. *Carcinogenesis*, **18**, 1833–1836.

Cooper,D.N. and Youssoufian,H. (1988) The CpG dinucleotide and human genetic disease. *Hum. Genet.*, **78**, 151–155.

Cox,L.S. and Lane,D.P. (1995) Tumour suppressors, kinases and clamps: how p53 regulates the cell cycle in response to DNA damage. *Bioessays*, **17**, 501–508.

Critchlow,S.E. and Jackson,S.P. (1998) DNA end-joining: from yeast to man. *Trends Biochem. Sci.*, **23**, 394–398.

Dedon,P.C., Plastaras,J.P., Rouzer,C.A. and Marnett,L.J. (1998) Indirect mutagenesis by oxidative DNA damage: formation of the pyrimidopurinone adduct of deoxyguanosine by base propenal. *Proc. Natl Acad. Sci. USA*, **95**, 11113–11116.

Degan,P., Stefano,B., De Caterina,M., Korkina,L.G., Pinto,L., Scopacasa,F., Zatterale,A., Calzone,R. and Pagano,G. (1995) *In vivo* accumulation of 8-hydroxy-2′-deoxyguanosine in DNA correlates with release of reactive oxygen species in Fanconi's anaemia families. *Carcinogenesis*, **16**, 735–742.

Deman,J. and van Larebeke,N. (2001) Carcinogenesis: mutations and mutagens. *Tumor Biol.*, **22**, 191–202.

Denissenko,M.F., Chen,J.X., Tang,M.S. and Pfeifer,G.P. (1997) Cytosine methylation determines hot spots of DNA damage in the human P53 gene. *Proc. Natl Acad. Sci. USA*, **94**, 3893–3898.

Denissenko,M.F., Pao,A., Pfeifer,G.P. and Tang,M. (1998) Slow repair of bulky DNA adducts along the nontranscribed strand of the human p53 gene may explain the strand bias of transversion mutations in cancers. *Oncogene*, **16**, 1241–1247.

Dianov,G., Price,A. and Lindahl,T. (1992) Generation of single-nucleotide repair patches following excision of uracil residues from DNA. *Mol. Cell. Biol.*, **12**, 1605–1612.

Dianov,G.L., Thybo,T., Dianova,I.I., Lipinski,J. and Bohr,V.A. (2000) Single nucleotide patch base excision repair is the major pathway for removal of thymine glycol from DNA in human cell extracts. *J. Biol. Chem.*, **16**, 11809–11813.

Doerge,D.R., Churchwell,M.I., Fang,J.L. and Beland,F.A. (2000) Quantification of etheno DNA adducts using liquid chromatography, on-line sample processing and electrospray tandem mass spectrometry. *Chem. Res. Toxicol.*, **13**, 1259–1264.

Doll,R. and Peto,R. (1981) The causes of cancer: quantitative estimates of avoidable risk of cancer in the United States today. *J. Natl Cancer Inst.*, **66**, 1191–1308.

Dosanjh,M.K., Chenna,A., Kim,E., Fraenkel-Conrat,H., Samson,L. and Singer,B. (1994) All four known cyclic adducts formed by the vinyl chloride metabolite chloroacetaldehyde are released by a human DNA glycosylase. *Proc. Natl Acad. Sci. USA*, **91**, 1024–1028.

Dwivedy,I., Devanesan,P., Cremonesi,P., Rogan,E. and Cavalieri,E. (1992) Synthesis and characterization of estrogen 2,3- and 3,4-quinones. Comparison of DNA adducts formed by the quinones versus horseradish peroxidase-activated catechol estrogens. *Chem. Res. Toxicol.*, **5**, 828–833.

Eide,I., Zhao,C., Kumar,R., Hemminki,K., Wu,K. and Swenburg,J.A. (1999) Comparison of (32)P-postlabeling and high-resolution GC/MS in quantifying N7-(2-Hydroxyethyl)guanine adducts. *Chem. Res. Toxicol.*, **12**, 979–984.

Epe,B. (2002) Role of endogenous oxidative DNA damage in carcinogenesis: what can we learn from repair-deficient mice? *Biol. Chem.*, **383**, 467–475.

Esterbauer,H., Eckl,P. and Ortner,A. (1990) Possible mutagens derived from lipids and lipid precursors. *Mutat. Res. Rev. Genet. Toxicol.*, **238**, 223–233.

Esterbauer,H., Schaur,R.J. and Zollner,H. (1991) Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic. Biol. Med.*, **11**, 81–128.

Evans,M.D., Cooke,M.S., Akil,M., Samanta,A. and Lunec,J. (2000) Aberrant processing of oxidative DNA damage in systemic lupus erythematosus. *Biochem. Biophys. Res. Commun.*, **273**, 894–898.

Fang,J.L., Vaca,C.E., Valsta,L.M. and Mutanen,M. (1996) Determination of DNA adducts of malonaldehyde in humans: effects of dietary fatty acid composition. *Carcinogenesis*, **17**, 1035–1040.

