From the Institute of Environmental Medicine,
Division of Biochemical Toxicology, Karolinska Institutet,
Stockholm, Sweden

Carcinogenic Polycyclic Aromatic Hydrocarbons

Theoretical, molecular, in vitro and cellular characterization of biotransformation and DNA damage

Kristian Dreij

Stockholm 2005

Published and printed by Karolinska University Press
Box 200, S-171 77 Stockholm, Sweden

ISBN 91-7140-372-8

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are widespread mutagenic and carcinogenic environmental pollutants, which require metabolic activation to electrophilic intermediates and subsequent covalent binding to critical targets in DNA to elicit their biological activity. Bay- and fjord-region diol epoxides (DEs) have been identified as the ultimate mutagenic and carcinogenic metabolites of PAHs. The balance between metabolic activation and detoxification routes influences the extent of DE-DNA adduct-formation. The most important detoxification pathway of DEs is glutathione transferase (GST) catalyzed conjugation with glutathione (GSH).

Human GSTs of Alpha class have been assayed with the ultimate carcinogenic (–)-*anti*- and (+)-*syn*-DEs derived from the nonplanar dibenzo[*a*,*l*]pyrene (DBPDE) and the (+)-*anti*-DE of the planar benzo[*a*]pyrene [(+)-*anti*-BPDE]. In general, the activities were in the order: (+)-*syn*-DBPDE > (–)-*anti*-DBPDE > (+)-*anti*-BPDE. GSTA1-1 was found to be the most efficient enzyme and demonstrated a remarkable catalytic efficiency (k_{cat}/K_m) of 464 $mM^{-1}s^{-1}$ with (+)-*syn*-DBPDE. The higher activity of GSTA1-1 with (+)-*syn*-DBPDE relative to (–)-*anti*-DBPDE was explained by molecular modeling showing the formation of more favorable interactions between the substrate and the enzyme-GSH complex. The results showed that the spatial orientation of the hydroxyl groups are important determinants for the catalytic efficiency and thus responsible for the observed difference in catalytic activity. Investigating preferences in structure of (+)-*syn*- and (–)-*anti*-DBPDE using DFT showed several levels of flexibility. Because of the distorted structure, the molecule can readily flip its DE moiety relative to the aromatic ring system ("in" and "out"). Furthermore, the hydroxyl groups on the saturated DE ring were found to be either in a diequatorial or in a diaxial conformation. Our results showed a lower energy profile and thus a preference for the in-diequatorial conformation for both DEs. The possibility of transversions on different levels might have biological consequences, both in detoxication and in DNA adduct formation, adapting to DNA and thus escape recognition/repair by the DNA repair machinery.

To study individual GSTs in a more biologically relevant system, V79 cells stably over-expressing different human GSTs were constructed and characterized. Factors governing the accessibility of lipophilic DE substrates for GSTs in the cell were investigated. With the highly reactive (+)-*anti*-BPDE, 1-2 % of the expected activity was observed, whereas the corresponding values for the less reactive (–)-*anti*-DBPDE were up to 13 %. Furthermore, the protective effect of individual GSTs against DE induced DNA adduct formation was determined. In general, an increase in GST activity was concomitant with a decrease in DNA adduct formation. DBPDE showed the highest DNA binding capacity among the DEs tested. GSTA1-1 showed the highest GSH conjugating capacity and offered best protection against DBPDE induced DNA adduct formation. With BPDE, GSTP1-1 was most active in GSH conjugate formation whereas GSTM1-1 displayed the most effective protection against formation of DNA adducts. Overall, this demonstrates the difficulty in extrapolating data obtained with pure enzymes to the complex situation in the intact cell.

We have compared the formation and removal of adducts as a function of time formed by (–)-*anti*-DBPDE and (+)-*anti*-BPDE in A549 human epithelial lung carcinoma cells. The treatment with DBPDE resulted in an initial increase of adducts to a maximal level of adducts after 1 hr of incubation. This was followed by an apparent, although not statistically significant, slow removal of adducts. In cells treated with BPDE the maximal level adducts was reached within 20 min of exposure. The formation was followed by an initial rapid decline in the adduct level and a later statistically significant 10-fold slower rate of adduct removal. Comparing the rate of removal of adducts derived from BPDE with those of DBPDE, the latter are obviously more refractory to the NER coupled repair than the former. Moreover, we observe a significant increase in ratio of dA/dG adducts for DBPDE, indicating that dA adducts are especially refractory to repair. The apparent resistance of adducts from DBPDE to be eliminated may reflect the ability of such adducts to escape recognition and/or the subsequent removal by the NER machinery.

LIST OF PUBLICATIONS

This thesis is based on the following original papers, which in the text will be referred to by their roman numerals:

I. Sundberg, K., **Dreij, K.**, Seidel, A. and Jernström, B. Glutathione conjugation and DNA adduct formation of dibenzo[*a,l*]pyrene and benzo[*a*]pyrene diol epoxides in V79 cells stably expressing different human glutathione transferases. Chem. Res. Toxicol. 15, 170-179, 2002.

II. **Dreij, K.**, Sundberg, K., Johansson, A-S., Nordling, E., Seidel, A., Persson, B., Mannervik, B., and Jernström, B. Catalytic activities of human Alpha class glutathione transferases toward carcinogenic dibenzo[*a,l*]pyrene diol epoxides. Chem. Res. Toxicol. 15, 825-831, 2002.

III. Cho, K-B., **Dreij, K.**, Jernström, B. and Gräslund, A. Conformations of benzene- and dibenzo[*a,l*]pyrene diol epoxides studied by density functional theory: ground states, transition states, dynamics, and solvent effects. Chem. Res. Toxicol. 16, 590-597, 2003.

IV. **Dreij, K.**, Bajak, E., Sundberg, K., Seidel A., Gusnanto A., Cotgreave, I. and Jernström B. DNA adducts of benzo[*a*]pyrene- and dibenzo[*a,l*]pyrene-diol epoxides in human lung epithelial cells: Kinetics of adduct-removal, effects on cell cycle check points and gene expression. Polycycl. Arom. Compd. 24, 549-566, 2004.

V. **Dreij, K.**, Seidel, A., Jernström, B. Differential removal of DNA-adducts derived from *anti*-diol epoxides of dibenzo[*a,l*]pyrene and benzo[*a*]pyrene in human cells. Chem. Res. Toxicol. 18, 655-664, 2005.

CONTENTS

LIST OF ABBREVIATIONS

3-MC	3-methylcholanthrene
aa	Amino acid
ARE	Antioxidant response element
ATM	Ataxia-telangiectasia mutated
ATR	ATM-Rad3-related
BcC	Benzo[*c*]chrysene
BcPh	Benzo[*c*]phenanthrene
BDE	Benzene diol epoxide
BER	Base excision repair
BgC	Benzo[*g*]chrysene
BP	Benzo[*a*]pyrene
C	Chrysene
CDNB	1-chloro-2,4-dinitrobenzene
CRE	cAMP response element
CSA	Cockayne syndrome protein A
CSB	Cockayne syndrome protein B
CYP450	Cytochrome P450
dA	Deoxyadenosine
DBA	Dibenz[*a,h*]anthracene
DBP	Dibenzo[*a,l*]pyrene
DDB	Damaged DNA binding protein
DE	Diol epoxide
DFT	Density functional theory
dG	Deoxyguanosine
DMBA	7,12-dimethylbenz[*a*]anthracene
DNA pol δ/ε	DNA polymerase δ/ε
EH	Epoxide hydrolase
GGR	Global genomic repair
GRE	Glucocorticoid response element
GSH	Glutathione
G-site	Glutathione binding site
GST	Glutathione transferase
H-site	Hydrophobic binding site
IARC	International agency for research on cancer
IUPAC	International union of pure and applied chemistry
MAPEG	Membrane associated proteins involved in eicosanoid and glutathione metabolism
MRP	Multidrug resistance protein
NER	Nucleotide excision repair
PAH	Polycyclic aromatic hydrocarbons
PCNA	Proliferating cell nuclear antigen
RNA pol	RNA polymerase II
RPA	Replication protein A
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TCR	Transcription coupled repair
TFIIH	Transcription factor IIH
XPA	Xeroderma pigmentosum group A
XPC-hHR23B	Xeroderma pigmentosum protein C-human homolog of RAD23B
XPF	Xeroderma pigmentosum group F
XPG	Xeroderma pigmentosum group G
XRE	Xenobiotic response element

An expert is a man who has made all the mistakes
that can be made in a very narrow field

Niels Bohr

GENERAL BACKGROUND

Introduction

All organisms are continuously exposed to a broad range of foreign substances, also referred to as xenobiotica. Examples of xenobiotics are man-made compounds such as products or waste products from chemical and pharmaceutical industries but also substances of natural source. Most xenobiotics are lipophilic, which may result in accumulation in cellular compartments to toxic concentrations. In order to prevent toxicity they need to be excreted, something made possible by making these molecules more water-soluble. This is performed by a special kind of metabolism evolved through evolution and specialized to be involved in detoxication called biotransformation. These systems are usually divided into three separate phases (I-III). Phase I metabolism involves an initial oxidation, reduction or hydrolysis introducing a functional group into the lipophilic compound to serve as a target for phase II-reactions, and are catalyzed by a number of enzymes, the most important being cytochrome P450 (CYP450) (Parkinson, 1996). Phase II metabolism frequently involves conjugation reactions catalyzed by glutathione transferase, UDP-glucuronosyl transferase and sulfotransferases, or reduction reactions catalyzed by epoxide hydrolase and quinone reductase (Parkinson, 1996). In general, phase II reactions result in a large increase in xenobiotic hydrophilicity, hence a great promotion of the excretion of foreign chemicals. Phase III is often referred to as the active transport of the polar conjugates out of the cell by the multidrug resistant protein (MRP) (Ishikawa, 1992; Ishikawa et al., 2000).
A large number of harmful substances exert their biological effect through covalent interactions with the cellular blueprint, DNA (Pitot and Dragan, 1996). Consequently, if a compound escapes detoxication and/or because of the metabolism in phase I and II becomes biological active, toxic effects due to the formation of DNA damage may arise. Luckily, we have a last line of intracellular defense in form of the DNA repair machinery, an apparatus capable of surveilling both active and inactive DNA for covalent modifications (DNA-adducts), and at sighting remove the lesion and restore DNA (Mitchell et al., 2003). Unfortunately, this process is not foolproof which may lead to manifested DNA damages, and as a result acute toxicity, apoptosis or mutations and subsequently development of cancer.

Cancer – a multistep process

Development of cancer is generally considered a multistep process driven by carcinogen-induced genetic and epigenetic damage in susceptible cells, which as a result gain a selective growth advantage. Subsequently, the cells may undergo clonal expansion as the result of activation of protooncogenes and/or inactivation of tumor suppressor genes (Harris, 1991; Barrett, 1993). Carcinogenesis is generally a slow process, with the period between insult and appearance of a tumor of several years and often as long as 30 years. The stages of carcinogenesis are commonly divided into initiation, promotion, and progression leading to the establishment of a malignant tumor. The first phase, tumor initiation, involves the interaction of a carcinogen

(chemical, radiation or viral) with DNA causing a genetic change also called mutation. If the alteration occurs in a sequence that encodes a growth regulatory protein, for example, this may, under certain circumstances provide that cell with a selective clonal growth advantage. Those circumstances include exposure to a class of compounds known as promoters. Thus, the promotion phase involves a selective clonal expansion of altered cells to form benign tumor cells. During the subsequent progression, further genetic alterations accumulate in the cells, causing the benign phenotype of the cells to change or convert into ones that are malignant and capable of metastasis. Molecular genetic studies of human cancer cells have revealed that human cancer cells carry multiple genetic alterations, including alterations of oncogenes and tumor suppressor genes (Harris, 1991; Bertram, 2000).

There are more than 100 distinct types of cancer, and subtypes of tumors can be found within specific organs giving rise to an almost infinite number of possible combinations of genetic and epigenetic modifications. However, Hanahan and Weinberg (Hanahan and Weinberg, 2000) have suggested six essential alterations in cell physiology responsible for the vast catalogue of cancer types. These alterations are:

1. Independence from growth signals
2. Insensitivity to growth-inhibitory mechanisms
3. Evasion of the apoptotic machinery
4. Unlimited replicative potential
5. Sustained angiogenesis
6. Tissue invasion and metastasis

The authors further propose that most if not all cancers have acquired the same set of functional capabilities during their development, although through various mechanistic strategies.

Chemical carcinogenesis

Paracelsus, active at the end of the 16th century, was the first to describe the fact that chemical exposure from the environment or occupation can lead to cancer. This was followed by the English physician sir John Percivall Pott who in 1775 described the occurrence of cancer alterations at the skin of the scrotum in a few patients, working as chimney sweeps, and traced it to repetitive exposure of soot. However, it was not until the end of the 19th century that it became clear that occupational exposure to certain chemicals or mixtures had carcinogenic effects. After many failures to induce cancer in laboratory animals, Yamagiwa and Ichikawa succeeded in inducing tumors by repeated application of coal tar to the ear of rabbits in 1915. Research over the following years for the active component of tar led to the synthesis of dibenz[*a,h*]anthracene (DBA) in 1929. The presence of DBA in coal tar was suggested by comparing the fluorescence spectrum of synthetic DBA with that of the tar. As a result, Kennaway and coworkers presented in 1930 the first proof that pure polycyclic aromatic hydrocarbons (PAHs) are capable of inducing malignant skin tumors in mice. In 1933, the active component isolated from 2 tons of coal tar pitch turned out to be another carcinogenic PAH, benzo[*a*]pyrene (BP). The isolation of a pure carcinogenic polycyclic aromatic hydrocarbon from coal tar is one of the milestones in studies on chemical carcinogens and BP has since served as a model compound (reviewed in Phillips, 1983; Luch,

2005). In the following years, many other classes and types of chemicals were found to be carcinogenic in animals (**Table 1**).

Early experimental results from Miller pointed to a link between metabolism, covalent binding to protein, DNA or RNA and biological activity (Miller, 1951). The link was further strengthen by the fact that the carcinogen itself was not sufficiently reactive or electrophilic to directly interact with these macromolecules. As a result, it was stated that these chemicals do not react directly with cellular constituents; they require enzymatic conversion into their ultimate carcinogenic forms, thus metabolic activation (Miller and Miller, 1975). The Miller's concept of metabolic activation or bioactivation to reactive intermediates has since been verified and extended to other classes of chemical carcinogens, including aromatic amines and nitrosamines (**Table 1**). Such activation of chemicals is now known to be catalyzed by almost all of the enzymes involved in biotransformation of xenobiotics. The majority of bioactivation reactions involve oxidation, and a number of oxidases, hydrogenases and oxygenases catalyze some of these detrimental oxidations. However, under the appropriate conditions, phase II enzymes may be involved in bioactivation as well as detoxication reactions. Still, the majority of oxidative bioactivation reactions can probably be attributed to CYP450, which also is true in the case of PAHs (Guengerich and Shimada, 1991).

Table 1. Examples of chemical carcinogens*

Compounds	**Main sources/uses**	**Affected organs/cancer**
Aromatic amines/amides		
2-Acetylaminofluorene	Model compound; pesticide	Liver, bladder
Aromatic hydrocarbons		
Benzo[*a*]pyrene	Coal tar; roofing; cigarette smoke	Skin, lung, stomach
2,3,7,8-TCDD	No commercial use; pesticide	Lung, liver
Polychlorinated biphenyls	Flame retardants; hydraulic fluids	Liver, skin
Metals		
Arsenic	Natural ores; alloys	Skin, lung, liver
Cadmium	Natural ores; pigments; batteries	Lung, kidney
Nickel	Natural ores; alloys; electrodes	Lung, nasal cavity
Natural carcinogens		
Aflatoxin B1	A mycotoxin	Liver
Asbestos	Thermal insulation; gaskets	Lung

*Adapted from (Luch, 2005)

Chemical carcinogens can be classified as genotoxic or epigenetic (nongenotoxic) (Weisburger and Williams, 1983). Genotoxic agents usually refer to carcinogens that either directly bind to, or damage genomic DNA, which in turn can result in mutations, often a genotoxic compound also exert tumor-promoting activity. Nongenotoxic carcinogens are characterized by their promoting activity, hormone modifying, immunosuppressive, cytotoxic, or peroxisome proliferating activity (Klaunig et al., 2000). Recently a new hypothesis has been suggested, by which the initial step of carcinogenesis is the induction of genetic instability by a direct and nonmutational inhibition of enzymes that proofread newly synthesized DNA during mitosis of local stem cells (Bignold, 2003).

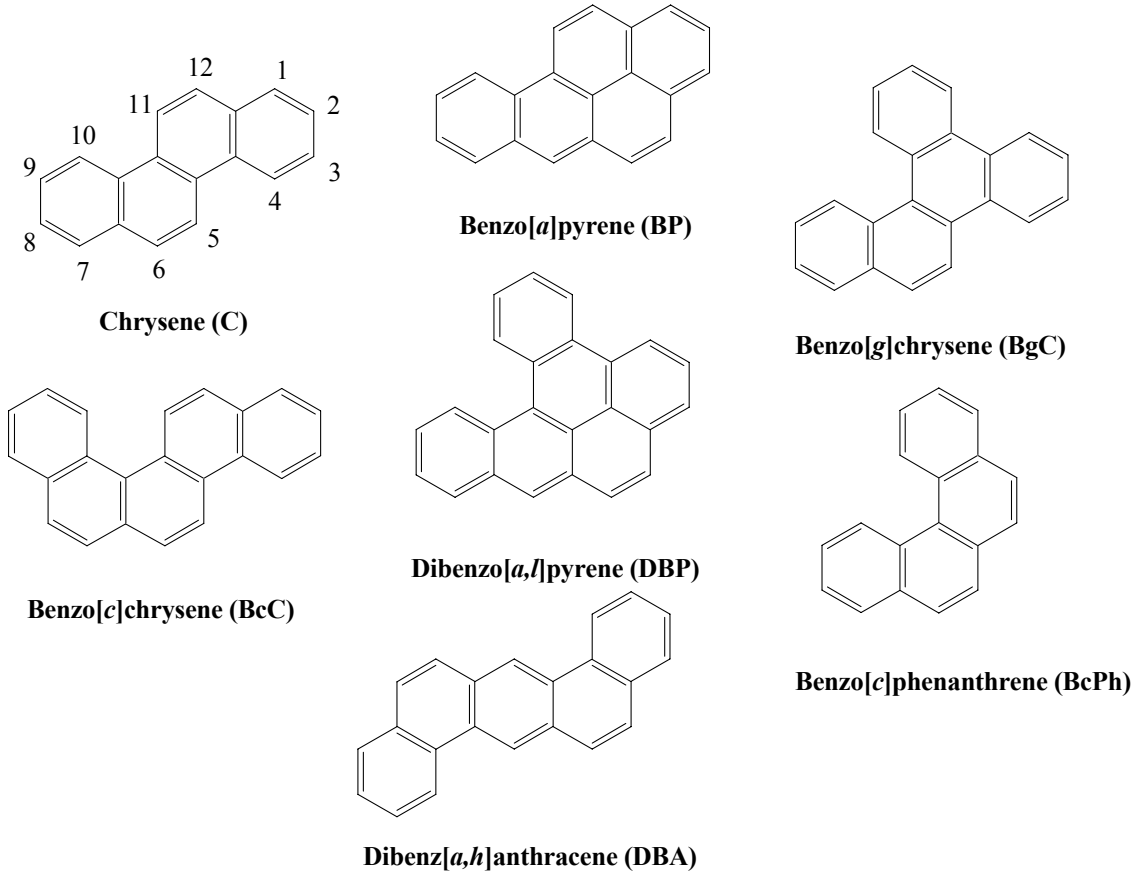


Figure 1. Structures and names (with abbreviation) of some commonly studied PAHs. The numbering system is exemplified on chrysene.

Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a group of highly lipophilic organic compounds containing two or more condensed benzo rings representing a wide range of molecular size and structural complexity (**Figure 1**). PAHs are formed during incomplete combustion or pyrolysis of organic matter and the major sources are industrial handling of coal, oil, and aluminium, domestic and residential heating but also natural sources such as forest fires and volcano activity. Although not volatile, they easily spread over great distances bound to soot particles and are ubiquitous environmental pollutants found in food, water and in the atmosphere. The general population is exposed to PAHs primarily through the air and by food-intake, with high levels in leafy plants, smoked fish and meat, where especially frying or charcoal broiling increases the total PAH content (Phillips, 1999). Certain life-style factors, such as cigarette smoking, and some occupations, such as gas and coke production, may greatly increase the exposure (IPCS, 1998).
The PAHs have been shown to be very potent carcinogens in experimental animals and several epidemiological studies have associated human tumors, especially in lung, urinary bladder, renal pelvis, mouth, pharynx, larynx and esophagus, with exposure to PAHs (IPCS, 1998). Furthermore, the DNA binding products in human cells and tissue are very similar to those formed in animals (Dipple, 1994). For these reasons PAH exposure are considered a substantial carcinogenic risk for humans and classified as probable or possible carcinogenic to human by the International Agency for Research on Cancer (IARC) (IARC, 1983; IARC, 1987). Animal experiments indicate that PAHs may also give rise to, for example, immunological and reproductive effects (IPCS, 1998). Tobacco smoke is an especially important source of PAHs because of its close association with tumor formation. The particulate phase from one cigarette contains at least 3500 compounds, of which more than 50 are known as carcinogens according to IARC. Of these substances, at least 10 were PAHs shown to induce pulmonary tumors in rodents (Hecht, 1999).

The nomenclature of PAHs has changed over the years but was standardized 1979 by the International Union of Pure and Applied Chemistry (IUPAC) (Dipple et al., 1984; Harvey, 1997). The currently accepted IUPAC rules in numbering the carbon backbone of the PAH is dictated by placing the polycyclic system so that 1) the maximum number of rings lies in a horizontal row, and 2) as many rings as possible are above and to the right of the horizontal row. The system is then numbered in a clockwise direction starting with the carbon atom not engaged in ring fusion in the most counter-clockwise position of the uppermost ring or the uppermost ring that is farthest to the right (**Figure 1**).

Metabolism

As with all lipophilic xenobiotics, PAHs must be metabolized into more water-soluble products in order to be excreted. Principally, the metabolism is initiated by the action of certain monooxygenases, cytochrome P450 (CYP450) catalyzing an oxidation (reviewed in Thakker et al., 1985; Grover, 1986). The primary products are arene oxides, i.e. epoxides across an aromatic double bond (**Figure 2**). This oxidation can in principal take place at any of the numerous double bonds. The epoxide can then either be hydrated by the addition of water, catalyzed by epoxide hydrolases forming *trans*-dihydrodiols or rearrange to phenols via the NIH-shift (Jerina and Daly, 1974). A third possibility of reaction for the epoxides is glutathione conjugation catalyzed by the glutathione transferases, which in humans may lead to the subsequent conversion into mercapturic acid and excretion. Dihydrodiols and phenols are not sufficiently polar to be excreted and are therefore metabolized to a variety of metabolites by conjugation reactions catalyzed by glucuronosyltransferases and sulfotransferases. Dihydrodiols can also be further oxidized by CYP450 to dihydrodiol epoxides, or more commonly diol epoxides (DEs), diol phenols and quinones. The diol epoxide can, in turn, either be enzymatically conjugated to glutathione or spontaneously react with water to form tetraols. Phenols are further transformed to polyphenolic compounds as well as to quinones. Taken together, PAHs are metabolized to a wide range of metabolites, some which represents detoxification products whereas others are mutagenic and carcinogenic, and responsible for the serious biological effects caused by PAHs. This is further presented in the following sections.

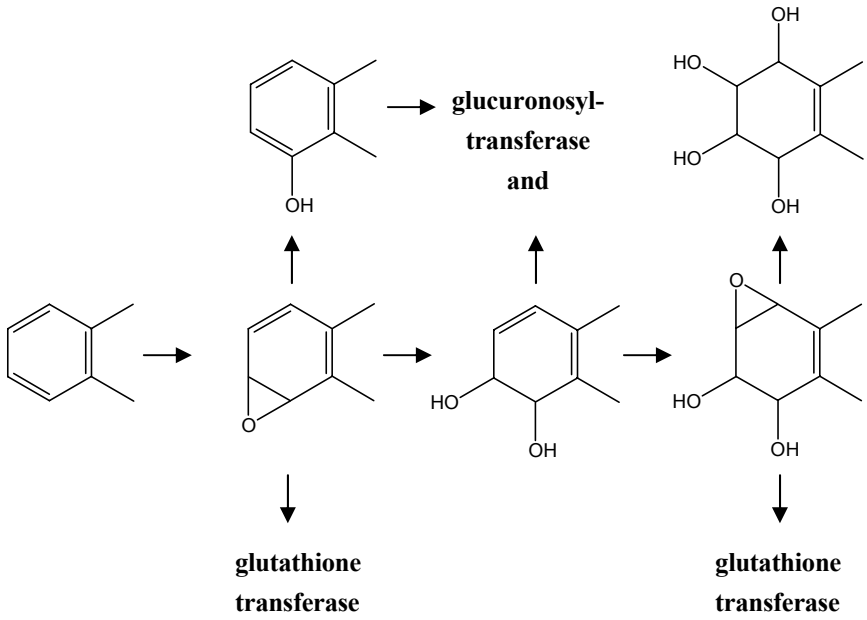


Figure 2. The major reactions involved in the metabolism of PAHs.

Metabolic activation

At about the same time as Miller reported the concept of bioactivation into ultimate carcinogens in the early 1950's Boyland suggested the possibility that epoxides (arene oxides) were reactive metabolites of PAHs (Miller, 1951; Boyland, 1950). But it was not until 1968 the first evidence of arene oxides as the biologically active metabolites of PAH was provided by demonstrating the metabolism of naphthalene to naphthalene 1,2-oxide by liver microsomes (Jerina et al., 1968). This was followed by a study of Borgen et al. in 1973 showing that the BP *trans*-7,8-dihydrodiol, following metabolic activation by hamster liver microsomes, was the major DNA-binding intermediate of BP (Borgen et al., 1973). Finally, in 1974 Sims and his colleagues identified the ultimate carcinogenic metabolite of BP from incubations with cultured cells, the BP 7,8-dihydrodiol 9,10-epoxide (BPDE) (Sims et al., 1974). These studies led to the almost explosive increase of studies investigating DEs of other PAHs, a field of research active up to date. In 1982, based on the preceding work by the above mentioned authors and others (Lehr and Jerina, 1977; Gelboin, 1980), Conney concluded the three step process responsible for producing the ultimate metabolites of BP (**Figure 3**) (Conney, 1982), a process shown to be true for all tested PAHs.

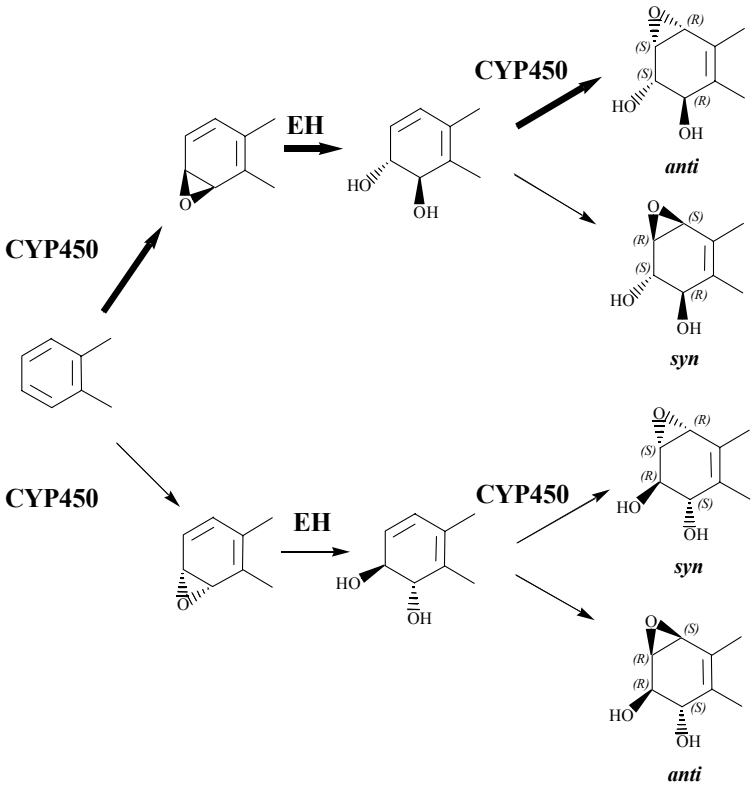


Figure 3. The major pathway for metabolic activation of PAHs to optically active DEs. Thicker arrows depict the preferred pathway of BP.

The first step in the bioactivation involves a CYP450 (in particular CYP1A1 and CYP1B1 (Shimada and Fujii-Kuriyama, 2004)) catalyzed epoxidation resulting in the formation of an arene oxide. Secondary, the epoxide is hydrolyzed to a *trans*-dihydrodiol by microsomal epoxide hydrolase (EH). The final step is a second CYP catalyzed epoxidation, where two diastereomeric DEs are formed. These two diastereomers may form four stereochemically different DEs, two with the *anti*-configuration, in which the epoxide is located opposite to the benzylic hydroxyl group, and two with the *syn*-configuration, in which the epoxide and the benzylic hydroxyl group is located in the same plane of the molecule. Although four stereochemically different DEs can be formed, studies have shown that, the activation pathway displays stereoselectivity. This is demonstrated by the fact that for example BP is preferentially converted to (–)-*trans*-BP 7,8-dihydrodiol which in turn predominantly forms the (+)-*anti* DE isomer [(+)-*anti*-BPDE] (**Figure 3**) (Yang et al., 1976).

The bay-region theory

After the discovery of BP 7,8-diol 9,10-epoxide, or BPDE, being the ultimate carcinogen of BP the focus was changed from the earlier intensively studied K-region epoxides, e.g. position 4,5 in BP, towards the so called bay-region epoxides. A bay-region is the region formed by angular benzo rings, whereas the region between two angular benzo rings is a special case of a bay-region and often refers to as a fjord-region (**Figure 4**). The following years, researchers focused on synthesizing and identifying potential metabolites of BP and other PAHs in order to determine their mutagenic and carcinogenic activities (reviewed in Conney, 1982). These studies led to a generalized theory, the bay-region theory (Jerina and Daly, 1976) as a theoretical basis to explain the carcinogenic potency of PAHs. The theory predicts that for DEs of a given PAH, those compounds in which the epoxide group forms part of a bay-region would have the highest chemical reactivity and the highest biological activity. Studies of different PAHs with respect to their DNA-binding, mutagenicity and carcinogenicity have all repeatedly confirmed the remarkable activity of bay- or fjord-region DEs (Thakker et al., 1985; Jerina et al., 1986; Glatt et al., 1991).

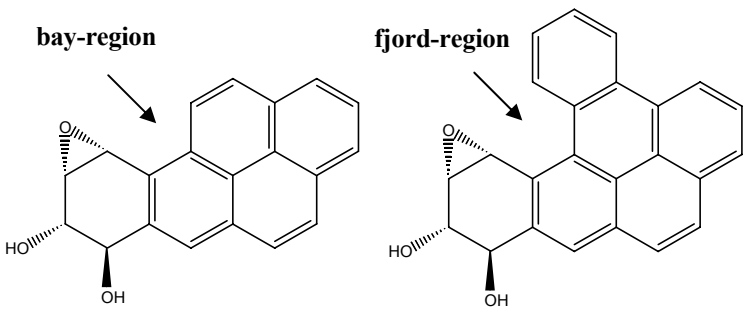


Figure 4. Example of a bay- and fjord-region PAH DE, BPDE (left) and DBPDE (right).

As indicated above and seen in **Figure 2**, the bay- and fjord-region DEs only make out a small part of the total metabolites of PAHs, but some important properties specific for bay-region DEs separates them from the other. DEs localized on saturated angular benzo rings located in the bay-region exhibit greater electrophilicity and are thus more

reactive towards nucleophiles than DEs located on other positions of the molecule (Lowe and Silverman, 1981; Borosky, 1999). Furthermore, molecular orbital calculations predict a more easily formation and stabilization of a carbonium ion at the benzylic carbon atom for bay-region epoxides or DEs than in a non bay-region. In the former case, the positive charge delocalizes into the aromatic system, whereas in the latter case, it cannot (Jerina et al., 1976; Jerina and Lehr, 1977). Their high activity is also in part due to a relatively higher resistance to enzymatic hydrolysis or conjugation (Thakker et al., 1985). The resistance arises from a steric inaccessibility of the bay- and fjord-region DEs. The three properties of reactivity and yet stability and the detoxification resistance contributes to the unique biological properties seen with bay- and fjord-region DEs.

Structure and activity

Soon after the establishment of the bay-region theory, it became obvious that in order for a full explanation for differences in DNA-binding, mutagenicity and carcinogenicity to be explained one more level of structural complexity was required. In other words, the bay-region theory could not explain the differences in biological activity seen between diastereomeric and enantiomeric DEs.

As seen in **Figure 3**, four stereoisomeric DEs can be formed during the metabolism to DEs. In the case of BP those are the diastereomeric pairs; (+)-/(–)-*anti*- and (+)-/(–)-*syn*-BPDE with the absolute configurations (*RSSR/SRRS*) and (*SRSR/RSRS*), respectively (Conney, 1982). The most common DEs formed in a biological system are the ones with (*R,S*)-diol (*S,R*)-epoxide absolute configuration (Pruess-Schwartz et al., 1984; Pruess-Schwartz et al., 1987; Ralston et al., 1995; Agarwal et al., 1997). The absolute configuration denoted *R* and *S* describe the spatial orientation of the functional groups, i.e. the diol and the epoxide. Because of the optical activity of the enantiomers, they will interact differently with optically active biomolecules, such as DNA, resulting in differences in chemical and biological activity. Interestingly, systematic studies of a number of bay-region DEs have demonstrated that those associated with high biological activity are in general the *anti* diastereomers, in particular the enantiomers with (*R,S*)-diol (*S,R*)-epoxide absolute configurations, corresponding to (+)-*anti*-DEs in bay-region and (–)-*anti*-DEs in fjord-region PAHs, respectively (Dipple, 1985; Jerina et al., 1991; Harvey, 1991).

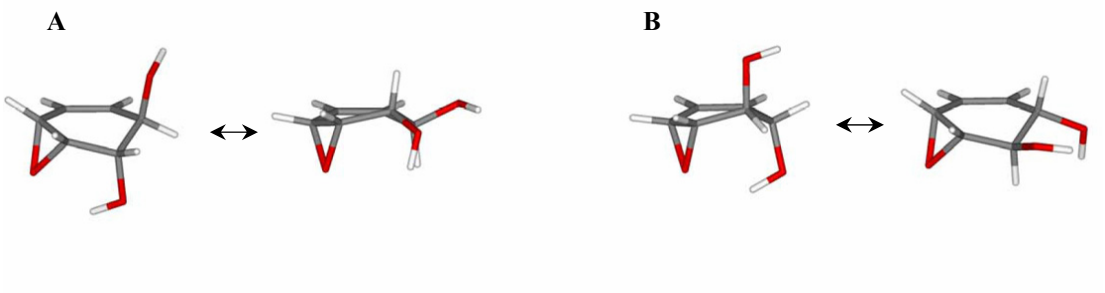


Figure 5. Diaxial and diequatorial orientation of the hydroxyl groups in *anti*- (A) and *syn*-DEs (B), respectively.

What seems to be responsible for the distinction in biological activity between *anti* and *syn* diastereomers is yet another structural feature. For each of the four conformations mentioned above, there are two preferred orientations of the hydroxyl groups relative to one another. The arrangement of the hydroxyl groups can be either pseudo-diequatorial (nearly in the plane of the molecule) or pseudo-diaxial (nearly perpendicular to the plane) (**Figure 5**) (Yagi et al., 1975). The *anti*-isomers of bay-region DEs prefer the diequatorial conformation whereas the *syn*-isomers prefer the diaxial conformation (Yagi et al., 1975). The latter conformation is believed to result in intramolecular hydrogen bonding between the benzylic hydroxyl group and the epoxide oxygen. As a result, this will lead to a higher chemical reactivity, i.e. hydrolysis, thus a smaller fraction capable of reacting with DNA etc. In contrast, the hydroxyl groups in the fjord-region DEs are diequatorially oriented in both the *anti*- and the *syn*-diastereomers (Sayer et al., 1981). As a result, also *syn*-enantiomers of the fjord-region DEs demonstrate high carcinogenic potency in contrast to bay-region ones (Wood et al., 1984; Glatt et al., 1991; Luch et al., 1994).

One of the most obvious structural differences between bay- and fjord-region PAH DEs is the difference in planarity. The molecular overcrowding between the rings located in the fjord-region leads to a distorted and more flexible molecule in contrast to the rigid and planar bay-region DEs (**Figure 6**) (Hirshfeld et al., 1963; Lewis-Bevan et al., 1995; Katz et al., 1998; Little et al., 1999). This was also confirmed during the present work and reported in paper III. Studies comparing the biological activity of bay- and fjord-region DEs have shown that the latter in general have a higher mutagenicity and carcinogenicity (Jerina et al., 1986; Glatt et al., 1991; Amin et al., 1995b; Amin et al., 1995a). The reason for this discrepancy is the relatively low solvolytic reactivity demonstrated by the fjord-region DEs compared to bay-region ones. Furthermore, fjord-region DEs also show a higher reactivity against nucleophilic macromolecules, e.g. DNA (Jerina et al., 1986; Jerina et al., 1991; Glatt et al., 1991).

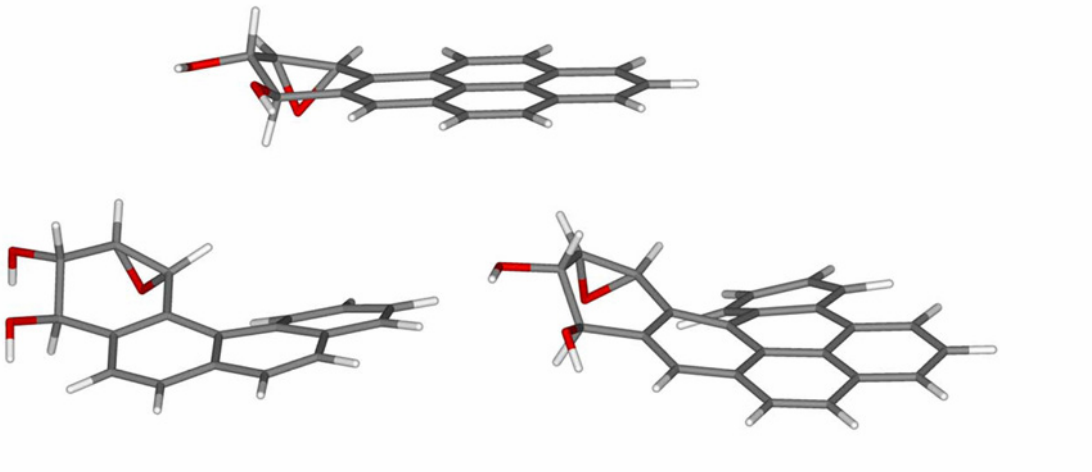


Figure 6. BPDE (top), CDE (left) and DBPDE (right) as examples of planar bay- and nonplanar fjord-region DEs.