Feig,D.I., Sowers,L.C. and Loeb,L.A. (1994) Reverse chemical mutagenesis: identification of the mutagenic lesions resulting from reactive oxygen species-mediated damage to DNA. *Proc. Natl Acad. Sci. USA*, **91**, 6609–6613.

Fink,S.P., Reddy,G.R. and Marnett,L.J. (1997) Mutagenicity in *Escherichia coli* of the major DNA adduct derived from the endogenous mutagen malondialdehyde. *Proc. Natl Acad. Sci. USA*, **94**, 8652–8657.

Fraga,C.G., Motchnik,P.A., Shigenaga,M.K., Helbock,H.J., Jacob,R.A. and Ames,B.N. (1991) Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. *Proc. Natl Acad. Sci. USA*, **88**, 11003–11006.

Frenkel,K. (1992) Carcinogen-mediated oxidant formation and oxidative DNA damage. *Pharmacol. Ther.*, **53**, 127–166.

Frosina,G., Fortini,P., Rossi,O., Carrozzino,F., Abbondandolo,A. and Dogliotti,E. (1994) Repair of abasic sites by mammalian cell extracts. *Biochem. J.*, **304**, 699–705.

Gentil,A., Renault,G., Madzak,C., Margot,A., Cabral-Neto,J.B., Vasseur,J.J., Rayner,B., Imbach,J.L. and Sarasin,A. (1990) Mutagenic properties of a unique abasic site in mammalian cells. *Biochem. Biophys. Res. Commun.*, **73**, 704–710.

Gerchman,L.L. and Ludlum,D.B. (1973) The properties of O^6-methylguanine in templates for RNA polymerase. *Biochim. Biophys. Acta*, **308**, 310–316.

Golding,B.T., Slaich,P.K., Kennedy,G., Bleasdale,C. and Watson,W.P. (1996)

Mechanisms of formation of adducts from reactions of glycidaldehyde with 2′-deoxyguanosine and/or guanosine. *Chem. Res. Toxicol.*, **9**, 147–157.

Hang,B., Medina,M., Fraenkel-Conrat,H. and Singer,B. (1998) A 55-kDa protein isolated from human cells shows DNA glycosylase activity toward 3,N^4-etheno-cytosine and G/T mismatch. *Proc. Natl Acad. Sci. USA*, **95**, 13561–13566.

Harrison,K.L., Wood,M., Lees,N.P., Hall,C.N., Margison,G.P. and Povey,A.C. (2001) Development and application of sensitive and rapid immunoassay for the quantitation of N7-methyldeoxyguanosine in DNA samples. *Chem. Res. Toxicol.*, **14**, 295–301.

Hecht,S.S., McIntee,E.J. and Wang,M. (2001) New DNA adducts of crotonaldehyde and acetaldehyde. *Toxicology*, **166**, 31–36.

Henle,E.S. and Linn,S. (1997) Formation, prevention and repair of DNA damage by iron/hydrogen peroxide. *J. Biol. Chem.*, **272**, 19095–19098.

Higuchi,Y. and Linn,S. (1995) Purification of all forms of HeLa cell mitochondrial DNA and assessment of damage to it caused by hydrogen peroxide treatment of mitochondria or cells. *J. Biol. Chem.*, **270**, 7950–7956.

Hill-Perkins,M., Jones,M.D. and Karran,P. (1986) Site-specific mutagenesis *in vivo* by single methylated or deaminated purine bases. *Mutat. Res.*, **162**, 153–163.

Hiraku,Y., Yamashita,N., Nishiguchi,M. and Kawanishi,S. (2001) Catechol estrogens induce oxidative DNA damage and estradiol enhances cell proliferation. *Int. J. Cancer*, **92**, 333–337.

Holliday,R. and Ho,T. (1998) Gene silencing and endogenous DNA methylation in mammalian cells. *Mutat. Res.*, **400**, 361–368.

Huff,A.C. and Topal,M.D. (1987) DNA damage at thymine N-3 abolishes base-pairing capacity during DNA synthesis. *J. Biol. Chem.*, **262**, 12843–12850.

Iida,T., Furuta,A., Kawashima,M., Nishida,J., Nakabeppu,Y. and Iwaki,T. (2001) Accumulation of 8-oxo-2′-deoxyguanosine and increased expression of hMTH1 protein in brain tumors. *Neuro-oncology*, **3**, 73–81.

Jackson,A.L., Chen,R. and Loeb,L.A. (1998) Induction of microsatellite instability by oxidative DNA damage. *Proc. Natl Acad. Sci. USA*, **95**, 12468–12473.

Jackson,A.L. and Loeb,L.A. (2001) The contribution of endogenous sources of DNA damage to the multiple mutations in cancer. *Mutat. Res.*, **477**, 7–21.

Jaiswal,M., LaRusso,N.F., Nishioka,N., Nakabeppu,Y. and Gores,G.J. (2001) Human Ogg1, a protein involved in the repair of 8-oxoguanine, is inhibited by nitric oxide. *Cancer Res.*, **61**, 6388–6393.