In 1981 Jerina et al. reported findings on the remarkable tumorigenic activity of the fjord-region DEs from benzo[*c*]phenanthrene (BcPh) suggesting that the high degree of crowding in the fjord-region could account for the remarkable biological activity (Jerina et al., 1981). A decade later the list of highly active fjord-region DEs was extended with benzo[*c*]chrysene (BcC) and benzo[*g*]chrysene (BgC) (Glatt et al., 1991; Phillips et al., 1991).
Up to date the most carcinogenic PAH identified in the environment (Sauvain et al., 2001; Seidel et al., 2004; Yu and Campiglia, 2005) is the fjord-region PAH dibenzo[*a,l*]pyrene (DBP) (**Figure 6**). DBP is several orders of magnitude more potent in experimental animals than for example the earlier proved most potent PAHs BP and 7,12-dimethylbenz[*a*]anthracene (DMBA) (Cavalieri et al., 1991; LaVoie et al., 1993; Higginbotham et al., 1993). DBP has in biological systems shown to be stereoselectively metabolized to (–)-*anti*- and (+)-*syn*-DBPDE, both with *R*-configuration at the benzylic carbon (Ralston et al., 1995; Baird et al., 1996). These two DEs have further been shown as exceptionally active mutagens in bacteria as well as mammalian cells (Luch et al., 1994; Luch et al., 1997; Yoon et al., 2004).

In summary, for a given PAH the tumorigenic activity seems at least in part to be dependent upon the following:

1. Location of the DE (bay- or fjord-region)
2. Absolute configuration at the benzylic carbon (*R*-absolute configuration)
3. Conformation of the molecule (planar or nonplanar)
4. Conformation of the hydroxyl groups (diequatorial conformation)

Detoxication of PAH diol epoxides

The known mechanisms of inactivation of PAH DEs include spontaneous hydrolysis to less harmful tetraols, further epoxidation by CYP450 to triol epoxides and pentaols (Dock et al., 1986; Grover, 1986). However, the glutathione transferase (GST) catalyzed conjugation with glutathione (GSH) is believed to be the most important enzymatic pathway for its inactivation (Robertson et al., 1986; Robertson and Jernström, 1986; Jernström et al., 1996). This has been further confirmed in cellular systems, that expression of GSTs significantly reduces the formation of DNA adducts (Romert et al., 1989; Hesse et al., 1990; Fields et al., 1994; Fields et al., 1998; Hu et al., 1999; Sundberg et al., 2000). A brief overview of the GST family is presented later. At least in humans, the GSH conjugate is subsequently converted into mercapturic acid and excreted via the kidneys.

PAH diol epoxide DNA adducts

PAH DEs can form adducts with DNA, RNA and proteins but their mutagenic and tumorigenic effects are thought to be related to the covalent interaction with DNA (Gräslund and Jernström, 1989; Szeliga and Dipple, 1998). The majority of DE derived DNA adducts has been found to result from reaction of the benzylic oxiranyl carbon of the molecule with the exocyclic amino groups of the DNA bases deoxyguanosine (dG) and deoxyadenosine (dA) (Jerina et al., 1991; Jernström and Gräslund, 1994; Szeliga and Dipple, 1998; Geacintov et al., 1997). Research looking

at preferences in formation of DNA adducts shows that bay-region DEs prefer forming adducts at the exocyclic N^2-amino group of dG, whereas the sterically hindered fjord-region DEs favor reaction with the N^6-amino group of dA (Koreeda et al., 1978; Meehan and Straub, 1979; Dipple et al., 1987; Ralston et al., 1995). For instance, the bay-region DE (+)-*anti*-BPDE (with *R* configuration at the benzylic carbon) has a >90 % preference of forming DNA-adducts with dG (Koreeda et al., 1978; Meehan and Straub, 1979), whereas the corresponding *R*-configured fjord-region (–)-*anti*-DBPDE shows a corresponding ~75 % preference for dA adducts (Ralston et al., 1995). The differences in preference in DNA binding between bay- and fjord-region DEs is, as mentioned above, also probably due the structural dissimilarities. Moreover, the pronounced preference of fjord-region DEs for dA has been suggested to, in part, explain the higher biologically potency observed with these DEs compared with the bay-region ones (Glatt et al., 1991; Ralston et al., 1995; Buterin et al., 2000).

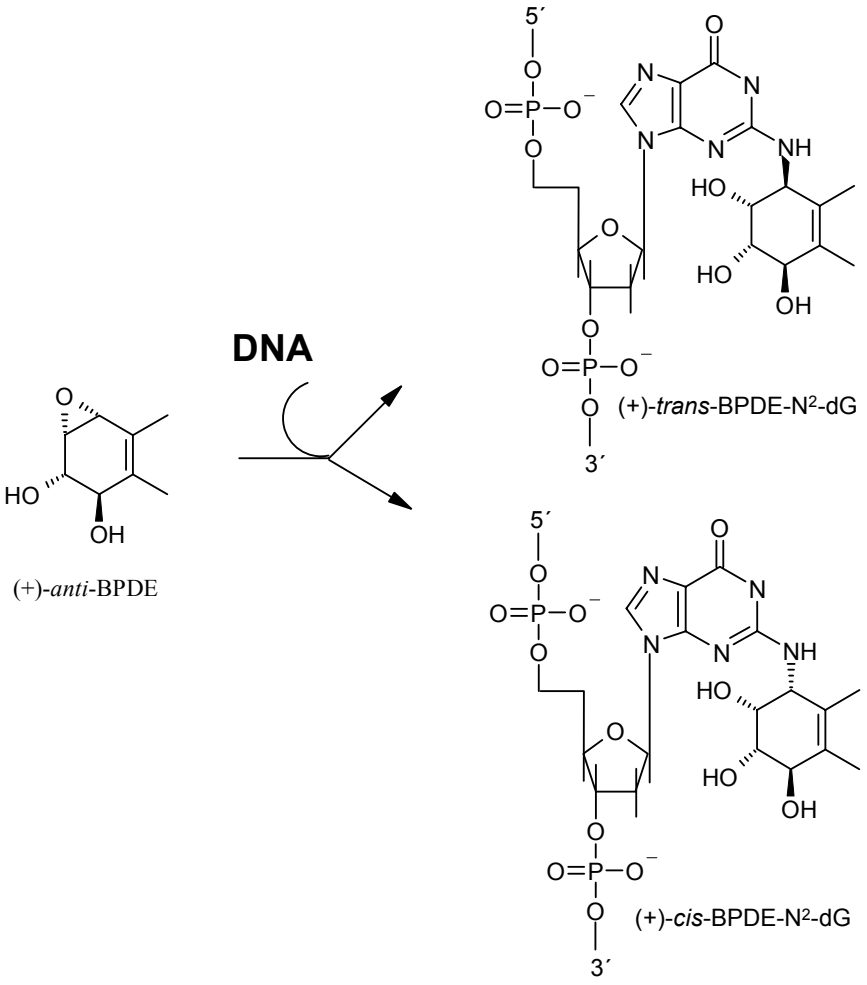


Figure 7. The formation and structure of *cis*- and *trans*-dG adducts from the reaction with BPDE.

The benzylic oxiranyl carbon in the DE binds covalently to the exocyclic amino groups of the bases preferably by *trans* addition. However, since all DNA-DE adducts result from reaction at this carbon with the opening of the epoxide ring both *cis* and *trans* products can be formed. *Cis* and *trans* refer to the relative orientation of the amino group of dA or dG bound to the benzylic carbon and the adjacent hydroxyl group (**Figure 7**). *Cis* adducts are less conformationally flexible than *trans* adducts because they are inherently more sterically crowded, with two hydroxyl groups located on the same side of the benzylic ring as the purine- and sugar-residues. In contrast, the two hydroxyl groups in the *trans* adducts are located on the opposite side of the purine- and sugar-residues, leading to a less crowded adduct (Geacintov et al., 1997). As a result, some of these stereoisomeric adducts may adopt different conformations in DNA. DE DNA adducts can, relative to the DNA helix, either be located intercalated or external and situated in the major or minor groove, all depending on the stereochemistry of the adduct (*trans* or *cis*) and base preferences (dA or dG). This in turn disturbs the DNA conformation to varying extent, leading to a more or less efficient recognition and subsequent removal by the DNA repair machinery (Hess et al., 1997a; Buterin et al., 2000; Lin et al., 2001; Geacintov et al., 2002; Wu et al., 2002).

A number of studies have shown that DE-DNA adducts can either lead to a complete block of replication, when the DNA polymerase is unable to pass a bulky adduct, or a misincorporation, when the adduct is bypassed. The latter case, also called translesion synthesis, has recently been ascribed to the DNA polymerase Y superfamily; a family of error prone DNA polymerases which can display low fidelity which in turn may result in mutations (Goodman, 2000). The low reliability of the members of this family has been demonstrated in various systems with adducts derived from BPDE (Zhang et al., 2002; Shen et al., 2002; Avkin et al., 2004). The major mutations resulting from the presence of DE-DNA adducts are G → T or A → T transversions (Dipple et al., 1987; Jernström and Gräslund, 1994). However, to initiate the carcinogenic process, the mutation must take place at critical positions and in critical genes such as tumor suppressorgenes and protooncogenes.

Removal of bulky diol epoxide adducts

To handle the great number of different types of DNA adducts/damage that may arise in a cell a versatile and sophisticated cellular machinery comprised of a number of different pathways has been developed (Sancar, 1996; Wood, 1996; Hoeijmakers, 2001). The two main mechanisms for excision of DNA damage are base excision repair (BER) and nucleotide excision repair (NER). A third mechanism, mismatch repair, has as main function to correct errors of DNA replication and to act on heteroduplexes formed during recombination. BER mainly acts on “smaller” DNA damages caused by endogenously hydrolysis, oxygen free radicals, and simple alkylating agents. NER is the most versatile pathway among these three systems with the capability to remove a large variety of structural unrelated lesions. The most obvious function of NER in humans is to remove those photoproducts from DNA caused by UV-irradiation (e.g. sunlight). This is especially apparent in individuals with NER defects, e.g. the disorder xeroderma pigmentosum (XP) where patients show a high incidence of UV-related skin

cancers (reviewed in Berneburg and Lehmann, 2001). However, NER has also been shown to be the major DNA repair strategy for eliminating a vast number of bulky DNA adducts. Accordingly, bulky PAH DE DNA adducts are primarily repaired by NER (Wood, 1999; Braithwaite et al., 1999; Mitchell et al., 2003), although both the DNA mismatch repair and BER have been reported to be involved (Wu et al., 2003; Braithwaite et al., 1998).

Nucleotide excision repair

NER further divides into two sub pathways. The first pathway is termed global genomic repair (GGR) and involves repair activity against DNA lesions across the genome. The second pathway coupled to active transcription and DNA lesions in the transcribed strand of active genes is termed transcription-coupled repair (TCR) (Mitchell et al., 2003; Hanawalt et al., 1994). This highly specific function of TCR is reflected in a higher rate of adduct removal relative to GGR (Bohr et al., 1985; Mellon et al., 1987).
The upstream regulation of NER is controlled by the action/activation of the DNA repair kinases ATM and ATR, which upon stalled replication or transcription, arrests cell cycle progression and recruits DNA repair machinery via activation, i.e. phosphorylation, of a number of modulators. One of the key players in the subsequent signaling is the tumor suppressor protein, p53. Activated p53 regulates a number of downstream cellular processes such as cell cycle arrest, apoptosis and DNA repair (Costa et al., 2003; Bassal and El-Osta, 2005; Adimoolam and Ford, 2003). Delaying the progression of the cell cycle provides the repair machinery with additional time to remove the damage before continuing the cycle. Regarding the role of p53 in NER, it is well accepted that p53 is involved in the transactivation of proteins partitioning in GGR, while the role of p53 in TCR is controversial (reviewed in Sengupta and Harris, 2005).

NER in human cells essentially involves the steps; recognition of DNA damage, incision and subsequent excision of the DNA strand containing the lesion, and finally DNA synthesis and ligation, a process including more than 30 proteins (**Figure 8**) (Lindahl and Wood, 1999; de Laat et al., 1999; Riedl et al., 2003; Dip et al., 2004). The most critical step in NER is the recognition of the DNA lesion, a mechanism principally different in GGR and TCR. While the protein complexes DDB and XPC-hHR32B are responsible for the rate-limiting step in GGR, the arrest of RNA polymerase II upon encountering a DNA adduct during translation initiates the repair in TCR (**Figure 8**) (Dip et al., 2004; van Hoffen et al., 2003). Although the initiation of the two different pathways is different, they both lead to the recruitment of the entire repair protein apparatus, in the latter case with the help of the Cockayne syndrome proteins CSA and CSB. Subsequently, the lesions are opened by the concerted action of XPA, replication protein A (RPA), and the helicase subunits of the transcription factor IIH (TFIIH) complex. During incision of the damaged DNA, the XPF complex cuts at the single-strand to double-strand transition on the 5′ side of the damage, and XPG cuts at the 3′ side of the open complex.

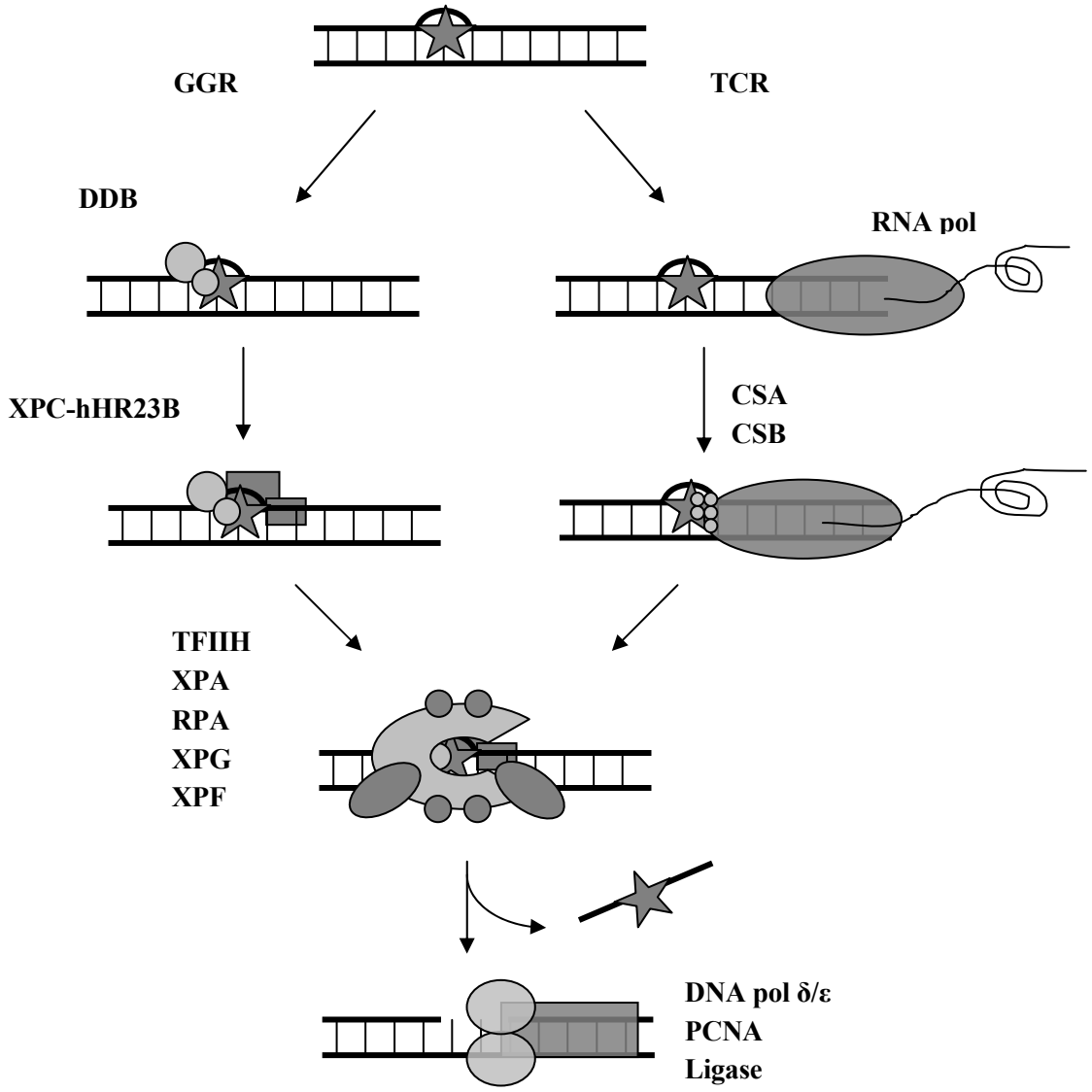


Figure 8. Schematic process of the different pathways in NER, from the detection of lesion to the new synthesis of DNA. Adapted from (Costa et al., 2003; van Hoffen et al., 2003).

Finally, DNA excision and de novo synthesis is accomplished by mammalian DNA replication factors such as the heterotrimeric replication protein A (RPA), proliferating cell nuclear antigen (PCNA), and DNA polymerase δ and ε. The reaction is completed by ligation of the newly synthesized DNA (Costa et al., 2003; van Hoffen et al., 2003) (**Figure 8**).

The recognition of adducts by the NER enzymes are dependent on adduct conformation and the associated helix distortion. Geacintov et al. (Geacintov et al., 2002) have summarized this in a multi-partite model, as an extension of the bi-partite model earlier advanced by Naegeli and co-workers (Naegeli et al., 1992; Hess et al., 1997b), describing the different characteristics determining the fate of the adduct, i.e. being recognized and repaired or not. The multi-partite model includes among other factors the presence of a chemical alteration of DNA, distortions such as disruption of the Watson-Crick hydrogen bonding, base displacement, and helix bending.

When it comes to DNA adducts derived from the reaction with PAH DEs, striking differences in relative repair susceptibilities between stereoisomeric adducts have been observed. This is also the case with two of the most studied DNA adducts concerning the relationship between structure and repair activity, i.e. those derived from the *anti* enantiomers of BPDE (bay) and BcPhDE (fjord). Based on studies using oligonucleotides specifically modified with these two DEs and in vitro NER assays, high resolution NMR, molecular dynamics and studies on thermal stabilities, Geacintov et al. have summarized the effect of adduct conformation on the efficiency of NER (Geacintov et al., 2005). The adducts removed with highest efficiency are those intercalated and showing a base displacing effect, e.g. *cis*-BP-dG (**Figure 9A**). In this case, the intercalated position of the aromatic residue leads to severe rupture of proper Watson-Crick bonds between the modified guanine and the adjacent cytosine and subsequently perturbation of the helix. Second most easily repaired are intercalated adducts but without a base displacing effect, e.g. *trans*-BP-dA. In this case, the aromatic moiety does not affect the hydrogen bonding or severely distort the helix structure. This is most likely due to the structural discrepancy between *cis* and *trans* adducts as mentioned above. Further, adducts localized externally and in the minor groove, as in the case of *trans*-BP-dG, are to some degree removed with less efficiency. This difference is most likely attributed to the lower degree of distortion of the DNA helices compared to the stereoisomeric *cis*-BP-dG.

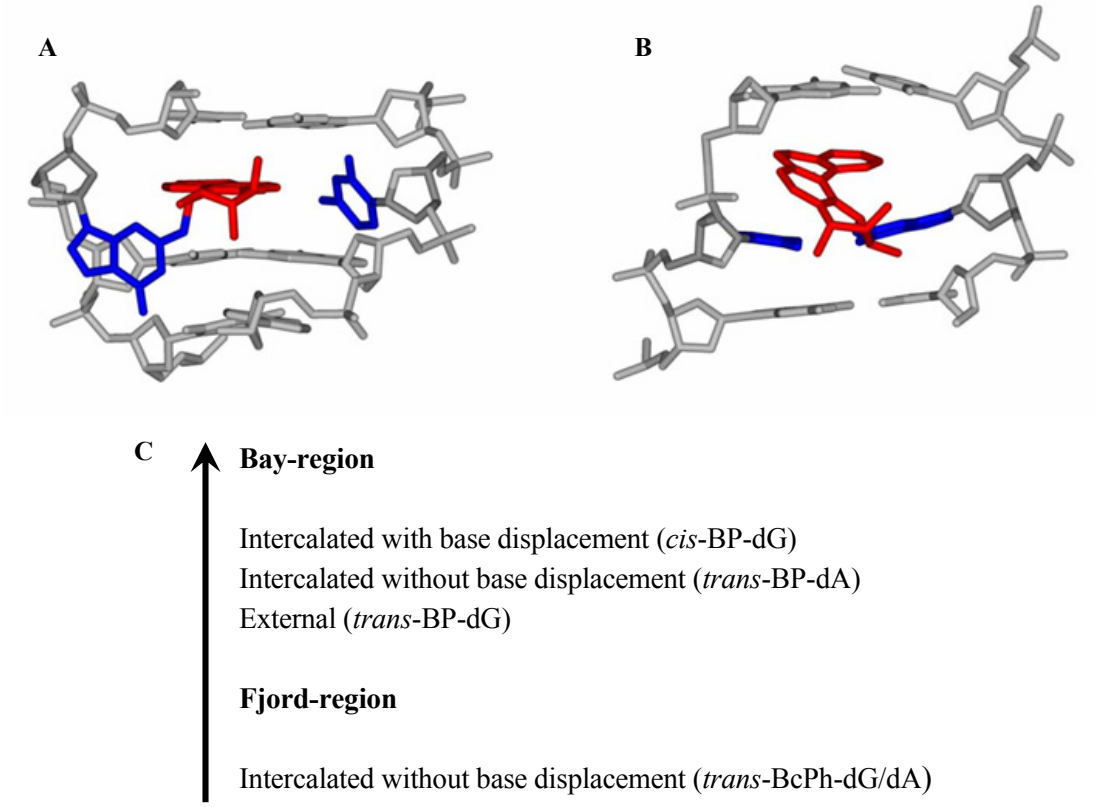


Figure 9. Structure of adducts derived from PAH DEs. A) *cis*-BP-dG; an adduct with base-displaced intercalative conformation, as clearly can be seen by the heavily displaced base pair (Cosman et al., 1993). B) *trans*-BcPh-dG; an intercalated adduct without base-displacing as a result of optimal base stacking (Lin et al., 2001). PAH DE adducts in red and the affected base pairs in blue. C) PAH DE adducts placed in order of precedence of being recognized and removed by NER.

Interestingly, studies in mouse skin indicate that externally localized DNA adducts of both BPDE and DBPDE are removed more rapidly than adducts with internal localization (Suh et al., 1995; Jankowiak et al., 1998). This implies that there is no simple correlation between activities obtained from in vitro experiments and the activities observed in complex cellular systems. Comparing adducts derived from BPDE and BcPhDE, the most refractory adducts are those derived from the fjord-region DE *anti*-BcPhDE. Even though both *trans*-BcPh-dA and -dG adducts have an intercalated conformation (**Figure 9B**) they are removed with much lower efficiency. The explanation for this remarkable difference is the different topologies of the aromatic ring systems of the fjord BcPh and bay BP residues. Whereas the latter is planar and rigid (**Figure 9A**), the flexible distortion of fjord-region DEs allows the residue to adapt according to the bases and thus optimize base stacking (**Figure 9B**). This in turn leads to intact Watson-Crick base pairs, undisturbed helix structure and consequently less efficient recognition and subsequent repair by the NER machinery (Geacintov et al., 2005). Whether this also is true for larger fjord-region DEs is still unclear. However, thermal melting experiments, looking at the effect of the DE lesion on the "melting temperature" T_m of the DNA duplex, indicates that also the larger BgCDE and DBPDE to a very small extent destabilize the base pairing and helix structure (Ruan et al., 2002). In addition, available information from experiments looking at differential repair of fjord- vs. bay-region DE-DNA adducts in cell-free human systems as well as mammalian systems indicates the presence of a comparable preference (Luch et al., 1999; Buterin et al., 2000; Lloyd and Hanawalt, 2002). This will be discussed in more detail in the section **Present investigation** and in conjunction to papers IV and V.

To conclude, the susceptibility of different PAH-DNA lesions to NER depend on; the stereochemistry of the adduct and the base modified (dG or dA), adduct conformation, and finally the extent of distortion on DNA caused by the adduct.

Glutathione transferases

Glutathione transferases (GSTs) catalyze the nucleophilic attack of glutathione (GSH) on an electrophilic center. The GST enzyme family is mainly involved in detoxification, and in some cases, activation of a wide variety of chemicals. The first report on enzyme-catalyzed GSH conjugation dates back to 1961 when cytosolic fractions of rat liver were observed to catalyze nucleophilic substitution reactions of aromatic halogen compounds (Booth et al., 1961; Combes and Stackelum, 1961). The GSTs catalyze numerous reactions including nucleophilic aromatic substitution reactions, epoxide ring openings, reversible Michael additions, isomerizations, and peroxidase reactions, to name a few (Eaton and Bammler, 1999). These enzymes have been found in almost every organism examined, including bacteria, plants and animals (Hayes and Pulford, 1995). The GST system is multi enzymatic and fall into two distinct superfamilies: the soluble GSTs and the microsomal membrane-bound GSTs. In the soluble family, the cytosolic and mitochondrial GSTs, comprise soluble enzymes that are structurally and evolutionary distantly related (Ladner et al., 2004; Robinson et al., 2004).

The microsomal GSTs are members of the family of membrane bound enzymes called MAPEG (Membrane Associated Proteins involved in Eicosanoid and Glutathione metabolism) (Jakobsson et al., 1999). The first enzyme of the MAPEG family was discovered in 1980 (Morgenstern et al., 1980). To date, six family members have been identified (Jakobsson et al., 1999). Two of the members are crucial for the endogenous metabolism of physiologically important reactive lipophilic intermediates (leukotrienes) (Dixon et al., 1990; Miller et al., 1990; Mancini and Evans, 1993), and three are cytoprotective exhibiting glutathione transferase and peroxidase activities in the detoxification of highly reactive lipophilic compounds of exogenous and endogenous origin (Jakobsson et al., 1996; Jakobsson et al., 1997).

Soluble human glutathione transferases

Classification and nomenclature

The human cytosolic GSTs occur in multiple forms, thus a concise classification and nomenclature is needed (Mannervik, 1985; Mannervik et al., 1992). The classification is based on the proteins primary structure, i.e. their amino acid sequence. It is generally accepted that GSTs that share more than 40-50% identity are included in the same class, and those that possess less than 25-30% identity are assigned to separate classes. Among the soluble GSTs up to date at least eight distinct gene classes are known: Alfa, Kappa, Mu, Omega, Pi, Sigma, Theta, Zeta and more than 16 proteins have been characterized in human (Hayes et al., 2005). They can form either hetero- or homodimers whereas heterodimers only can be formed between subunits within a class (Mannervik and Widersten, 1995; Rowe et al., 1997). The subunits are denoted by Arabic numerals. For example, GSTA1-1 describes the homodimer of two GSTA1 subunits. Lower case letters (h for human, m for mouse etc) preceding GST denotes the

species. In the instance of allelic variants of isoenzymes, a lower case letter is used for designation, e.g. GSTM1a-1a.

Tissue distribution and expression

GSTs have been found in almost all organs in the human body, with the highest levels in the testis and the liver (Rowe et al., 1997; Awasthi et al., 1994). The profiles of the individual isoenzymes differ significantly among different tissues. Alpha class GSTs are, in general, relatively highly expressed in kidney, testis and liver, where it can represent as much as 2-3 % of the total cytosolic protein. Depending on the specific sub-family, Mu class GSTs are expressed to different extents in different tissues. GSTP1-1 is expressed in relatively high levels in brain and lung, but not in liver. Theta class GSTs are expressed predominantly in liver and kidney, and only at relatively low levels in lung (Rowe et al., 1997), whereas Sigma and Omega classes are mainly expressed in liver (Hayes and Strange, 2000). Kappa class GSTs, so far only found in mitochondria and peroxisomes, appear to be widely expressed in human tissue (Morel et al., 2004). In addition, different GST subunits are expressed in a tissue-specific manner, giving raise to a very complex enzyme pattern.
In common with other phase II enzymes, levels of expression of GST can be significantly induced by exposure to xenobiotica. The induction of GST by xenobiotics is mediated through different transcriptional mechanisms. Several GST genes contain antioxidant response element (ARE), xenobiotic response element (XRE), glucocorticoid response element (GRE) and cAMP response element (CRE) (Hayes and Pulford, 1995; Jhaveri and Morrow, 1998). PAHs, such as 3-methylcholanthrene (3-MC) and BP, have been shown to induce the expression of GSTs in rodents via binding to the Ah-receptor which in turn interacts with the XRE of the gene in question, the mechanism is most likely the same in humans (Hayes and Pulford, 1995).

3D structure

Chronologically, the following human GSTs have been determined by X-ray diffraction analysis; GSTP1-1 (Reinemer et al., 1992), GSTA1-1 (Sinning et al., 1993), GSTM2-2 (Raghunathan et al., 1994), GSTT2-2 (Rossjohn et al., 1998), GSTA4-4 (Bruns et al., 1999), GSTM3-3 (Patskovsky et al., 1999b), GSTM1a-1a (Patskovsky et al., 1999a), GSTO1-1 (Board et al., 2000), GSTZ1-1 (Polekhina et al., 2001), and GSTA3-3 (Gu et al., 2004). Up to date more than 130 different structures of GSTs have been determined, originating from all species spanning from yeast to human, including point mutated, fused, and proteins with or without ligand etc (Berman et al., 2000). A number of GST structures containing PAH related ligands have been determined, including two hGSTP1-1 structures with GSH conjugate of (+)-*anti*-BPDE (Ji et al., 1999) and one with conjugated phenanthrene DE (Ji et al., 1997).
As a common feature, the cytosolic GSTs are composed of two subunits related to one another by a 2-fold symmetry axis. Between the two domains is a short variable linker region of 5-10 residues. In spite of differences in amino acid (aa) sequences all soluble GST has basically the same protein fold, which consist of two structural domains. The N-terminal domain is composed of mixed α/β-structures and contains the G-site. The C-terminal domain contains many, though not all, of the aa residues forming the H-site

and consists of α-helices. The active site on each subunit of the GST molecule consists of a G-site (GSH-binding site) and an H-site (hydrophobic substrate binding site). The G-site is well conserved between GST-classes with a high specificity for GSH whereas the H-site shows a great variation between the classes, and thus allows interactions with different electrophilic substrates, resulting in a broader and more variable substrate binding specificity (Mannervik and Widersten, 1995; Armstrong, 1997). Looking closer at the three most common GSTs, classes Alpha, Mu and Pi, some notable differences are evident in the region of the active site. The Alpha class GST contain an extra C-terminal α-helix, this helix covers the substrate bound in the H-site (**Figure 10A**), which is thought to explain the preference of Alpha class GSTs for more hydrophobic compounds (Allardyce et al., 1999). The Mu class enzyme contains an extra loop, restricting the active site from the environment (**Figure 10B**). In contrast, GSTP1-1 lacks both of these structural features and has an active site, which appears to be more accessible for substrates (**Figure 10C**) (Mannervik and Widersten, 1995; Armstrong, 1997).

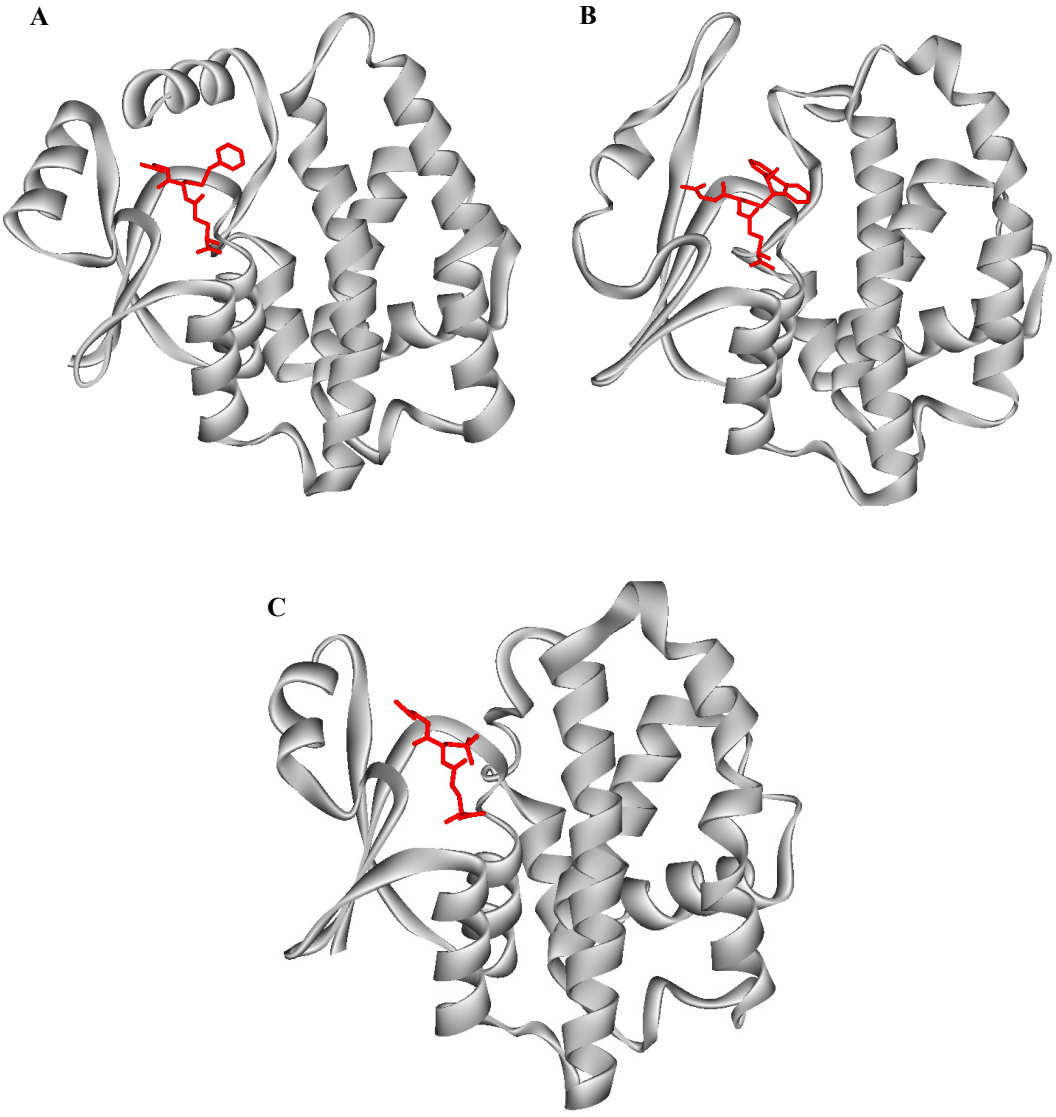


Figure 10: Comparison of the active sites and their structures between monomeric hGSTA1-1 (A), hGSTM1-1 (B), and hGSTP1-1 (C). The structures are obtained from references mentioned above.

GSH conjugation

All GSTs possess the ability to conjugate GSH with compounds containing an electrophilic centre with the general reaction:

$$R\text{-}X + GSH \rightarrow GSR + HX$$

The G-site is responsible for the most fundamental aspect of the catalytic mechanism, namely binding the first substrate GSH and activating its sulfur for nucleophilic attack. The nucleophilic sulfhydryl group of cysteine in GSH, -SH, plays an important role in reactions with electrophilic compounds. The cloud of electrons surrounding the nucleus of the sulfur atom is highly polarizable and thus making the sulfhydryl group a soft and efficient nucleophile for reactions with electrophilic compounds. This is accomplished via a number of electrostatic and hydrogen-bonding interactions and a lowering of the pK_a of the thiol group of GSH from 9.0 to about 6.5 (Armstrong, 1997). The class Alpha, Mu, and Pi enzymes have recruited the hydroxyl group of a tyrosyl residue located near the polypeptide to activate the sulfhydryl group of bound GSH (Liu et al., 1992; Kolm et al., 1992; Wang et al., 1992). Additionally, the class Alpha enzyme has recruited yet another residue into the inner coordination sphere of the sulfur, the side chain of Arg15 (Björnestedt et al., 1995). The at least as important role of the H-site is to harbor the electrophilic substrate mainly through hydrophobic interactions facilitating the conjugation reaction (Armstrong, 1997).

Although the conjugation of GSH to lipophilic substrates increases the water solubility, and as a result facilitates the removal from the cell, this might not be the most important function of GSTs. According to Hayes et al. (Hayes and Pulford, 1995), the major biological benefit of GSH conjugation of lipophilic compounds lies in the tagging event, signaling an export of the conjugate from the cell, rather than an increase of the compounds solubility. The export of GSH conjugates from the cell is actively performed by the multidrug resistance protein (MRP) family, an ATP dependent efflux pump with a broad specificity of ionic conjugates e.g. GSH conjugates (Ishikawa, 1992; Commandeur et al., 1995; Ishikawa et al., 2000). Nine MRP proteins have to date been identified (Haimeur et al., 2004), and these are all members of the C family of the ABC transporters. Among these, MRP1 and MRP2 can export GSH conjugates (Paumi et al., 2001; Morrow et al., 2000). The conjugates are then excreted in bile, via the kidney, mostly as mercapturic derivatives.
Studies by Srivastava et al. have shown that the MRP2 (cMOAT/MDR2) pump is responsible for the efflux of (+)-*anti*-BPDE GSH-conjugates and that this event is important for avoiding product inhibition of GST activity as well as protection against BPDE induced DNA damage (Srivastava et al., 1998; Srivastava et al., 1999; Srivastava et al., 2002).

Substrates and specificities

The GST substrates share three common features; they are usually lipophilic, possess an electrophilic centre, and are to some extent conjugated non-enzymatically with GSH (Parkinson, 1996). In general, the transferases display overlapping substrate specificities, with known substrates spanning from environmental pollutants to drugs and endogenous molecules (Eaton and Bammler, 1999).

One of the first substrates to be discovered, and still of great importance is 1-chloro-2,4-dinitrobenzene (CDNB) (Clark et al., 1973), which since has been regarded as the typical GST substrate and a great aid in the early protein characterization (Habig and Jakoby, 1981). As mentioned, catalyzed reactions include conjugation/substitution, conjugation/addition, reduction, isomerization and denitrosation (Mannervik and Widersten, 1995). GSTs are of special interest to toxicologists because they metabolize cancer chemotherapeutic agents such as thiotepa and cyclophosphamide, pesticides and environmental pollutants DDT and lindane, carcinogens such as aflatoxin B_1 and BPDE, and by-products of oxidative stress, for example acrolein and fatty acid hydroperoxides (Hayes and Pulford, 1995). GSTs also show a selenium-independent GSH peroxidase activity toward organic hydroperoxides. Preferences in substrate and reactions catalyzed by human GSTs are summarized in **Table 2**.

Table 2. Substrate or reaction preferences of human glutathione transferases*

Class	Substrates or reaction
Alpha	CDNB, CuOOH, PAH DEs, EA, 4-hydroxynonenal
Kappa	CDNB, CuOOH, (*S*)-15-hydroperoxy-5,8,11,13-eicosatetraenoic acid
Mu	CDNB, AFB_1-epoxide, PAH DEs, styrene-7,8-oxide, $PGH_2 \rightarrow PGE_2$
Pi	CDNB, acrolein, PAH DEs, EA, thiotepa
Sigma	PGD2 synthase ($PGH_2 \rightarrow PGD_2$)
Theta	BCNU, CuOOH, butadiene epoxide, menaphthyl sulphate
Zeta	Dichloroacetate, fluoroacetate, maleylacetoacetate
Omega	Thioltransferase, dehydroascorbate reductase

*adapted from (Hayes and Strange, 2000; Hayes et al., 2005). For abbreviations, please see refs.