Jansson,G. (1991) Oestrogen-induced enhancement of myeloperoxidase activity in human polymorphonuclear leukocytes—a possible cause of oxidative stress in inflammatory cells. *Free Radic. Res. Commun.*, **14**, 195–208.

Jaruga,P. and Dizdaroglu,M. (1996) Repair of products of oxidative DNA base damage in human cells. *Nucleic Acids Res.*, **24**, 1389–1394.

Kadlubar,F.F., Anderson,K.E., Häussermann,S. *et al*. (1998) Comparison of DNA adduct levels associated with oxidative stress in human pancreas. *Mutat. Res.*, **405**, 125–133.

Kang,H., Konishi,C., Kuroki,T. and Huh,N. (1995) Detection of O^6-methylguanine, O^4-methylthymine and O^4-ethylthymine in human liver and peripheral blood leukocyte DNA. *Carcinogenesis*, **16**, 1277–1280.

Karran,P. and Lindahl,T. (1980) Hypoxanthine in deoxyribonucleic acid: generation by heat-induced hydrolysis of adenine residues and release in free form by a deoxyribonucleic acid glycosylase from calf thymus. *Biochemistry*, **19**, 6005–6011.

Kato,S., Petruzzelli,S., Bowman,E.D., Turteltaub,K.W., Blömeke,B., Weston,A. and Shields,P.G. (1993) 7-Alkyldeoxyguanosine adduct detection by two-step HPLC and the ^{32}P-postlabelling assay. *Carcinogenesis*, **14**, 545–550.

Kiyosawa,H., Suko,M., Okudaira,H., Murata,K., Miyamoto,T., Chung,M.H., Kasai,H. and Nishimura,S. (1990) Cigarette smoking induces formation of 8-hydroxydeoxyguanosine, one of the oxidative DNA damages in human peripheral leukocytes. *Free Radic. Res. Commun.*, **11**, 23–27.

Kono,Y. and Fridovich,I. (1982) Superoxide radical inhibits catalase. *J. Biol. Chem.*, **257**, 5751–5754.

Kumar,R. and Hemminki,K. (1996) Separation of 7-methyl- and 7-(2-hydroxyethyl)-guanine adducts in human DNA samples using a combination of TLC and HPLC. *Carcinogenesis*, **17**, 485–492.

Langouët,S., Müller,M. and Guengerich,F.P. (1997) Misincorporation of dNTPs opposite **1**, N^2-ethenoguanine and 5,6,7,9-tetrahydro-7-hydroxy-9-oxoimidazo[1,2-a]purine in oligonucleotides by *Escherichia coli* polymerases I exo$^-$ and II exo$^-$, T7 polymerase exo$^-$, human immunodeficiency virus-1 reverse transcriptase and rat polymerase β. *Biochemistry*, **36**, 6069–6079.

Laval,F., Wink,D.A. and Laval,J. (1997) A discussion of mechanisms of NO genotoxicity: implication of inhibition of DNA repair proteins. *Rev. Physiol. Biochem. Pharmacol.*, **131**, 175–191.

Lawrence,C.W., Borden,A., Banerjee,S.K. and LeClerc,J.E. (1990) Mutation frequency and spectrum resulting from a single abasic site in a single-stranded vector. *Nucleic Acids Res.*, **18**, 2153–2157.

Lenton,K.J., Therriault,H., Fulop,T., Payette,H. and Wagner,J.R. (1999) Glutathione and ascorbate are negatively correlated with oxidative DNA damage in human lymphocytes. *Carcinogenesis*, **20**, 607–613.

Li,D., Firozi,P.F., Zhang,W., Shen,J., DiGiovanni,J., Lau,S., Evans,D., Friess,H., Hassan,M. and Abbruzzese,J.L. (2002) DNA adducts, genetic polymorphisms and K-ras mutation in human pancreatic cancer. *Mutat. Res.*, **513**, 37–48.

Li,E., Bestor,T.H. and Jaenisch,R. (1992) Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell*, **69**, 915–926.

Li,K.M., Devanesan,P.D., Rogan,E.G. and Cavalieri,E.L. (1998) Formation of the depurinating 4-hydroxyestradiol (4-OHE2)-1-N7Gua and 4-OHE2-1-N3Ade adducts by reaction of E2-3,4-quinone with DNA. *Proc. Am. Assoc. Cancer Res.*, **39**, 636.

Liehr,J.G. (2000) Is estradiol a genotoxic mutagenic carcinogen? *Endocr. Rev.*, **21**, 40–54.

Lindahl,T. (1979) DNA glycosylases, endonucleases for apurinic/apyrimidinic sites and base excision-repair. *Prog. Nucleic Acid Res. Mol. Biol.*, **22**, 135–192.

Lindahl,T. (1982) DNA repair enzymes. *Annu. Rev. Biochem.*, **51**, 61–89.