As mentioned earlier the major detoxication route of the ultimate carcinogenic bay- and fjord-region PAH DEs is GSH conjugation catalyzed by GSTs. In general, Alpha, Mu and Pi class enzymes are able to conjugate PAH DEs with varying capability. Those that are metabolized by GST include DEs produced from chrysene, methylchrysene, benzo[*c*]chrysene, benzo[*g*]chrysene, benzo[*c*]phenanthrene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, and dibenzo[*a,l*]pyrene (Jernström et al., 1996; Sundberg et al., 1997; Hu et al., 1998; Sundberg et al., 1999). As reported in paper II, Alpha class GSTs, and in particular, A1-1 was shown to be exceptionally efficient in conjugating and detoxifying the highly potent DEs from dibenzo[*a,l*]pyrene. The broad ability of

GSTs to conjugate PAH DEs will be discussed in more detail in the section **Present investigation** in conjunction to papers I and II.

In some cases, conjugation with GSH enhances the toxicity of a xenobiotic. Examples of this are GSH conjugates of dihalomethanes, 1,2-dihaloalkanes and haloalkenes, which in all cases lead to the formation of reactive intermediates (Anders, 2004). This fact has been exploited in cancer chemotherapy using drugs activated by GSTs in efforts targeting tumors overexpressing particular transferases (Lyttle et al., 1994; Morgan et al., 1998). On the other hand, over expression of GST in mammalian tumor cells has been implicated with resistance to various anticancer agents and chemical carcinogens (Hayes and Pulford, 1995).

Another aspect of substrates is those that are not conjugated with GSH but bind covalently or noncovalently to GST. It has been known for many years that GSTs interact more or less covalently with hydrophobic compounds such as 3-MC, steroids, certain hormones, bile acids, heme, and fatty acids. The noncovalent binding activity led to class Alpha GST originally being called Ligandin. The biological significance of these interactions is still unclear but functions such as suicide behavior and intracellular/organ transporters or buffers have been suggested (Hayes and Pulford, 1995).

Polymorphism in GST genes

As mentioned earlier GSTs have been found in almost all organs and tissue in the human body, with the highest levels in the testis and the liver (Rowe et al., 1997; Zimniak et al., 1994), consequently the presence of GST polymorphisms is likely to contribute to interindividual differences in response to xenobiotics. Among the seven cytosolic GST classes, identified genetic polymorphisms have been detected in every one of them (reviewed in Hayes and Strange, 2000; Hayes et al., 2005). The enzymes GSTM1-1 and GSTT1-1 both have a frequently occurring null phenotype, with a incidence of around 50 and 15% in Caucasian populations, respectively (Warholm et al., 1980; Board, 1981; Pemble et al., 1994). This has lead to a number of studies of whether individuals lacking these two enzymes have a higher incidence of bladder, breast, colorectal, head/neck, and lung cancer. In general, individual GST genes do not seem to have a significant impact on susceptibility to cancer, while combinations of polymorphisms (null or "regular") may have modest effects (Benhamou et al., 2002; Hashibe et al., 2003; van Lieshout et al., 1999).

Looking at the studies focusing on the relationship between toxic effects from PAHs, often in correlation to smoking or occupational exposure, and genetic variations in GST genes, the majority of the studies have been focusing on BPDE DNA adducts and the *GSTM1*0* genotype. In a summary of published data on the modulation of (+)-*anti*-BPDE DNA adduct levels in smokers' lung by *CYP1A1* and *GSTM1* genotypes, the correlation was more pronounced in lung parenchyma from persons with the *GSTM1*0* genotype. Furthermore, carriers of the *CYP1A1*2-GSTM1*0* genotype had higher BPDE DNA adduct levels than those with *CYP1A1*1/*1-GSTM1*0* (Alexandrov et al., 2002). Studying coke-oven workers and the effect of *GSTM1*0* genotype on (±)-*anti*-

BPDE DNA adducts in mononuclear white blood cells showed that individuals with null genotype had a significantly higher risk of having high BPDE adduct levels than individuals with active *GSTM1* genotype (Pavanello et al., 2004). Another study looked at the correlation between levels of BPDE DNA adducts in leukocytes from smokers and polymorphism of *GSTM1*, *GSTP1* and *CYP1A1*. Their results showed that subjects in the "high-risk" group, *i.e.* polymorphic in all three genes, had higher levels of BPDE DNA adducts compared to those having a "normal" genotype (Lodovici et al., 2004). In summary, individuals smoking or with a "high exposure" occupation which also are carriers of genetic polymorphisms in GST genes generally show a higher level of PAH derived DNA adducts compared to controls. It is possible to assume that subjects with enhanced hereditary capacity to activate, and/or low capacity to deactivate, PAHs are at higher risk of developing cancer when exposed to cigarette smoke or other PAH-polluted sources.

PRESENT INVESTIGATION

Aim of the study

The aim of the study was to gain further knowledge about the biotransformation of PAH DEs catalyzed by human GSTs and in addition to learn more about differences in DNA adduct formation and removal. These were studied separately and in conjunction to each other, i.e. the impact of GSH conjugation on levels of DNA damage. We have used methods representing different scientific approaches; theoretical, molecular, in vitro, and cellular, in order to elucidate the area in different aspects. By using BPDE and DBPDE, two of the most potent PAH DEs with distinct biophysical and structural features, as our test compounds have we not only studied a bay- and a fjord-region DE, but also coherently throughout the project studied the effect of lipophilicity, reactivity, and structure on biological activity, i.e. conjugation and DNA adduct formation and removal.

Results and discussion

As mentioned earlier, GSTs and more specifically Alpha, Mu and Pi class enzymes are capable of conjugating PAH DEs, although with varying efficiency. A number of articles have been published by our laboratory and others on the activity of purified human GSTs A1-1, M1-1, and P1-1 towards a number of diastereomeric and enantiomeric bay- and fjord-region DEs (Jernström et al., 1996; Sundberg et al., 1997; Sundberg et al., 1998a; Sundberg et al., 1998b; Hu et al., 1998). Although the information from experiments with cells expressing GSTs is limited, evidence points to a key role in DE-detoxification and, thus, protection against DNA adduct-formation (Romert et al., 1989; Hesse et al., 1990; Fields et al., 1994; Hu et al., 1999). The results presented in papers I, II, and III extend and verify knowledge in this field regarding activities, preferences, and the effect of GSH conjugation on formation of DNA adducts. Moreover, the papers discuss the impact of biophysical properties on activity and preference.

Apart from variations in detoxication, the difference in biological activity of bay- and fjord-region PAHs can be ascribed to the following facts:

1. The fjord-region category is expected to be distorted due to the inherent steric crowding of the molecule (Katz et al., 1998; Little et al., 1999).
2. Fjord-region DEs generally bind more extensively to DNA than the bay-region analogues and predominantly react with adenine residues rather than guanine in DNA (Koreeda et al., 1978; Giles et al., 1995; Ralston et al., 1995).
3. Adducts of fjord-region DEs distort DNA less than the more rigid bay-region DEs leading to a less efficient adduct recognition and removal by NER (Geacintov et al., 1997; Guo et al., 2003; Geacintov et al., 2005).
4. p53-mediated adduct recognition and cell cycle arrest seem to be different with fjord-region DEs as compared with bay-region ones (Binkova et al., 2000; Lloyd and Hanawalt, 2002).

The correlations as well as the effect of the four points is presented and discussed in papers III, IV, and V.

GSH conjugation

Structure rather than reactivity as a determining factor of activity and preference
GSTA1-1 has in previous studies been shown to have substantially higher activity than that of human GSTs P1-1 and M1-1 against the potent fjord-region DEs of benzo[*c*]phenanthrene, benzo[*c*]chrysene and benzo[*g*]chrysene (Jernström et al., 1996; Sundberg et al., 1997). Recent work has also shown that alpha class GSTs from mouse are highly active with fjord-region DEs (Hu et al., 1998; Xia et al., 1998). In paper II, we investigated the catalytic activities of GSTs A1-1, A2-2, A3-3, and A4-4, toward the ultimate carcinogenic (+)-*syn*- and (–)-*anti*-DBPDE, and (+)-*anti*-BPDE (with *R* configuration at the benzylic carbon), respectively. Our results showed that GSTA1-1 demonstrated activity with all DEs tested whereas A2-2 and A3-3 only were active with the DBPDE enantiomers (**Table 3**). With GSTA4-4, no detectable activity was observed. This was explained by molecular modeling clearly indicating that the main reason for the lack of activity with the DEs and GSTA4-4 is a shallower and narrow H-site, which cannot accommodate substrates of this class. In a more recent study, activity against (+)-*anti*-BPDE of both hGSTA1-1 and A2-2 was reported. However, the activity of GSTA2-2 was 5-fold lower compared to GSTA1-1 (1.1 vs. 0.2 $mM^{-1}s^{-1}$) (Singh et al., 2004). By using site-directed mutagenesis, the authors showed that the single aa of Ile-11 was responsible for the differences in catalytic activities.

Table 3. Kinetic constants for human GSTA variants A1-1, A2-2, and A3-3 toward (–)-*anti*- and (+)-*syn*-DBPDE

	k_{cat}/K_m ($mM^{-1}s^{-1}$)		
	(+)-*anti*-BPDE	(–)-*anti*-DBPDE	(+)-*syn*-DBPDE
GSTA1-1	2.6	66.3	464
GSTA2-2	nd[a]	3.4	30.4
GSTA3-3	nd	16.2	190

[a]nd; not detectable.

As shown previously with GSTA1-1 and the fjord-region DEs discussed above, a marked preference for catalyzing *S*-glutathionylation of the (+)-*syn*-enantiomers was observed (Jernström et al., 1996). This was also the case with DBPDE, (+)-*syn*-DBPDE was superior as substrate compared to the (–)-*anti*-enantiomer with GSTA1-1 as the most efficient enzyme with a notable k_{cat}/K_m of 464 $mM^{-1}s^{-1}$, followed by A3-3 and A2-2. With (–)-*anti*-DBPDE, the k_{cat}/K_m values were in general about 10-fold lower. Molecular modeling was used in order to reveal factors governing substrate-enzyme interactions and catalytic efficiencies. The docking calculations of free DBPDE enantiomers or their GSH conjugates in GSTA1-1 show that the large aromatic residue of DBPDE fits well into the H-site where it interacts with the aromatic side chains of Phe10 and Phe220. In addition, a number of key amino acids have been identified lining the rest of the active site creating a hydrophobic cavity, large enough to accommodate the bulky DBPDE molecules. Interestingly this is not in line with what has been indicated by another study suggesting the narrow H-site of hGSTA1-1 as the

structural basis for the high difference in activity of human and mouse GSTA1-1 towards *anti*-BPDE (Gu et al., 2000).
Comparing the GSH conjugates derived from (+)-*syn*-DBPDE and (–)-*anti*-DBPDE, respectively, with molecular modeling, explained the higher activity of GSTA1-1 for the former by demonstrating more favorable interactions between the substrate and the enzyme-GSH complex. Comparing the conformation of the molecules reveals that the position of the hydroxyl group at position 12 is drastically affected depending on the absolute configuration of the hydroxyl groups i.e. *R,S* or *S,R* and, in the case of (+)-*syn*-DBPDE (*S,R*) this exposes the benzylic oxirane carbon for a more favorable S-glutathionylation. These results show that the spatial orientation of the hydroxyl groups are important determinants for the catalytic efficiency and thus responsible for the observed 10-fold difference in catalytic activity.
Replacing (–)-*anti*-DBPDE with the less structurally distorted (+)-*anti*-BPDE, revealed that GSTA1-1 was 25-fold less active. Compared to BPDE, DBPDE is considerably more lipophilic due to the additional benzo ring and, due to the structural distortion of the molecule more bulky. Previous work with human GSTA1-1 and the less complex fjord-region DEs from benzo[*c*]phenanthrene, benzo[*c*]chrysene and benzo[*g*]chrysene indicated that increased complexity of the aromatic ring system and thus, increased lipophilicity of the substrate was associated with increased catalytic efficiency (Jernström et al., 1996). A correlation between increased lipophilicity and activity has been observed with mammalian GSTs and 4-hydroxyalkenals with an increasing number of methylene groups (Danielson et al., 1987). Accordingly, such factors, rather than the inherent chemical reactivity of the benzylic oxirane carbon, seem to promote the interaction and the rate of the subsequent conjugation reaction.

What determines the efficiency of GST in the intact cell?
In paper I we constructed and characterized mammalian V79 cells stably overexpressing human GSTs A1-1, M1-1, and P1-1 in order to study the function of GSTs in a biologically more relevant system. V79 Chinese hamster lung fibroblast cells have been used extensively as indicator cells in mutagenicity and cytotoxicity studies. V79 cells lack CYP450 expression but contain significant amounts of Pi class GST. However, despite this the cells lack the ability to conjugate (+)-*anti*-BPDE with GSH (Swedmark et al., 1996). In our study, the V79MZ clone was used. This clone has frequently been used in studies on various xenobiotic-metabolizing enzymes (Glatt et al., 1991).
The three GST overexpressing clones used in this study were denoted V79MZhA1-1, M1-1, and P1-1, respectively. These cell lines were characterized with respect to total and cytosolic protein contents, and amounts and concentrations of GSH and active GST (Paper I Table 2). The V79 clones were further characterized by estimating the cytosolic GST activity toward (+)- and (–)-*anti*-DBPDE.
The cytosolic fractions obtained from the V79MZhA1-1 and V79MZhM1-1 cells catalyzed GSH conjugation of both enantiomers, whereas cytosol from the V79MZhP1-1 cells was only active toward (–)-*anti*-DBPDE, the enantiomer with *R*-absolute configuration at the benzylic oxirane carbon (**Table 4**). The exclusive preference observed with GSTP1-1 is fully consistent with previous results using the pure enzyme (Sundberg et al., 1997). For comparison, the results from experiments using pure enzymes were included. Surprisingly, the apparent k_{cat}/K_m observed in the

GSTA1-1 cytosol was substantially reduced. In fact, only 10 % of the activity was observed with both *anti*-DBPDE enantiomers. However, upon isolation from the cytosolic fraction GSTA1-1 regained full activity indicating that factors present in the V79MZhA1-1 cytosol are responsible for the suppressed activity rather than a defective protein. Activity of GSTA1-1 against DBPDE and BPDE in presence of proteins (bovine serum albumin, cell extract, and cytosol) has been measured (unpublished data). Rather than inhibiting the GSTA1-1 catalyzed conjugation reaction a marked stimulation was observed. In contrast to what was seen above, the results from these relatively simple in vitro experiments indicate that the possible GSTA1-1 inhibitor is not of cytosolic nor cell extract origin.

In agreement with previous results with the potent fjord-region DEs of benzo[*c*]phenanthrene, benzo[*c*]chrysene and benzo[*g*]chrysene (Jernström et al., 1996; Sundberg et al., 1997), the activity of recombinant GSTA1-1 toward the *anti*-DBPDE enantiomers was substantially higher than that of human GSTs P1-1 and M1-1 (**Table 4**).

Table 4. Catalytic efficiencies toward DBPDE in cytosolic fractions and human GSTs isolated from V79MZ transfectants

	GST activity			
	(+)-*anti*-DBPDE ($mM^{-1}s^{-1}$)		(–)-*anti*-DBPDE ($mM^{-1}s^{-1}$)	
	cytosolic fraction	pure enzyme	cytosolic fraction	pure enzyme
V79MZhA1-1	1.1	10.6	6.1	66.3
V79MZhM1-1	1.4	2.3	4.0	5.9
V79MZhP1-1	nd[a]	nd	1.6	1.5

[a]nd; not detectable.

The V79 cells in paper I were also incubated with the *R*-configured *anti*-enantiomers of DBPDE and BPDE, to study the effect of GSH conjugation on DNA adduct formation (see below). Furthermore, the cells were used to attempt to identify factors governing the accessibility of lipophilic DE substrates for GSTs in the intact cell. Lipophilicity and chemical reactivity are factors likely to affect both the accessibility and availability of a compound for GST catalyzed reactions. This was done by calculating the expected rate of GSH conjugation based on the catalytic activity of the pure enzymes and estimated intracellular available amount of BPDE and DBPDE, which allowed us to compare these rates with those observed in the cells. Since the former is about 15-fold more lipophilic than the latter, the concentration of (–)-*anti*-DBPDE in the polar phase is correspondingly lower and, thus less DE is expected to be available for conjugation. On the other hand, (+)-*anti*-BPDE is more chemically reactive than (–)-*anti*-DBPDE and will react more extensively with various nucleophilic targets in the cell. With (+)-*anti*-BPDE it was calculated that up to 2 % of available compound forms GSH conjugates whereas with (–)-*anti*-DBPDE the corresponding figure was about 13 %. The low observed rate with (+)-*anti*-BPDE is most likely due to competing reactions such as hydrolysis and/or more unspecific reactions with cellular constituents. Consistent with this interpretation is the results with the less reactive and more lipophilic DBPDE. Other factors likely to restrict the accessibility of GST and lipophilic substrates in the intact cells are macromolecular crowding and associated

reduced rates of diffusion (Luby-Phelps, 2000; Ellis, 2001). Another factor is the distribution of GSH and GST in different cellular compartments.

Altogether, our data show that GSTA1-1 is the most efficient Alpha class enzyme toward DBPDE with (+)-*syn*-DBPDE as the superior substrate with a k_{cat}/K_m of 464 $mM^{-1}s^{-1}$. The high efficiency was demonstrated by a number of favorable interactions as shown by molecular modeling. This activity is the highest ever observed for a human GST toward a DE substrate and exceeds the values usually obtained with fjord-region DEs by 2 orders of magnitudes (Jernström et al., 1996; Sundberg et al., 1997). Moreover, considering the extraordinary high activity of human Alpha class GSTs, and in particular, the A1-1 isoenzyme, toward the extremely potent carcinogenic DBPDE enantiomers, both the presence and the level of expression may be an important determinant for an individual's susceptibility to this type of compounds. The poor reflection between expected rates of GSH conjugation in our intact V79 cells and those observed underline that there is no simple correlation between activities obtained from experiments with purified enzymes and the activities observed in complex cellular systems.

Structural preferences of DBPDE and biological implications

As seen in paper II, structure is an important determinant of efficiency in detoxication. Moreover, the structural dissimilarities between bay and fjord-region DEs are believed to at least in part be responsible for the observed differences in biological activity (Jerina et al., 1986; Jerina et al., 1991; Glatt et al., 1991). To gain additional information about the structural preferences of fjord-region DEs we used accurate quantum chemical methods (density functional theory, DFT) to calculate energy values associated with different conformations of (+)-*syn*- and (–)-*anti*-DBPDE. To verify the applicability of this method to study PAH DEs, we optimized the geometry on the parent compound DBP for which a crystal structure is known (Katz et al., 1998). To study the transitions between diequatorial and diaxial conformations of the hydroxyl groups on the saturated ring of DBPDE and other PAH DEs we used the smaller benzene DEs (BDEs) as a model compound.

In agreement with recent studies (Ariese et al., 1996; Jankowiak et al., 1997) it was found that DBPDE is distorted due to overcrowding in the fjord-region. In addition, we found that due to the nonplanarity the arene oxide of the fjord-region DEs may be either on the same side of the saturated ring as the distal ring (in) or on the opposite side (out). The results of the calculations on the eight possible DBPDE conformers (Paper III, Figure 4) show that the structures with the lowest energy is in-diequatorial in both *anti* and *syn*. This is in agreement with earlier results on less complex fjord-region DE (Sayer et al., 1981). Calculations of energy profiles on the BDEs showed that the *anti*-diastereomer prefers the diequatorial conformation (>99.99 %), while the *syn*-form has a weak preference for the diaxial conformation (~60 %).