Lindahl,T. (1993) Instability and decay of the primary structure of DNA. *Nature*, **362**, 709–715.

Lindahl,T. (1996) The Croonian Lecture, 1996: Endogenous damage to DNA. *Phil. Trans. R. Soc. Lond. B*, **351**, 1529–1538.

Lindahl,T. and Karlström,O. (1973) Heat-induced depyrimidination of deoxyribonucleic acid in neutral solution. *Biochemistry*, **11**, 5151–5154.

Lindahl,T. and Nyberg,B. (1972) Rate of depurination of native deoxyribonucleic acid. *Biochemistry*, **11**, 3610–3618.

Loft,S. and Poulsen,H.E. (1996) Cancer risk and oxidative DNA damage in man. *J. Mol. Med.*, **74**, 297–312.

Lutz,W.K. (1990) Endogenous genotoxic agents and processes as a basis of spontaneous carcinogenesis. *Mutat. Res.*, **238**, 287–295.

Lutz,W.K., Fekete,T. and Vamvakas,S. (1998) Position- and base pair-specific comparison of p53 mutation spectra in human tumors: elucidation of relationships between organs for cancer etiology. *Environ. Health Perspect.*, **106**, 207–211.

Marnett,L.J. (1999) Chemistry and biology of DNA damage by malondialdehyde. *IARC Sci. Publ.*, **150**, 17–27.

Marnett,L.J. (2000) Oxyradicals and DNA damage. *Carcinogenesis*, **21**, 361–370.

Marnett,L.J. (2002) Oxy radicals, lipid peroxidation and DNA damage. *Toxicology*, **181/182**, 219–222.

Marnett,L.J. and Plastaras,J.P. (2001) Endogenous DNA damage and mutation. *Trends Genet.*, **17**, 214–221.

Martucci,C.P. and Fishman,J. (1993) P450 enzymes of estrogen metabolism. *Pharmacol. Ther.*, **57**, 237–257.

Mecocci,P., MacGarvey,U. and Beal,M.F. (1994) Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. *Ann. Neurol.*, **36**, 747–751.

Mecocci,P., MacGarvey,U., Kaufman,A.E., Koontz,D., Shoffner,J.M., Wallace,D.C. and Beal,M.F. (1993) Oxidative damage to mitochondrial DNA shows marked age-dependent increases in human brain. *Ann. Neurol.*, **34**, 609–616.

Mistry,N., Podmore,I., Cooke,M., Butler,P., Griffiths,H., Herbert,K. and Lunec,J. (2003) Novel monoclonal antibody recognition of oxidative DNA damage adduct, deoxycytidine-glyoxal. *Lab. Invest.*, **83**, 241–250.

Mizutari,K., Ono,T., Ikeda,K., Kayashima,K. and Horiuchi,S. (1997) Photo-enhanced modification of human skin elastin in actinic elastosis by N$^\varepsilon$-(carboxymethyl)-L-lysine, one of the glycoxidation products of the Maillard reaction. *J. Invest. Derm.*, **108**, 797–802.

Moriya,M., Zhang,W., Johnson,F. and Grollman,A.P. (1994) Mutagenic potency of exocyclic DNA adducts: marked differences between *Escherichia coli* and simian kidney cells. *Proc. Natl Acad. Sci. USA*, **91**, 11899–11903.

Murata-Kamiya,N., Kamiya,H., Kaji,H. and Kasai,H. (1997) Glyoxal, a major product of DNA oxidation, induces mutations at G:C sites on a shuttle vector plasmid replicated in mammalian cells. *Nucleic Acids Res.*, **25**, 1897–1902.

Mustonen,R., Försti,A., Hietanen,P. and Hemminki,K. (1991) Measurement by ^{32}P-postlabelling of 7-methylguanine levels in white blood cell DNA of healthy individuals and cancer patients treated with decarbazine and procarbazine. Human data and method development for 7-alkylguanines. *Carcinogenesis*, **12**, 1423–1431.

Mustonen,R. and Hemminki,K. (1992) 7-Methylguanine levels in DNA of smokers' and non-smokers' total white blood cells, granulocytes and lymphocytes. *Carcinogenesis*, **13**, 1951–1955.

Mustonen,R., Schoket,B. and Hemminki,K. (1993) Smoking-related DNA adducts: ^{32}P-postlabelling analysis of 7-methylguanine in human bronchial and lymphocyte DNA. *Carcinogenesis*, **14**, 151–154.

Nair,V. and Offerman,R.J. (1985) Ring-extended products from the reaction of epoxy carbonyl compounds and nucleic acid bases. *J. Org. Chem.*, **50**, 5627–5631.

Nair,J., Barbin,A., Guichard,Y. and Bartsch,H. (1995) 1,N^6-ethenodeoxyadenosine and 3,N^4-ethenodeoxycytidine in liver DNA from humans and untreated rodents detected by immunoaffinity/^{32}P-postlabelling. *Carcinogenesis*, **16**, 613–617.

Nair,J., Vaca,C.E., Velic,I., Mutanen,M., Valsta,L.M. and Bartsch,H. (1997) High dietary omega-6 polyunsaturated fatty acids drastically increase the formation of etheno-DNA base adducts in white blood cells of female subjects. *Cancer Epidemiol. Biomarkers Prev.*, **6**, 597–601.

Nair,J., Gal,A., Tamir,S., Tannenbaum,S., Wogan,G. and Bartsch,H. (1998) Etheno adducts in spleen DNA of SJL mice stimulated to overproduce nitric oxide. *Carcinogenesis*, **19**, 2081–2084.

Nair,J., Barbin,A., Velic,I. and Bartsch,H. (1999) Etheno DNA-base adducts from endogenous reactive species. *Mutat. Res.*, **424**, 59–69.

Nakamura,J., La,D.K. and Swenberg,J.A. (2000) 5′-nicked apurinic/apyrimidinic sites are resistant to β-elimination by β-polymerase and are persistent in human cultured cells after oxidative stress. *J. Biol. Chem.*, **275**, 5323–5328.

Nakamura,J. and Swenberg,J.A. (1999) Endogenous apurinic/apyrimidinic sites in genomic DNA of mammalian tissues. *Cancer Res.*, **59**, 2522–2526.

Nakazawa,H., English,D., Randell,P.L., Nakazawa,K., Martel,N., Armstrong,B.K. and Yamasaki,H. (1994) UV and skin cancer: specific p53 gene mutation in normal skin as a biologically relevant exposure measurement. *Proc. Natl Acad. Sci. USA*, **91**, 360–364.

Nath,R.G. and Chung,F.L. (1994) Detection of exocyclic 1,N2-propanodeoxyguanosine adducts as common DNA lesions in rodents and humans. *Proc. Natl Acad. Sci. USA*, **91**, 7491–7495.

Nath,R.G., Ocando,J.E. and Chung,F.L. (1996) Detection of 1,N^2-propanodeoxyguanosine adducts as potential endogenous DNA lesions in rodent and human tissues. *Cancer Res.*, **56**, 452–456.

Nath,R.G., Ocando,J.E., Guttenplan,J.B. and Chung,F.L. (1998) 1,N^2-propanodeoxyguanosine adducts: potential new biomarkers of smoking induced DNA damage in human oral tissue. *Cancer Res.*, **58**, 581–584.

Ochs,K., Sobol,R.W., Wilson,S.H. and Kaina,B. (1999) Cells deficient in DNA polymerase beta are hypersensitive to alkylating agent-induced apoptosis and chromosomal breakage. *Cancer Res.*, **59**, 1544–1551.

O'Driscoll,M., Macpherson,P., Xu,Y.Z. and Karran,P. (1999) The cytotoxicity of DNA carboxymethylation and methylation by the model carboxymethylating agent azaserine in human cells. *Carcinogenesis*, **9**, 1855–1862.

Ono,T., Negishi,K. and Hayatsu,H. (1995) Spectra of superoxide-induced mutations in the lacI gene of a wild-type and a mutM strain of *Escherichia coli* K-12. *Mutat. Res.*, **326**, 175–183.

Palejwala,V.A., Rzepka,R.W., Simha,D. and Humayun,M.Z. (1993) Quantitative multiplex sequence analysis of mutational hot spots. Frequency and specificity of mutations induced by a site-specific ethenocytosine in M13 viral DNA. *Biochemistry*, **32**, 4105–4111.

Pan,J. and Chung,F.L. (2002) Formation of cyclic deoxyguanosine adducts from ω-3 and ω-6 polyunsaturated fatty acids under oxidative conditions. *Chem. Res. Toxicol.*, **15**, 367–372.

Pandya,G.A. and Moriya,M. (1996) 1,N^6-Ethenodeoxyadenosine, a DNA adduct highly mutagenic in mammalian cells. *Biochemistry*, **35**, 11487–11492.

Pegg,A.E. (1983) Alkylation and subsequent repair of DNA after exposure to dimethylnitrosamine and related carcinogens. *Rev. Biochem. Toxicol.*, **5**, 83–133.

Pegg,A.E., Dolan,M.E. and Moschel,R.C. (1995) Structure, function and inhibition of O^6-alkylguanine-DNA alkyltransferase. *Prog. Nucleic Acid Res. Mol. Biol.*, **51**, 167–223.

Perera,F.P. (1996) Molecular epidemiology: insights into cancer susceptibility, risk assessment and prevention. *J. Natl Cancer Inst.*, **88**, 496–509.

Plastaras,J.P., Riggins,J.N., Otteneder,M. and Marnett,L.J. (2000) Reactivity and mutagenicity of endogenous DNA oxopropenylating agents: base propenals, malondialdehyde and N(epsilon)-oxopropenyllysine. *Chem. Res. Toxicol.*, **13**, 1235–1242.