Comparing the DBPDE conformers with the BDE model, the general features of DBPDE are in agreement with the small-scale model. The *anti*-diequatorial conformation is clearly favored over the *anti*-diaxial, and the *syn*-diaxial form is about the same in energy as the *syn*-diequatorial form and seems to depend on the solvent and the molecular conformation. Furthermore, since no crystal structures of DBPDEs are yet available, the present results represent the first attempt to determine the structure of

all of the conformations and configurations of these DEs. The biological implications of in vs. out conformations in fjord-region DEs are difficult to assess. However, apart from differences in detoxication as seen in paper II, the flexibility of DBPDE, due to the diequatorial-diaxial hydroxyl group transitions and the in-out interconversions, makes it likely that this large molecule can be accommodated in DNA without causing any major distortions. This hypothesis is in agreement with recent results on smaller fjord-region DEs, showing the ability of adaptation to the DNA helical structure (Geacintov et al., 2005)

DNA adduct formation and removal

GSH conjugation reduces the formation of DNA adducts

The impact of GSH conjugation on the formation of DNA adducts and tumorigenicity in mammalian cell systems has not been extensively studied (Romert et al., 1989; Hesse et al., 1990; Fields et al., 1994). A previous report from our laboratory presented preliminary results on the GSH conjugation and DNA adduct formation of PAH DEs in mammalian cells overexpressing human GST (Sundberg et al., 2000). To extend previous studies and study the proposed effect of GSH conjugation by individual human GSTs isoenzymes on DNA protection in a biologically relevant system the V79 cells overexpressing individual GSTs (as mentioned above) were incubated with the *R*-configured *anti*-enantiomers of DBPDE and BPDE (Paper I).

The rates of conjugation found in the cells fairly well reflected the order of catalytic efficiencies obtained with the pure enzymes (Jernström et al., 1996; Sundberg et al., 1997), although GSTA1-1 was found to be strongly inhibited when expressed in cells (10 % of fully functional protein). Increased GSH conjugation of (–)-*anti*-DBPDE was associated with a reduction in DNA adduct formation. GSTA1-1 inhibited the formation of adducts more than 6-fold and GSTM1-1 and GSTP1-1 about 2-fold. Relative to control cells, GSTM1-1 was found to inhibit DNA adduct formation of (+)-*anti*-BPDE most effectively followed by GSTP1-1 and GSTA1-1. The higher biological activity of DBPDE was clearly reflected in the 10-fold higher amount of adducts compared to BPDE observed in the V79MZ control cells. This is consistent with previous findings showing that fjord-region DEs bind more extensively to DNA than bay-region ones (Koreeda et al., 1978; Giles et al., 1995; Ralston et al., 1995). The result on GSH conjugation and DNA adduct formation are summarized in **Figure 11**.

Although GSTP1-1 showed the highest capacity to form GSH conjugates with (+)-*anti*-BPDE (30-fold), GSTM1-1 offered the best protection against DNA adduct formation (12-fold), followed by GSTP1-1 and GSTA1-1. The reason for the discrepancy between the extent of GSH conjugate formation and protection against DNA adducts is not known, but factors such as differences in intracellular distribution of different GSTs, e.g. cytosol vs. nuclei and the ability to trap/transport (+)-*anti*-BPDE by binding at other sites than the H-site may be involved. Taken together, although strongly inhibited GSTA1-1 was found to be most efficient in protection against (–)-*anti*-DBPDE induced DNA damage in cells whereas GSTM1-1 was most efficient with (+)-*anti*-BPDE.

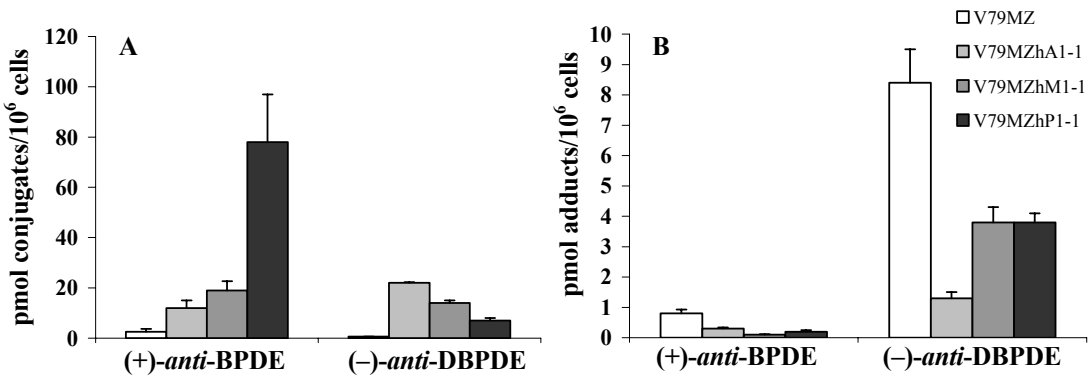


Figure 11. GSH conjugation (A) and DNA adduct formation (B) in control and GST overexpressing cells incubated with (+)-*anti*-BPDE or (–)-*anti*-DBPDE.

Are DNA adducts differently repaired between bay- and fjord-region DEs?
Returning to the points presented above listing the probable reasons for the difference in biological potency between bay- and fjord-region PAHs, they all involve the interaction with DNA and the subsequent cellular response, i.e. DNA adduct recognition and repair, and the effect on the cell cycle etc. Previous findings in cell-free human systems, cultured cells, and in rodents (Buterin et al., 2000; Luch et al., 1999; Jankowiak et al., 1998) propose a difference in NER-coupled removal of DNA adducts derived from fjord- or bay-region DEs. In addition, adduct recognition and p53-mediated cell cycle arrest in human fibroblasts seem to be different with fjord-region DEs (dA adducts) as compared with bay-region ones (dG adducts) (Binkova et al., 2000; Lloyd and Hanawalt, 2002). In order to further investigate and establish the suggested differences we compared the formation and removal of adducts as a function of time formed by the carcinogenic metabolites derived from DBPDE and BPDE in A549 human epithelial lung carcinoma cells (papers IV and V). The A549 cells were chosen since they are a stable human cell line of pulmonary origin and readily used in studies looking at different toxicological aspects of DNA adducts (Shellard et al., 1993; Khan and Anderson, 2001; Tan et al., 2001; Hsia et al., 2002; Chen et al., 2003). Moreover, important in the context of cell cycle regulation A549 cells express wild-type p53 (Jia et al., 1997).

The treatment with 0.1 μM (–)-*anti*-DBPDE in paper V resulted in an initial increase of adducts to a maximal level of adducts after 1 hr of incubation (**Figure 12**). This was followed by an apparent, although not statistically significant, slow removal of adducts. In cells treated with 1.0 μM (+)-*anti*-BPDE the maximal level adducts was reached within 20 min of exposure (**Figure 12**). The formation was followed by an initial rapid decline in the adduct level and a later statistically significant 10-fold slower rate of adduct removal. The biphasic pattern of BPDE removal has been observed previously in mammalian cells (Shinohara and Cerutti, 1977; Feldman et al., 1978; Yang et al., 1980) and, at least in part, may reflect the action of TCR and the subsequent GGR.

As seen in paper I, we observed a 10-fold higher potency in forming DNA adducts of DBPDE compared to BPDE at equal concentrations. Comparing the rate of removal of adducts derived from BPDE with those of DBPDE, the latter are obviously more refractory to the NER coupled repair than the former. This is consistent with results

from *in vitro* experiments using well-defined DE modified oligonucleotides and human cell extracts showing that adducts derived from fjord PAH DEs are, in contrast to analogous adducts derived from the bay-region BPDE, refractory to NER-mediated repair (Hess et al., 1997a; Buterin et al., 2000; Lin et al., 2001; Geacintov et al., 2002; Wu et al., 2002). Differential repair of fjord- vs. bay-region DE-DNA adducts in mammalian systems has not been extensively studied. However, except our studies available information indicates that DE-DNA adducts derived from fjord-region PAHs such as BgC and DBP are removed at lower rates than adducts from BPDE (Luch et al., 1999; Lloyd and Hanawalt, 2002).

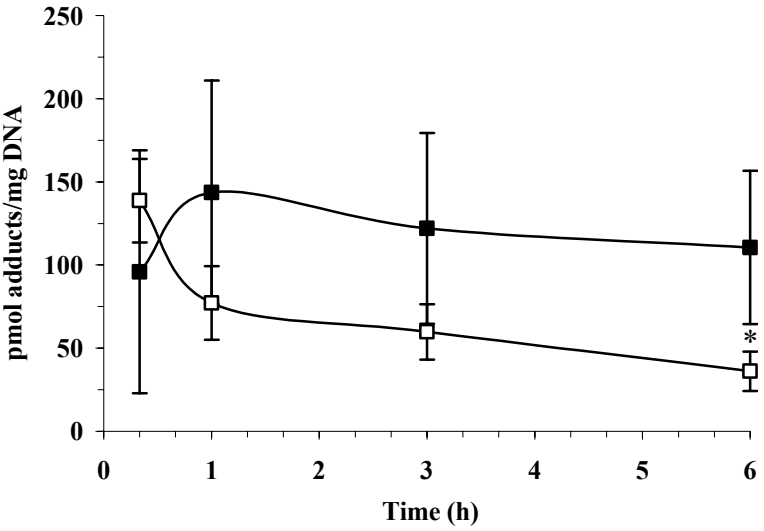


Figure 12. Time-dependent removal of DNA adducts from (+)-*anti*-BPDE (□) and (–)-*anti*-DBPDE (■) in A549 cells.

Prior to paper V we published a preliminary report (paper IV) on differences in DNA formation and removal between BPDE and DBPDE. In this study we observed no significant difference in rates of adduct removal between (+)-*anti*-BPDE and (±)-*anti*-DBPDE. Furthermore, in paper IV an enzymatic DNA hydrolysis and a HPLC procedure was developed making it possible to quantify and identify all possible dA- and dG-adducts. This procedure was further developed in paper V.

Consistent with earlier studies (Koreeda et al., 1978; Giles et al., 1995; Ralston et al., 1995) we observed a pronounced preference for adduct formation on dA for DBPDE and dG for BPDE. In addition, we observed a significant increase in relative contribution of the R-*cis*-DBPDE-N^6-dA adduct as a function of time. Further analysis of DNA adduct distribution as a function of incubation time revealed that the ratio of dA or dG-adduct, respectively, for BPDE was independent of time, 96 % dG, whereas the corresponding ratio for DBPDE was significantly increased from 74 % dA at 20 min to 80 % dA after 6 hrs of incubation. This is an interesting observation, since the exceptional high carcinogenicity of fjord PAHs is believed to arise from these compounds' preference to form dA adducts (Dipple and Bigger, 1991; Ralston et al., 1995).

Preliminary results in paper IV from global gene expression analysis, with focus on genes involved in DNA-maintenance and cell cycle checkpoints, demonstrate interesting differences in the stress response elicited in the cells following exposure to the distorted and flexible non-planar DBPDE or the rigid and planar BPDE molecule. As expected some major common induction events were clearly related to the activation of p53-dependent cell cycle checkpoint. Others included damage-specific damaged DNA binding protein 2 (DDB2) and XP-C repair complementing protein p125 (XPC).

The results presented here on DNA adduct removal in mammalian cells demonstrate that adducts derived from (–)-*anti*-DBPDE, a fjord-region DE, is significantly more refractory to NER coupled repair than the adducts derived from the bay-region (+)-*anti*-BPDE. Moreover, we observe a significant increase in ratio of dA/dG adducts for DBPDE, indicating that dA adducts are especially refractory to repair. Furthermore, the data obtained from the gene expression analysis clearly underline the importance of adduct structure and conformation in dictating the biological response.

Conclusions

From our results using purified human GSTs and potent fjord-region DEs it can be concluded that lipophilicity and structural features are factors that are more important rather than reactivity in the GST mediated detoxication. This was shown by comparing the enzymatic activities of human Alpha GST class against ultimate carcinogenic DEs of DBP and BP. The impact of structure was explained by using molecular modeling, showing significant differences in interactions between diastereomeric DEs and the enzyme-GSH complex. Furthermore, our data show that GSTA1-1 is the most efficient enzyme toward DBPDE with (+)-*syn*-DBPDE as the superior substrate with a remarkable high k_{cat}/K_m of 464 $mM^{-1}s^{-1}$.

In attempts to identify factors governing the accessibility of lipophilic DE substrates for GSTs in the intact cell, we observed a surprisingly low fraction of DBPDE and BPDE available for conjugation. This was calculated by comparing the observed rate of conjugation with the expected, based on catalytic activities obtained from pure enzymes and estimated intracellular available amount of substrate. Possible explanations are likely differences in GST distribution and macromolecular crowding leading to reduced rates of diffusion.

Investigating preferences in structure of (+)-*syn*- and (–)-*anti*-DBPDE showed several levels of flexibility. Because of the distorted structure, the molecule can readily flip its DE moiety relative to the aromatic ring system (“in” and “out”). Furthermore, the hydroxyl groups on the saturated DE ring were found to be either in a diequatorial or in a diaxial conformation, also with low energy barriers indicating an easy transition from one to the other. Our results showed a lower energy profile and thus a preference for the in-diequatorial conformation for both DEs. The possibility of transversions on different levels might have biological consequences, both in detoxication and in DNA adduct formation, adapting to DNA and thus escape recognition/repair by the DNA repair machinery.

In the V79 cells, an increase in GST activity was generally associated with a decrease in DNA adduct formation. Compared to BPDE, DBPDE demonstrated a 10 fold higher DNA binding at equal concentrations, in accord with the high biological activity observed by others. GSTA1-1 showed the highest GSH conjugation capacity and inhibited the formation of DBPDE adducts to the highest degree. With BPDE, GSTP1-1 was most active in GSH conjugate formation while GSTM1-1 offered the best protection against DNA adducts. In conclusion, this point to a correlation between GSH conjugation and protection against DNA adducts, although not with a direct correlation between conjugate formation and DNA adduct levels.

Our results on differences in DNA adduct formation and removal by the NER machinery demonstrate that DBPDE, as an example of a fjord-region DE, obviously is more refractory to NER than bay-region DEs, in this case BPDE. This is due to the obvious resistance of dA adducts, as shown as the significant increase in dA/dG adduct ratio. Compared to results from in vitro systems we observed less impact of DNA adduct conformation on NER coupled removal. A fact possibly reflecting the more complex situation in cellular DNA or chromatin both with regard to adduct structural features and increased complexity of the DNA repair machinery.

ACKNOWLEDGEMENT

The studies presented in this thesis have been carried out at the Division of Biochemical Toxicology, Institute of Environmental Medicine, Karolinska Institutet. This work would not have been possible without the support of my dear and skilled colleagues and supportive friends and family.

First, I would like to express my warmest thanks to my supervisor Professor Bengt Jernström for giving me the opportunity to work in his laboratory. For always keeping your door open and showing patience and encouragement. Good luck with your Alvis, I am looking forward for a ride!

I would also like to thank my co-supervisor Professor Ian Cotgreave for your endless source of ideas and inspiration.

My co-authors, Prof. Albrecht Seidel for providing PAH DEs and your scientific contribution over the years. Dr. Kyung-Bin Cho and Prof. Astrid Gräslund at Stockholms University, for the theoretical, dense but functional collaboration. :-)
Dr. Ann-Sofie Johansson and Prof. Bengt Mannervik at Uppsala University, for providing enzymes and scientific input. Dr. Erik Nordling and Prof. Bengt Persson at MBB, KI for helping me with the *in silico* experiments. Thank you all for a very nice and fruitful collaboration.

My fantastic students, Linda Stjernström, Donald Besong and Nina Sommerfeld; it has been a pleasure working with you and good luck in the future.

The members of the Biochemical Toxicology group in plan 3: my roommates Therese and Katarina for all the laughter and for almost never being quiet, Åse, Edyta, Ylva, and Louise, for sharing labs and letting me doing it my way…, Ralf for your enthusiasm, Roland, Annci, Johan, Miyuki, Elena, Claudia, Gerd L, Christina and Anna-Lena for creating the best possible working atmosphere. All the former members of the group, particularly Kathrin for introducing me to lab and being the best collaborator imaginable, Richard, Lena, Tiziana, Lina, Jennie, Janne, and Annette.

I would also like to thank the rest of the people in plan 3 for making these years a pleasant journey. Particularly Salomon, David, Ulla, Johan, Helen, Monica, Annika H, Mattias Ö, Sara, Emilie, Gerd P, Annika S, Natasha, Kina, Ingalill, Nicholas, and probably several others…

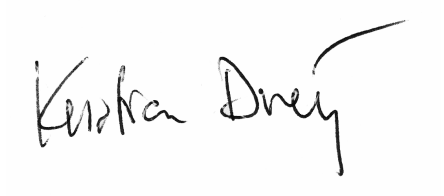
The rest of the friends and colleagues at IMM for spring parties, IMM parties, kräftskivor, IMM pubs, and the occasional scientific(?) TEM conference…

Anders Bergendorff and Toxikologutbildningen for having a great time and for introducing me to toxicology.

My dear friends, for giving me a life outside science, if that's possible…
Especially my JLL friends, for all the beers at MF or in Riga, deep discussions (with party hats…), and PS2/XBOX orgies: Andreas for never dropping a beer and making the best långajlandajstee… Martin, for trying to get me appreciating "obscure" music… Jonas, for always being 100% sure, maybe, and your generous and appreciated drinkparties! Stefan, for being the master of red drinks and a great job as Magnus Härenstam. Bartek, for your never ending source of R-rated stories…
But of course also Ilona, Martina, Maria, Martin and Tanja for your support and friendship.

My family Håkan, AnnMarie, AnnSofie and Pontus for your never-ending support and love, thank you. My sister AnnSofie also for helping me with the layout of the cover.

Last, but not least, *the first the last my everything,* Linda, for being supportive, loving, patient, loving, and wonderful. I love you my gos.



REFERENCES

Adimoolam, S., and Ford, J. M. (2003) p53 and regulation of DNA damage recognition during nucleotide excision repair. *DNA Repair (Amst) 2*, 947-954.

Agarwal, R., Coffing, S. L., Baird, W. M., Kiselyov, A. S., Harvey, R. G., and Dipple, A. (1997) Metabolic activation of benzo[*g*]chrysene in the human mammary carcinoma cell line MCF-7. *Cancer Res 57*, 415-419.

Alexandrov, K., Cascorbi, I., Rojas, M., Bouvier, G., Kriek, E., and Bartsch, H. (2002) CYP1A1 and GSTM1 genotypes affect benzo[*a*]pyrene DNA adducts in smokers' lung: comparison with aromatic/hydrophobic adduct formation. *Carcinogenesis 23*, 1969-1977.

Allardyce, C. S., McDonagh, P. D., Lian, L. Y., Wolf, C. R., and Roberts, G. C. (1999) The role of tyrosine-9 and the C-terminal helix in the catalytic mechanism of Alpha-class glutathione S-transferases. *Biochem J 343 (Pt 3)*, 525-531.

Amin, S., Desai, D., Dai, W., Harvey, R. G., and Hecht, S. S. (1995a) Tumorigenicity in newborn mice of fjord region and other sterically hindered diol epoxides of benzo[*g*]chrysene, dibenzo[*a,l*]pyrene (dibenzo[*def,p*]chrysene), 4H-cyclopenta[*def*]chrysene and fluoranthene. *Carcinogenesis 16*, 2813-2817.

Amin, S., Krzeminski, J., Rivenson, A., Kurtzke, C., Hecht, S. S., and el-Bayoumy, K. (1995b) Mammary carcinogenicity in female CD rats of fjord region diol epoxides of benzo[*c*]phenanthrene, benzo[*g*]chrysene and dibenzo[*a,l*]pyrene. *Carcinogenesis 16*, 1971-1974.