Podlutsky,A., Osterholm,A.M., Hou,S.M., Hofmaier,A. and Lambert,B. (1998) Spectrum of point mutations in the coding region of the hypoxanthine-guanine phosphoribosyltransferase (hprt)gene in human T-lymphocytes *in vivo*. *Carcinogenesis*, **19**, 557–566.

Podmore,I.D., Griffiths,H.R., Herbert,K.E., Mistry,N., Mistry,P. and Lunec,J. (1998) Vitamin C exhibits pro-oxidant properties (letter). *Nature*, **392**, 559.

Povey,A.C. (2000) DNA adducts: endogenous and induced. *Toxicol. Pathol.*, **3**, 405–414.

Prieto-Alamo,M.J., Jurado,J., Abril,N., Diaz-Pohl,C., Bolcsfoldi,G. and Pueyo,C. (1996) Mutational specificity of aflatoxin B1. Comparison of *in vivo* host-mediated assay with *in vitro* S9 metabolic activation. *Carcinogenesis*, **17**, 1997–2002.

Prolla,T.A. (1998) DNA mismatch repair and cancer. *Curr. Opin. Cell Biol.*, **10**, 311–316.

Pryor,W.A. (1986) Oxy-radicals and related species: their formation, lifetimes and reactions. *Annu. Rev. Physiol.*, **48**, 657–667.

Purmal,A.A., Kow,Y.K. and Wallace,S.S. (1994) Major oxidative products of cytosine, 5-hydroxycytosine and 5-hydroxyuracil, exhibit sequence context-dependent mispairing *in vitro*. *Nucleic Acids Res.*, **22**, 72–78.

Richeson,C.E., Mulder,P., Bowry,V.W. and Ingold,K.U. (1998) The complex chemistry of peroxynitrite decomposition: new insights. *J. Am. Chem. Soc.*, **120**, 7211–7219.

Rideout,W.M., Coetzee,G.A., Olumi,A.F. and Jones,P.A. (1990) 5-Methylcytosine as an endogenous mutagen in the human LDL receptor and p53 genes. *Science (Wash.)*, **249**, 1288–1290.

Roberts,M.J., Wondrak,G.T., Laurean,D.C., Jacobson,M.K. and Jacobson,E.L. (2003) DNA damage by carbonyl stress in human skin cells. *Mutat. Res.*, **522**, 45–56.

Robinson,D.R., Goodall,K., Albertini,R.J., O'Neill J.P., Finette,B., Sala-Trepat,M., Moustacchi,E., Tates,A.D., Beare,D.M. and Green,M.H. (1994) An analysis of *in vivo* hprt mutant frequency in circulating T-lymphocytes in the normal human population: a comparison of four data-sets. *Mutat. Res.*, **313**, 227–247.

Rogstad,D.K., Liu,P., Burdzy,A., Lin,S.S. and Sowers,L.C. (2002) Endogenous DNA lesions can inhibit the binding of the AP-1 (C-Jun) transcription factor. *Biochemistry*, **41**, 8093–8102.

Rouzer,C.A., Chaudhary,A.K., Nokubo,M., Ferguson,D.M., Reddy,G.R., Blair,I.A. and Marnett,L.J. (1997) Analysis of the malondialdehyde-2′-deoxyguanosine adduct pyrimidopurinone in human leukocyte DNA by gas chromatography/electron capture/negative chemical ionization/mass spectrometry. *Chem. Res. Toxicol.*, **10**, 181–188.

Roy,D., Weisz,J. and Liehr,J.G. (1990) The O-methylation of 4-hydroxyestradiol is inhibited by 2-hydroxyestradiol: implications for estrogen-induced carcinogenesis. *Carcinogenesis*, **11**, 459–462.

Rydberg,B. and Lindahl,T. (1982) Nonenzymatic methylation of DNA by the intracellular methyl group donor *S*-adenosyl-L-methionine is a potentially mutagenic reaction. *EMBO J.*, **1**, 211–216.

Rydberg,D., Hewer,A., Phillips,D.H. and Haugan,A. (1994) Different susceptibility to smoking-induced DNA damage among male and female lung cancer patients. *Cancer Res.*, **54**, 5801–5803.

Saffhill,R. (1984) Differences in the promutagenic nature of 3-methylcytosine as revealed by DNA and RNA polymerising enzymes. *Carcinogenesis*, **5**, 691–693.

Saffhill,R. (1985) *In vitro* miscoding of alkylthymines with DNA and RNA polymerases. *Chem. Biol. Interact.*, **53**, 121–130.

Sancar,A. (1995) DNA repair in humans. *Annu. Rev. Genet.*, **29**, 69–105.

Saparbaev,M. and Laval,J. (1998) 3,N^4-ethenocytosine, a highly mutagenic adduct, as a primary substrate for *Escherichia coli* double-strand uracil-DNA glycosylase and human mismatch-specific thymine-DNA glycosylase. *Proc. Natl Acad. Sci. USA*, **95**, 8508–8513.

Saparbaev,M., Langouët,S., Privezentzev,C.V., Guengerich,F.P., Cai,H., Elder,R.H. and Laval,J. (2002) 1,N^2-ethenoguanine, a mutagenic DNA adduct, is a primary substrate of *Escherichia coli* mismatch-specific uracil-DNA glycosylase and human alkylpurine-DNA-*N*-glycosylase. *J. Biol. Chem.*, **277**, 26987–26993.

Schuler,D., Otteneder,M., Sagelsdorff,P., Eder,E., Gupta,R.C., Lutz,W.K., Schuler,D., Otteneder,M., Sagelsdorff,P., Eder,E., Gupta,R.C. and Lutz,W.K. (1997) Comparative analysis of 8-oxo-2′-deoxyguanosine in DNA by 32P- and 33P-postlabeling and electrochemical detection. *Carcinogenesis*, **18**, 2367–2371.

Seeberg,E., Eide,L. and Bjoras,M. (1995) The base excision repair pathway. *Trends Biochem. Sci.*, **20**, 391–397.

Shen,Z., Wu,W. and Hazen,S.L. (2000) Activated leukocytes oxidatively damage DNA, RNA and the nucleotide pool through halide-dependent formation of hydroxyl radical. *Biochemistry*, **39**, 5474–5482.

Shields,P.G., Povey,A.C., Wilson,V.L., Weston,A. and Harris,C.C. (1990) Combined high-performance liquid chromatography/^{32}P-postlabelling assay of N^7-methylguanosine. *Cancer Res.*, **50**, 6580–6584.

Shinohara,A. and Ogawa,T. (1995) Homologous recombination and the roles of double-strand breaks. *Trends Biochem. Sci.*, **20**, 387–391.