Anders, M. W. (2004) Glutathione-dependent bioactivation of haloalkanes and haloalkenes. *Drug Metab Rev 36*, 583-594.

Ariese, F., Small, G. J., and Jankowiak, R. (1996) Conformational studies of depurinating DNA adducts from *syn*-dibenzo[*a,l*]pyrene diol epoxide. *Carcinogenesis 17*, 829-837.

Armstrong, R. N. (1997) Structure, catalytic mechanism, and evolution of the glutathione transferases. *Chem Res Toxicol 10*, 2-18.

Awasthi, Y. C., Sharma, R., and Singhal, S. S. (1994) Human glutathione S-transferases. *Int J Biochem 26*, 295-308.

Avkin, S., Goldsmith, M., Velasco-Miguel, S., Geacintov, N., Friedberg, E. C., and Livneh, Z. (2004) Quantitative analysis of translesion DNA synthesis across a benzo[*a*]pyrene-guanine adduct in mammalian cells: the role of DNA polymerase kappa. *J Biol Chem 279*, 53298-53305.

Baird, W. M., Ralston, S., Luch, A., Platt, K. L., and Seidel, A. (1996) Stereoselective metabolic activation of dibenzo[*a,l*]pyrene in the human mammary carcinoma cell line MCF-7 results in formation of (–)-*anti*-11,12-diol-13,14-epoxide

deoxyadenosine and (+)-*syn*-11,12-diol-13,14-epoxidedeoxyadenosine adducts in DNA. *Polycycl Arom Compd 10*, 235-242.

Barrett, J. C. (1993) Mechanisms of multistep carcinogenesis and carcinogen risk assessment. *Environ Health Perspect 100*, 9-20.

Bassal, S., and El-Osta, A. (2005) DNA damage detection and repair, and the involvement of epigenetic states. *Hum Mutat 25*, 101-109.

Benhamou, S., Lee, W. J., Alexandrie, A. K., Boffetta, P., Bouchardy, C., Butkiewicz, D., Brockmöller, J., Clapper, M. L., Daly, A., Dolzan, V., et al. (2002) Meta- and pooled analyses of the effects of glutathione S-transferase M1 polymorphisms and smoking on lung cancer risk. *Carcinogenesis 23*, 1343-1350.

Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, L. N., and Bourne, P. E. (2000) The protein data bank. *Nucleic Acids Res 28*, 235-242.

Berneburg, M., and Lehmann, A. R. (2001) Xeroderma pigmentosum and related disorders: defects in DNA repair and transcription. *Adv Genet 43*, 71-102.

Bertram, J. S. (2000) The molecular biology of cancer. *Mol Aspects Med 21*, 167-223.

Bignold, L. P. (2003) Initiation of genetic instability and tumour formation: a review and hypothesis of a nongenotoxic mechanism. *Cell Mol Life Sci 60*, 1107-1117.

Binkova, B., Giguere, Y., Rossner, P., Jr., Dostal, M., and Sram, R. J. (2000) The effect of dibenzo[*a,l*]pyrene and benzo[*a*]pyrene on human diploid lung fibroblasts: the induction of DNA adducts, expression of p53 and p21(WAF1) proteins and cell cycle distribution. *Mutat Res 471*, 57-70.

Björnestedt, R., Stenberg, G., Widersten, M., Board, P. G., Sinning, I., Jones, T. A., and Mannervik, B. (1995) Functional significance of arginine 15 in the active site of human class alpha glutathione transferase A1-1. *J Mol Biol 247,* 765-773.

Board, P. G. (1981) Gene deletion and partial deficiency of the glutathione S-transferase (ligandin) system in man. *FEBS Lett 135*, 12-14.

Board, P. G., Coggan, M., Chelvanayagam, G., Easteal, S., Jermiin, L. S., Schulte, G. K., Danley, D. E., Hoth, L. R., Griffor, M. C., Kamath, A. V., et al. (2000) Identification, characterization, and crystal structure of the Omega class glutathione transferases. *J Biol Chem 275*, 24798-24806.

Bohr, V. A., Smith, C. A., Okumoto, D. S., and Hanawalt, P. C. (1985) DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. *Cell 40*, 359-369.

Booth, J., Boyland, E., and Sims, P. (1961) An enzyme from rat liver catalyzing conjugations with glutathione. *Biochem J 79*, 516-524.

Borgen, A., Darvey, H., Castagnoli, N., Crocker, T. T., Rasmussen, R. E., and Wang, I. Y. (1973) Metabolic conversion of benzo[*a*]pyrene by Syrian hamster liver microsomes and binding of metabolites to deoxyribonucleic acid. *J Med Chem 16*, 502-506.

Borosky, G. L. (1999) Theoretical study related to the carcinogenic activity of polycyclic aromatic hydrocarbon derivatives. *J Org Chem 64*, 7738-7744.

Boyland, C. (1950) The biological significance of metabolism of polycyclic compounds. *Biochem Soc Symp 5*, 40-54.

Braithwaite, E., Wu, X., and Wang, Z. (1998) Repair of DNA lesions induced by polycyclic aromatic hydrocarbons in human cell-free extracts: involvement of two excision repair mechanisms in vitro. *Carcinogenesis 19*, 1239-1246.

Braithwaite, E., Wu, X., and Wang, Z. (1999) Repair of DNA lesions: mechanisms and relative repair efficiencies. *Mutat Res 424*, 207-219.

Bruns, C. M., Hubatsch, I., Ridderström, M., Mannervik, B., and Tainer, J. A. (1999) Human glutathione transferase A4-4 crystal structures and mutagenesis reveal the basis of high catalytic efficiency with toxic lipid peroxidation products. *J Mol Biol 288*, 427-439.

Buterin, T., Hess, M. T., Luneva, N., Geacintov, N. E., Amin, S., Kroth, H., Seidel, A., and Naegeli, H. (2000) Unrepaired fjord region polycyclic aromatic hydrocarbon-DNA adducts in ras codon 61 mutational hot spots. *Cancer Res 60*, 1849-1856.

Cavalieri, E. L., Higginbotham, S., RamaKrishna, N. V., Devanesan, P. D., Todorovic, R., Rogan, E. G., and Salmasi, S. (1991) Comparative dose-response tumorigenicity studies of dibenzo[*a,l*]pyrene versus 7,12-dimethylbenz-[*a*]anthracene, benzo[*a*]pyrene and two dibenzo[*a,l*]pyrene dihydrodiols in mouse skin and rat mammary gland. *Carcinogenesis 12*, 1939-1944.

Chen, Y. S., Ho, C. C., Cheng, K. C., Tyan, Y. S., Hung, C. F., Tan, T. W., and Chung, J. G. (2003) Curcumin inhibited the arylamines N-acetyltransferase activity, gene expression and DNA adduct formation in human lung cancer cells (A549). *Toxicol In Vitro 17*, 323-333.

Clark, A. G., Smith, J. N., and Speir, T. W. (1973) Cross specificity in some vertebrate and insect glutathione-transferases with methyl parathion (dimethyl p-nitrophenyl phosphorothionate), 1-chloro-2,4-dinitro-benzene and s-crotonyl-N-acetylcysteamine as substrates. *Biochem J 135*, 385-392.

Combes, B., and Stackelum, G. S. (1961) A liver enzyme that conjugates sulfobromophtalein sodium with glutathione. *J Clin Invest 40*, 981-988.

Commandeur, J. N., Stijntjes, G. J., and Vermeulen, N. P. (1995) Enzymes and transport systems involved in the formation and disposition of glutathione S-conjugates. Role in bioactivation and detoxication mechanisms of xenobiotics. *Pharmacol Rev 47*, 271-330.

Conney, A. H. (1982) Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes Memorial Lecture. *Cancer Res 42*, 4875-4917.

Cosman, M., de los Santos, C., Fiala, R., Hingerty, B. E., Ibanez, V., Luna, E., Harvey, R., Geacintov, N. E., Broyde, S., and Patel, D. J. (1993) Solution conformation of the (+)-*cis*-*anti*-[BP]dG adduct in a DNA duplex: intercalation of the covalently attached benzo[*a*]pyrenyl ring into the helix and displacement of the modified deoxyguanosine. *Biochemistry 32*, 4145-4155.

Costa, R. M., Chigancas, V., Galhardo Rda, S., Carvalho, H., and Menck, C. F. (2003) The eukaryotic nucleotide excision repair pathway. *Biochimie 85*, 1083-1099.

Danielson, U. H., Esterbauer, H., and Mannervik, B. (1987) Structure-activity relationships of 4-hydroxyalkenals in the conjugation catalysed by mammalian glutathione transferases. *Biochem J 247*, 707-713.

de Laat, W. L., Jaspers, N. G., and Hoeijmakers, J. H. (1999) Molecular mechanism of nucleotide excision repair. *Genes Dev 13*, 768-785.

Dip, R., Camenisch, U., and Naegeli, H. (2004) Mechanisms of DNA damage recognition and strand discrimination in human nucleotide excision repair. *DNA Repair (Amst) 3*, 1409-1423.

Dipple, A., Moschel, R. C., and Bigger, C. A. H. (1984) Polynuclear Aromatic Carcinogenes. In *Chemical Carcinogens 2* (Searle, C. E., Ed.), ACS Monograph 182, Washington D.C.

Dipple, A. (1985) Polycyclic aromatic hydrocarbons: an introduction. In *Polycyclic Hydrocarbons and Carcinogenesis* (Harvey, R. G., Ed.) pp 1-17, American Chemical Society, Washington DC.

Dipple, A., Pigott, M. A., Agarwal, S. K., Yagi, H., Sayer, J. M., and Jerina, D. M. (1987) Optically active benzo[*c*]phenanthrene diol epoxides bind extensively to adenine in DNA. *Nature 327*, 535-536.

Dipple, A., and Bigger, C. A. (1991) Mechanism of action of food-associated polycyclic aromatic hydrocarbon carcinogens. *Mutat Res 259*, 263-276.

Dipple, A. (1994) Reactions of polycyclic aromatic hydrocarbons with DNA. *IARC Sci Publ 125*, 107-129.

Dixon, R. A., Diehl, R. E., Opas, E., Rands, E., Vickers, P. J., Evans, J. F., Gillard, J. W., and Miller, D. K. (1990) Requirement of a 5-lipoxygenase-activating protein for leukotriene synthesis. *Nature 343*, 282-284.

Dock, L., Waern, F., Martinez, M., Grover, P. L., and Jernström, B. (1986) Studies on the further activation of benzo[*a*]pyrene diol epoxides by rat liver microsomes and nuclei. *Chem Biol Interact 58*, 301-318.

Eaton, D. L., and Bammler, T. K. (1999) Concise review of the glutathione S-transferases and their significance to toxicology. *Toxicol Sci 49*, 156-164.

Ellis, R. J. (2001) Macromolecular crowding: an important but neglected aspect of the intracellular environment. *Curr Opin Struct Biol 11*, 114-119.

Feldman, G., Remsen, J., Shinohara, K., and Cerutti, P. (1978) Excisability and persistence of benzo(*a*)pyrene DNA adducts in epithelioid human lung cells. *Nature 274*, 796-798.

Fields, W. R., Li, Y., and Townsend, A. J. (1994) Protection by transfected glutathione S-transferase isozymes against carcinogen-induced alkylation of cellular macromolecules in human MCF-7 cells. *Carcinogenesis 15*, 1155-1160.

Fields, W. R., Morrow, C. S., Doss, A. J., Sundberg, K., Jernström, B., and Townsend, A. J. (1998) Overexpression of stably transfected human glutathione S-transferase P1- 1 protects against DNA damage by benzo[*a*]pyrene diol-epoxide in human T47D cells. *Mol Pharmacol 54*, 298-304.

Geacintov, N. E., Cosman, M., Hingerty, B. E., Amin, S., Broyde, S., and Patel, D. J. (1997) NMR solution structures of stereoisometric covalent polycyclic aromatic carcinogen-DNA adduct: principles, patterns, and diversity. *Chem Res Toxicol 10*, 111-146.

Geacintov, N. E., Broyde, S., Buterin, T., Naegeli, H., Wu, M., Yan, S., and Patel, D. J. (2002) Thermodynamic and structural factors in the removal of bulky DNA adducts by the nucleotide excision repair machinery. *Biopolymers 65*, 202-210.

Geacintov, N. E., Naegeli, H., Dinshaw, J. P., and Broyde, S. (2005) Structural aspects of polycyclic aromatic carcinogen-damaged DNA and its recognition by NER proteins. In *DNA Damage Recognition* (Siede, W., Kow, Y. W., and Doetsch, P. W., Eds.), Marcel Dekker Inc.

Gelboin, H. V. (1980) Benzo[*a*]pyrene metabolism, activation and carcinogenesis: role and regulation of mixed-function oxidases and related enzymes. *Physiol Rev 60*, 1107-1166.

Giles, A. S., Seidel, A., and Phillips, D. H. (1995) In vitro reaction with DNA of the fjord-region diol epoxides of benzo[*g*]chrysene and benzo[*c*]phenanthrene as studied by 32P-postlabeling. *Chem Res Toxicol 8*, 591-599.

Glatt, H., Piee, A., Pauly, K., Steinbrecher, T., Schrode, R., Oesch, F., and Seidel, A. (1991) Fjord- and bay-region diol-epoxides investigated for stability, SOS induction in *Escherichia coli*, and mutagenicity in *Salmonella typhimurium* and mammalian cells. *Cancer Res 51*, 1659-1667.

Goodman, M. F. (2000) Coping with replication 'train wrecks' in Escherichia coli using Pol V, Pol II and RecA proteins. *Trends Biochem Sci 25*, 189-195.

Gräslund, A., and Jernström, B. (1989) DNA-carcinogen interaction: covalent DNA-adducts of benzo(*a*)pyrene 7,8- dihydrodiol 9,10-epoxides studied by biochemical and biophysical techniques. *Q Rev Biophys 22*, 1-37.

Grover, P. L. (1986) Pathways involved in the metabolism and activation of polycyclic hydrocarbons. *Xenobiotica 16*, 915-931.

Gu, Y., Singh, S. V., and Ji, X. (2000) Residue R216 and catalytic efficiency of a murine class alpha glutathione S-transferase toward benzo[*a*]pyrene 7(*R*),8(*S*)-diol 9(*S*), 10(*R*)-epoxide. *Biochemistry 39*, 12552-12557.

Gu, Y., Guo, J., Pal, A., Pan, S. S., Zimniak, P., Singh, S. V., and Ji, X. (2004) Crystal structure of human glutathione S-transferase A3-3 and mechanistic implications for its high steroid isomerase activity. *Biochemistry 43*, 15673-15679.

Guengerich, F. P., and Shimada, T. (1991) Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. *Chem Res Toxicol 4*, 391-407.

Guo, J., Geacintov, N. E., and Broyde, S. (2003) Structural and thermodynamic studies of dibenzo[*a,l*]pyrene diol epoxide DNA adducts. *American Association for Cancer Research proceeding*, pp 450 .

Habig, W. H., and Jakoby, W. B. (1981) Assays for differentiation of glutathione S-transferases. *Methods Enzymol 77*, 398-405.

Haimeur, A., Conseil, G., Deeley, R. G., and Cole, S. P. (2004) The MRP-related and BCRP/ABCG2 multidrug resistance proteins: biology, substrate specificity and regulation. *Curr Drug Metab 5*, 21-53.

Hanahan, D., and Weinberg, R. A. (2000) The hallmarks of cancer. *Cell 100*, 57-70.

Hanawalt, P. C., Donahue, B. A., and Sweder, K. S. (1994) Repair and transcription. Collision or collusion? *Curr Biol 4*, 518-521.

Harris, C. C. (1991) Chemical and physical carcinogenesis: advances and perspectives for the 1990s. *Cancer Res 51*, 5023-5044.

Harvey, R. G. (1991) Chemistry and Carcinogenicity. In *Polycyclic Aromatic Hydrocarbons*, Cambridge University Press, Cambridge.

Harvey, R. G. (1997) *Polycyclic Aromatic Hydrocarbons.* Wiley-VCH inc., New York.

Hashibe, M., Brennan, P., Strange, R. C., Bhisey, R., Cascorbi, I., Lazarus, P., Oude Ophuis, M. B., Benhamou, S., Foulkes, W. D., Katoh, T., et al. (2003) Meta- and pooled analyses of GSTM1, GSTT1, GSTP1, and CYP1A1 genotypes and risk of head and neck cancer. *Cancer Epidemiol Biomarkers Prev 12*, 1509-1517.

Hayes, J. D., and Pulford, D. J. (1995) The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol 30*, 445-600.

Hayes, J. D., and Strange, R. C. (2000) Glutathione S-transferase polymorphisms and their biological consequences. *Pharmacology 61*, 154-166.

Hayes, J. D., Flanagan, J. U., and Jowsey, I. R. (2005) Glutathione transferases. *Annu Rev Pharmacol Toxicol 45*, 51-88.

Hecht, S. S. (1999) Tobacco smoke carcinogens and lung cancer. *J Natl Cancer Inst 91*, 1194-1210.

Hess, M. T., Gunz, D., Luneva, N., Geacintov, N. E., and Naegeli, H. (1997a) Base pair conformation-dependent excision of benzo[*a*]pyrene diol epoxide-guanine adducts by human nucleotide excision repair enzymes. *Mol Cell Biol 17*, 7069-7076.

Hess, M. T., Schwitter, U., Petretta, M., Giese, B., and Naegeli, H. (1997b) Bipartite substrate discrimination by human nucleotide excision repair. *Proc Natl Acad Sci U S A 94*, 6664-6669.

Hesse, S., Cumpelik, O., Mezger, M., Kiefer, F., and Wiebel, F. J. (1990) Glutathione conjugation protects some, but not all, cell lines against DNA binding of benzo[*a*]pyrene metabolites. *Carcinogenesis 11*, 485-487.

Higginbotham, S., RamaKrishna, N. V., Johansson, S. L., Rogan, E. G., and Cavalieri, E. L. (1993) Tumor-initiating activity and carcinogenicity of dibenzo[*a,l*]pyrene versus 7,12-dimethylbenz[*a*]anthracene and benzo[*a*]pyrene at low doses in mouse skin. *Carcinogenesis 14*, 875-878.

Hirshfeld, F. L., Sandler, S., and Schmidt, G. M. (1963) Structure of overcrowded aromatic compounds part VI, the crystal structure of benzo[*c*]phenanthrene and of 1,12-dimethyl-benzo[*c*]phenanthrene. *J Chem Soc*, 2108-2125.

Hoeijmakers, J. H. (2001) Genome maintenance mechanisms for preventing cancer. *Nature 411*, 366-374.

Hsia, T. C., Chung, J. G., Lu, H. F., Ho, H. C., Yang, C. C., Lu, K. H., and Hung, C. F. (2002) The effect of paclitaxel on 2-aminofluorene-DNA adducts formation and arylamine N-acetyltransferase activity and gene expression in human lung tumor cells (A549). *Food Chem Toxicol 40*, 697-703.

Hu, X., Pal, A., Krzeminski, J., Amin, S., Awasthi, Y. C., Zimniak, P., and Singh, S. V. (1998) Specificities of human glutathione S-transferase isozymes toward anti-diol epoxides of methylchrysenes. *Carcinogenesis 19*, 1685-1689.

Hu, X., Herzog, C., Zimniak, P., and Singh, S. V. (1999) Differential protection against benzo[*a*]pyrene-7,8-dihydrodiol-9,10- epoxide-induced DNA damage in HepG2 cells stably transfected with allelic variants of pi class human glutathione S-transferase. *Cancer Res 59*, 2358-2362.

IARC (1983) *Polynuclear aromatic compounds, part 1, Chemical, Environmental and Experimental Data.* Lyon, France.