Singer,B., Spengler,S.J., Fraenkel-Conrat,H. and Kusmierek,J.T. (1986) O^4-

methyl, -ethyl, or isopropyl substituents on thymidine in poly(dA-dT) all lead to transitions upon replication. *Proc. Natl Acad. Sci. USA*, **83**, 28–32.
Slupphaug,G., Kavli,B. and Krokan,H.E. (2003) The interacting pathways for prevention and repair of oxidative DNA damage. *Mutat. Res.*, **531**, 231–251.
Snustad,D.P., Simmons,M.J. and Jenkins,J.B. (1997) *Principles of Genetics*. John Wiley & Sons, New York, NY.
Sodum,R.S. and Chung,F.L. (1988) 1,N^2-Ethenodeoxyguanosine as a potential marker for DNA adduct formation by trans-4-hydroxy-2-nonenal. *Cancer Res.*, **48**, 320–323.
Sodum,R.S. and Chung,F.L. (1991) Stereoselective formation of *in vitro* nucleic acid adducts by 2,3-epoxy-4-hydroxynonanal. *Cancer Res.*, **51**, 137–143.
Spalding,J.W. (1988) Toxicology and carcinogenesis studies of malondialdehyde sodium salt (3-hydroxy-2-propenal, sodium salt) in F344/N rats and B6C3F1 mice. *NTP Tech. Rep.*, **331**, 5–13.
Spencer,J.P., Jenner,A., Aruoma,O.I., Cross,C.E., Wu,R. and Halliwell,B. (1996) Oxidative DNA damage in human respiratory tract epithelial cells. Time course in relation to DNA strand breakage. *Biochem. Biophys. Res. Commun.*, **224**, 17–22.
Stack,D., Byun,J., Gross,M.L., Rogan,E.G. and Cavalieri,E. (1996) Molecular characteristics of catechol estrogen quinones in reactions with deoxyribonucleosides. *Chem. Res. Toxicol.*, **9**, 851–859.
Stern,L.L., Mason,J.B., Selhub,J. and Choi,S.W. (2000) Genomic DNA hypomethylation, a characteristic of most cancers, is present in peripheral leukocytes of individuals who are homozygous for the C677T polymorphism in the methylenetetrahydrofolate reductase gene. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 849–853.
Strathern,J.N., Shafer,B.K. and McGill,C.B. (1995) DNA synthesis errors associated with double-strand-break repair. *Genetics*, **140**, 965–972.
Swenberg,J.A., La,D.K., Scheller,N.A. and Wu,K.Y. (1995) Dose-response relationships for carcinogens. *Toxicol. Lett.*, **82/83**, 751–756.
Szyfter,K., Hemminki,K., Szyfter,W., Szmeja,Z., Banaszewski,J. and Pabiszczak,M. (1996) Tobacco smoke-associated N^7-alkylguanine in DNA of larynx tissue and leukocytes. *Carcinogenesis*, **17**, 501–506.
Tabatabaie,T. and Floyd,R.A. (1994) Susceptibility of glutathione peroxidase and glutathione reductase to oxidative damage and the protective effect of spin trapping agent. *Arch. Biochem. Biophys.*, **314**, 112–119.
Tates,A.D., van Dam,F.J., van Mossel,H., Schoenmaker,H., Thijssen,J.C., Woldring,V.M., Zwinderman,A.H. and Natarajan,A.T. (1991) Use of the clonal assay for the measurement of frequencies of HPRT mutants in T-lymphocytes from five control populations. *Mutat. Res.*, **253**, 199–213.
Törnqvist,M., Gustafsson,B., Kautiainen,A., Harms-Ringdahl,M., Granath,F. and Ehrenberg,L. (1989) Unsaturated lipids and intestinal bacteria as sources of endogenous production of ethene and ethylene oxide. *Carcinogenesis*, **10**, 39–41.
Trichopoulos,D. and Petridou,E. (1994) Epidemiologic studies and cancer etiology in humans. *Med. Exerc. Nutr. Health*, **3**, 206–225.
Tudek,B., Boiteux,S. and Laval,J. (1992) Biological properties of imidazole ring opened N7-methylguanine in M13mp18 phage DNA. *Nucleic Acids Res.*, **20**, 3079–3084.
Vaca,C.E., Fang,J.L., Mutanen,M. and Valsta,L. (1995) ^{32}P-postlabeling determination of DNA adducts of malonaldehyde in humans: total white blood cells and breast tissue. *Carcinogenesis*, **16**, 1847–1851.
Vaca,C.E., Nilsson,J.A., Fang,J.L. and Grafström,R.C. (1998) Formation of DNA adducts in human buccal epithelial cells exposed to acetaldehyde and methylglyoxal *in vitro*. *Chem. Biol. Interact.*, **108**, 197–208.
Vamvakas,S., Vock,E.H. and Lutz,W.K. (1997) On the role of DNA double-strand breaks in toxicity and carcinogenesis. *Crit. Rev. Toxicol.*, **27**, 155–174.
Van Delft,J.H.M., van Winden,M.J.M., Luiten-Schuite,A., Ribeiro,L.R. and Baan,R.A. (1994) Comparison of various immunochemical assays for the detection of ethylene oxide-DNA adducts with monoclonal antibodies against imidazole ring opened N^7-(2-hydroxyethyl)guanosine: application in a biological monitoring study. *Carcinogenesis*, **15**, 1867–1994.
Wagner,J.R., Hu,C.C. and Ames,B.N. (1992) Endogenous oxidative damage of deoxycytidine in DNA. *Proc. Natl Acad. Sci. USA*, **89**, 3380–3384.
Williams,G.M. and Jeffrey,A.M. (2000) Oxidative DNA damage: endogenous and chemically induced. *Regulat. Toxicol. Pharmacol.*, **32**, 283–292.
Wilson,V.L., Taffe,B.G., Shields,P.G., Povey,A.C. and Harris,C.C. (1993) Detection and quatification of 8-hydroxydeoxyguanosine adducts in peripheral blood of people exposed to ionizing radiation. *Environ. Health Perspect.*, **99**, 261–263.
Wood,R.D. (1996) DNA repair in eukaryotes. *Annu. Rev. Biochem.*, **65**, 135–137.
Yager,J.D. and Liehr,J.G. (1996) Molecular mechanisms of estrogen carcinogenesis. *Annu. Rev. Pharmacol. Toxicol.*, **36**, 203–232.
Ye,N., Holmquist,G.P. and O'Connor,T.R. (1998) Heterogeneous repair of N-methylpurines at the nucleotide level in normal human cells. *J. Mol. Biol.*, **284**, 269–285.
Yen,T.Y., Christova-Gueoguieva,N.I., Scheller,N., Holt,S., Swenberg,J.A. and Charles,M.J. (1996) Quantitative analysis of the DNA adduct N2,3-ethenoguanine using liquid chromatography/electrospray ionization mass spectrometry. *J. Mass Spectrom.*, **31**, 1271–1276.
Yi,P., Sun,X., Doerge,D.R. and Fu,P.P. (1998) An improved 32P-postlabeling/high-performance liquid chromatography method for the analysis of the malondialdehyde-derived 1,N^2-propanodeoxyguanosine DNA adduct in animal and human tissues. *Chem. Res. Toxicol.*, **11**, 1032–1041.
Yoshie,Y. and Ohshima,H. (1998) Synergistic induction of DNA strand breakage by catechol-estrogen and nitric oxide: implications for hormonal carcinogenesis. *Free Radic. Biol. Med.*, **24**, 314–318.
Zhao,C. and Hemminki,K. (2002) The *in vivo* levels of DNA alkylation products in human lymphocytes are not age dependent: an assay of 7-methyl- and 7-(2-hydroxyethyl)-guanine DNA adducts. *Carcinogenesis*, **23**, 307–310.
Zhao,C., Tyndyk,M., Eide,I. and Hemminki,K. (1999) Endogenous and background DNA adducts by methylating and 2-hydroxyethylating agents. *Mutat. Res.*, **424**, 117–125.

Received on August 1, 2003; revised and accepted on March 11, 2004