IARC (1987) *Overall evaluations of carcinogenicity:an updating of IARC Monographs volumes 1 to 42.* Lyon, France.

IPCS (1998) *Selected non-heterocyclic polycyclic aromatic hydrocarbons.* World Health Organisation, Geneva.

Ishikawa, T. (1992) The ATP-dependent glutathione S-conjugate export pump. *Trends Biochem Sci 17*, 463-468.

Ishikawa, T., Kuo, M. T., Furuta, K., and Suzuki, M. (2000) The human multidrug resistance-associated protein (MRP) gene family: from biological function to drug molecular design. *Clin Chem Lab Med 38*, 893-897.

Jakobsson, P. J., Mancini, J. A., and Ford-Hutchinson, A. W. (1996) Identification and characterization of a novel human microsomal glutathione S-transferase with leukotriene C4 synthase activity and significant sequence identity to 5-lipoxygenase-activating protein and leukotriene C4 synthase. *J Biol Chem 271*, 22203-22210.

Jakobsson, P. J., Mancini, J. A., Riendeau, D., and Ford-Hutchinson, A. W. (1997) Identification and characterization of a novel microsomal enzyme with glutathione-dependent transferase and peroxidase activities. *J Biol Chem 272*, 22934-22939.

Jakobsson, P. J., Thoren, S., Morgenstern, R., and Samuelsson, B. (1999) Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. *Proc Natl Acad Sci USA 96*, 7220-7225.

Jankowiak, R., Ariese, F., Zamzow, D., Luch, A., Kroth, H., Seidel, A., and Small, G. J. (1997) Conformational studies of stereoisomeric tetrols derived from *syn*- and *anti*-dibenzo[*a,l*]pyrene diol epoxides. *Chem Res Toxicol 10*, 677-686.

Jankowiak, R., Ariese, F., Hewer, A., Luch, A., Zamzow, D., Hughes, N. C., Phillips, D., Seidel, A., Platt, K. L., Oesch, F., et al. (1998) Structure, conformations, and repair of DNA adducts from dibenzo[*a,l*]pyrene: 32P-postlabeling and fluorescence studies. *Chem Res Toxicol 11*, 674-685.

Jerina, D. M., Daly, J. W., Witkop, B., Zaltmn-Nirenberg, P., and Udenfreind, S. (1968) The role of arene oxide-oxepin systems in the metabolism of aromatic substrates. 3. Formation of 1,2-naphthalene oxide from naphthalene by liver microsomes. *J Am Chem Soc 90*, 6525-6527.

Jerina, D. M., and Daly, J. W. (1974) Arene oxides: a new aspect of drug metabolism. *Science 185*, 573-582.

Jerina, D. M., and Daly, J. W. (1976) Oxidation at carbon. In *Drug metabolism* (Parke, D. V., and Smith, R. L., Eds.), Taylor and Frances, London.

Jerina, D. M., Lehr, R. E., Yagi, H., Hernandez, O., Dansette, P. M., Wislocki, P. G., Wood, A. W., Chang, R. L., Levin, W., and Conney, A. H. (1976) Mutagenicity of benzo[*a*]pyrene derivatives and the description of a quantum mechanical model which predicts the ease of carbonium ion formation from diol epoxides. In *In vitro Metabolic Activation and Mutagenesis Testing* (deSerres, F. J., Fouts,

J. R., Bend, J. R., and Philpot, R. M., Eds.) pp 159-177, Elsevier/North-Holland Biomedical Press, Amsterdam.

Jerina, D. M., and Lehr, R. E. (1977) The bay region theory: A quantum mechanical approach to aromatic hydrocarbon-induced carcinogenicity. In *Microsomes and drug oxidations* (Ullrich, V., Roots, I., Hildebrandt, A. G., Estabrook, R. W., and Conney, A. H., Eds.) pp 709-720, Pergamon, Oxford.

Jerina, D. M., Sayer, J. M., Yagi, H., Croisy-Delcey, M., Ittah, Y., Thakker, D. R., Wood, A. W., Chang, R. L., Levin, W., and Conney, A. H. (1981) Highly tumorigenic bay-region diol epoxides from the weak carcinogen benzo[*c*]phenanthrene. *Adv Exp Med Biol 136 (Pt A)*, 501-523.

Jerina, D. M., Sayer, J. M., Agarwal, S. K., Yagi, H., Levin, W., Wood, A. W., Conney, A. H., Pruess-Schwartz, D., Baird, W. M., Pigott, M. A., et al. (1986) Reactivity and tumorigenicity of bay-region diol epoxides derived from polycyclic aromatic hydrocarbons. *Adv Exp Med Biol 197*, 11-30.

Jerina, D. M., Chadha, A., Cheh, A. M., Schurdak, M. E., Wood, A. W., and Sayer, J. M. (1991) Covalent bonding of bay-region diol epoxides to nucleic acids. In *Biological Reactive Intermediates IV. Molecular and cellular effects and their impact on human health* (Withmer, C. M., Snyder, R., Jollow, D. J., Kalf, G. S., Kocsis, J. J., and Sipes, I. G., Eds.) pp 533-553, Plenum Press, New York.

Jernström, B., and Gräslund, A. (1994) Covalent binding of benzo[*a*]pyrene 7,8-dihydrodiol 9,10-epoxides to DNA: molecular structures, induced mutations and biological consequences. *Biophys Chem 49*, 185-199.

Jernström, B., Funk, M., Frank, H., Mannervik, B., and Seidel, A. (1996) Glutathione S-transferase A1-1-catalysed conjugation of bay and fjord region diol epoxides or polycyclic aromatic hydrocarbons with glutathione. *Carcinogenesis 17*, 1491-1498.

Jhaveri, M. S., and Morrow, C. S. (1998) Methylation-mediated regulation of the glutathione S-transferase P1 gene in human breast cancer cells. *Gene 210*, 1-7.

Ji, X., Tordova, M., O'Donnell, R., Parsons, J. F., Hayden, J. B., Gilliland, G. L., and Zimniak, P. (1997) Structure and function of the xenobiotic substrate-binding site and location of a potential non-substrate-binding site in a class pi glutathione S-transferase. *Biochemistry 36*, 9690-9702.

Ji, X., Blaszczyk, J., Xiao, B., O'Donnell, R., Hu, X., Herzog, C., Singh, S. V., and Zimniak, P. (1999) Structure and function of residue 104 and water molecules in the xenobiotic substrate-binding site in human glutathione S-transferase P1-1. *Biochemistry 38*, 10231-10238.

Jia, L. Q., Osada, M., Ishioka, C., Gamo, M., Ikawa, S., Suzuki, T., Shimodaira, H., Niitani, T., Kudo, T., Akiyama, M., et al. (1997) Screening the p53 status of human cell lines using a yeast functional assay. *Mol Carcinog 19*, 243-253.

Katz, A. K., Carrell, H. L., and Glusker, J. P. (1998) Dibenzo[*a,l*]pyrene (dibenzo[*def,p*]chrysene): fjord-region distortions. *Carcinogenesis 19*, 1641-1648.

Khan, Q. A., and Anderson, L. M. (2001) Hydrocarbon carcinogens evade cellular defense mechanism of G1 arrest in nontransformed and malignant lung cell lines. *Toxicol Appl Pharmacol 173*, 105-113.

Klaunig, J. E., Kamendulis, L. M., and Xu, Y. (2000) Epigenetic mechanisms of chemical carcinogenesis. *Hum Exp Toxicol 19*, 543-555.

Kolm, R. H., Sroga, G. E., and Mannervik, B. (1992) Participation of the phenolic hydroxyl group of Tyr-8 in the catalytic mechanism of human glutathione transferase P1-1. *Biochem J 285 (Pt 2)*, 537-540.

Koreeda, M., Moore, P. D., Wislocki, P. G., Levin, W., Yagi, H., and Jerina, D. M. (1978) Binding of benzo[*a*]pyrene 7,8-diol-9,10-epoxides to DNA, RNA, and protein of mouse skin occurs with high stereoselectivity. *Science 199*, 778-781.

Ladner, J. E., Parsons, J. F., Rife, C. L., Gilliland, G. L., and Armstrong, R. N. (2004) Parallel evolutionary pathways for glutathione transferases: structure and mechanism of the mitochondrial class kappa enzyme rGSTK1-1. *Biochemistry 43*, 352-361.

LaVoie, E. J., He, Z. M., Meegalla, R. L., and Weyand, E. H. (1993) Exceptional tumor-initiating activity of 4-fluorobenzo[*j*]fluoranthene on mouse skin: comparison with benzo[*j*]fluoranthene, 10-fluorobenzo[*j*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a,l*]pyrene and 7,12-dimethylbenz[*a*]anthracene. *Cancer Lett 70*, 7-14.

Lehr, R. E., and Jerina, D. M. (1977) Relationships of quantum mechanical calculations, relative mutagenicity of benzo[*a*]anthracene diol epoxides, and "bay region" concept of aromatic hydrocarbon carcinogenicity. *J Toxicol Environ Health 2*, 1259-1265.

Lewis-Bevan, L., Little, S. B., and Rabinowitz, J. R. (1995) Quantum mechanical studies of the structure and reactivities of the diol epoxides of benzo[*c*]-phenanthrene. *Chem Res Toxicol 8*, 499-505.

Lin, C. H., Huang, X., Kolbanovskii, A., Hingerty, B. E., Amin, S., Broyde, S., Geacintov, N. E., and Patel, D. J. (2001) Molecular topology of polycyclic aromatic carcinogens determines DNA adduct conformation: a link to tumorigenic activity. *J Mol Biol 306*, 1059-1080.

Lindahl, T., and Wood, R. D. (1999) Quality control by DNA repair. *Science 286*, 1897-1905.

Little, S. B., Rabinowitz, J. R., Wei, P., and Yang, W. (1999) A comparison of calculated and experimental geometries for crowded polycyclic aromatic hydrocarbons and their metabolites. *Polycycl Arom Compd 14&15*, 53-61.

Liu, S., Zhang, P., Ji, X., Johnson, W. W., Gilliland, G. L., and Armstrong, R. N. (1992) Contribution of tyrosine 6 to the catalytic mechanism of isoenzyme 3-3 of glutathione S-transferase. *J Biol Chem 267*, 4296-4299.

Lloyd, D. R., and Hanawalt, P. C. (2002) p53 controls global nucleotide excision repair of low levels of structurally diverse benzo(*g*)chrysene-DNA adducts in human fibroblasts. *Cancer Res 62*, 5288-5294.

Lodovici, M., Luceri, C., Guglielmi, F., Bacci, C., Akpan, V., Fonnesu, M. L., Boddi, V., and Dolara, P. (2004) Benzo(*a*)pyrene diolepoxide (BPDE)-DNA adduct levels in leukocytes of smokers in relation to polymorphism of CYP1A1, GSTM1, GSTP1, GSTT1, and mEH. *Cancer Epidemiol Biomarkers Prev 13*, 1342-1348.

Lowe, J. P., and Silverman, B. D. (1981) Simple molecular orbital explanation for "bay-region" carcinogenic reactivity. *J Am Chem Soc 103*, 2852-2855.

Luby-Phelps, K. (2000) Cytoarchitecture and physical properties of cytoplasm: volume, viscosity, diffusion, intracellular surface area. *Int Rev Cytol 192*, 189-221.

Luch, A., Glatt, H., Platt, K. L., Oesch, F., and Seidel, A. (1994) Synthesis and mutagenicity of the diastereomeric fjord-region 11,12-dihydrodiol 13,14-epoxides of dibenzo[*a,l*]pyrene. *Carcinogenesis 15*, 2507-2516.

Luch, A., Seidel, A., Glatt, H., and Platt, K. L. (1997) Metabolic activation of the (+)-*S,S*- and (–)-*R,R*-enantiomers of *trans*-11,12-dihydroxy-11,12-dihydro-dibenzo[*a,l*]pyrene: stereoselectivity, DNA adduct formation, and mutagenicity in Chinese hamster V79 cells. *Chem Res Toxicol 10*, 1161-1170.

Luch, A., Kudla, K., Seidel, A., Doehmer, J., Greim, H., and Baird, W. M. (1999) The level of DNA modification by (+)-*syn*-(11*S*,12*R*,13*S*,14*R*)- and (–)-*anti*-(11*R*,12*S*,13*S*,14*R*)-dihydrodiol epoxides of dibenzo[*a,l*]pyrene determined the effect on the proteins p53 and p21WAF1 in the human mammary carcinoma cell line MCF-7. *Carcinogenesis 20*, 859-865.

Luch, A. (2005) Nature and nurture - lessons from chemical carcinogenesis. *Nat Rev Cancer 5*, 113-125.

Lyttle, M. H., Satyam, A., Hocker, M. D., Bauer, K. E., Caldwell, C. G., Hui, H. C., Morgan, A. S., Mergia, A., and Kauvar, L. M. (1994) Glutathione-S-transferase activates novel alkylating agents. *J Med Chem 37*, 1501-1507.

Mancini, J. A., and Evans, J. F. (1993) Coupling of recombinant 5-lipoxygenase and leukotriene A4 hydrolase activities and transcellular metabolism of leukotriene A4 in Sf9 insect cells. *Eur J Biochem 218*, 477-484.

Mannervik, B. (1985) The isoenzymes of glutathione transferase. *Adv Enzymol Relat Areas Mol Biol 57*, 357-417.

Mannervik, B., Awasthi, Y. C., Board, P. G., Hayes, J. D., Di Ilio, C., Ketterer, B., Listowsky, I., Morgenstern, R., Muramatsu, M., Pearson, W. R., et al. (1992)

Nomenclature for human glutathione transferases [letter]. *Biochem J 282*, 305-306.

Mannervik, B., and Widersten, M. (1995) Human glutathione transferases: classification, tissue distribution, structure and functional properties. In *Advances in Drug Metabolism in Man* (Pacifici, G. M., and Francchia, G. N., Eds.) pp 408-459, European Commission.

Meehan, T., and Straub, K. (1979) Double-stranded DNA steroselectively binds benzo(*a*)pyrene diol epoxides. *Nature 277*, 410-412.

Mellon, I., Spivak, G., and Hanawalt, P. C. (1987) Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian DHFR gene. *Cell 51*, 241-249.

Miller, D. K., Gillard, J. W., Vickers, P. J., Sadowski, S., Leveille, C., Mancini, J. A., Charleson, P., Dixon, R. A., Ford-Hutchinson, A. W., Fortin, R., et al. (1990) Identification and isolation of a membrane protein necessary for leukotriene production. *Nature 343*, 278-281.

Miller, E. C. (1951) Studies on the formation of protein-bound derivatives of 3,4-benzpyrene in the epidermal fraction of mouse skin. *Cancer Res 11*, 100-108.

Miller, J. A., and Miller, E. C. (1975) Metabolic activation and reactivity of chemical carcinogens. *Mutat Res 33*, 25-26.

Mitchell, J. R., Hoeijmakers, J. H., and Niedernhofer, L. J. (2003) Divide and conquer: nucleotide excision repair battles cancer and ageing. *Curr Opin Cell Biol 15*, 232-240.

Morel, F., Rauch, C., Petit, E., Piton, A., Theret, N., Coles, B., and Guillouzo, A. (2004) Gene and protein characterization of the human glutathione S-transferase kappa and evidence for a peroxisomal localization. *J Biol Chem 279*, 16246-16253.

Morgan, A. S., Sanderson, P. E., Borch, R. F., Tew, K. D., Niitsu, Y., Takayama, T., Von Hoff, D. D., Izbicka, E., Mangold, G., Paul, C., et al. (1998) Tumor efficacy and bone marrow-sparing properties of TER286, a cytotoxin activated by glutathione S-transferase. *Cancer Res 58*, 2568-2575.

Morgenstern, R., Meijer, J., Depierre, J. W., and Ernster, L. (1980) Characterization of rat-liver microsomal glutathione S-transferase activity. *Eur J Biochem 104*, 167-174.

Morrow, C. S., Smitherman, P. K., and Townsend, A. J. (2000) Role of multidrug-resistance protein 2 in glutathione S-transferase P1-1-mediated resistance to 4-nitroquinoline 1-oxide toxicities in HepG2 cells. *Mol Carcinog 29*, 170-178.

Naegeli, H., Bardwell, L., and Friedberg, E. C. (1992) The DNA helicase and adenosine triphosphatase activities of yeast Rad3 protein are inhibited by DNA damage. A potential mechanism for damage-specific recognition. *J Biol Chem 267*, 392-398.

Parkinson, A. (1996) Biotransformation of xenobiotics. In *Casarett and Doull´s Toxicology: The basis science of Poisons* (Klaassen, C., Ed.) pp 113-186, McGraw-Hill, New York.

Patskovsky, Y. V., Patskovska, L. N., and Listowsky, I. (1999a) An asparagine-phenylalanine substitution accounts for catalytic differences between hGSTM3-3 and other human class mu glutathione S-transferases. *Biochemistry 38*, 16187-16194.

Patskovsky, Y. V., Patskovska, L. N., and Listowsky, I. (1999b) Functions of His107 in the catalytic mechanism of human glutathione S-transferase hGSTM1a-1a. *Biochemistry 38*, 1193-1202.

Paumi, C. M., Ledford, B. G., Smitherman, P. K., Townsend, A. J., and Morrow, C. S. (2001) Role of multidrug resistance protein 1 (MRP1) and glutathione S-transferase A1-1 in alkylating agent resistance. Kinetics of glutathione conjugate formation and efflux govern differential cellular sensitivity to chlorambucil versus melphalan toxicity. *J Biol Chem 276*, 7952-7956.

Pavanello, S., Siwinska, E., Mielzynska, D., and Clonfero, E. (2004) GSTM1 null genotype as a risk factor for *anti*-BPDE-DNA adduct formation in mononuclear white blood cells of coke-oven workers. *Mutat Res 558*, 53-62.

Pemble, S., Schroeder, K. R., Spencer, S. R., Meyer, D. J., Hallier, E., Bolt, H. M., Ketterer, B., and Taylor, J. B. (1994) Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. *Biochem J 300 (Pt 1)*, 271-276.

Phillips, D. H. (1983) Fifty years of benzo(*a*)pyrene. *Nature 303*, 468-472.

Phillips, D. H., Hewer, A., Seidel, A., Steinbrecher, T., Schrode, R., Oesch, F., and Glatt, H. (1991) Relationship between mutagenicity and DNA adduct formation in mammalian cells for fjord- and bay-region diol-epoxides of polycyclic aromatic hydrocarbons. *Chem Biol Interact 80*, 177-186.

Phillips, D. H. (1999) Polycyclic aromatic hydrocarbons in the diet. *Mutat Res 443*, 139-147.

Pitot, H. C., and Dragan, Y. P. (1996) Chemical carcinogenesis. In *Casarett and Doull´s Toxicology: The basis science of Poisons* (Klaassen, C., Ed.) pp 201-267, McGraw-Hill, New York.

Polekhina, G., Board, P. G., Blackburn, A. C., and Parker, M. W. (2001) Crystal structure of maleylacetoacetate isomerase/glutathione transferase zeta reveals the molecular basis for its remarkable catalytic promiscuity. *Biochemistry 40*, 1567-1576.

Pruess-Schwartz, D., Sebti, S. M., Gilham, P. T., and Baird, W. M. (1984) Analysis of benzo(*a*)pyrene:DNA adducts formed in cells in culture by immobilized boronate chromatography. *Cancer Res 44*, 4104-4110.

Pruess-Schwartz, D., Baird, W. M., Yagi, H., Jerina, D. M., Pigott, M. A., and Dipple, A. (1987) Stereochemical specificity in the metabolic activation of benzo(*c*)phenanthrene to metabolites that covalently bind to DNA in rodent embryo cell cultures. *Cancer Res 47*, 4032-4037.

Raghunathan, S., Chandross, R. J., Kretsinger, R. H., Allison, T. J., Penington, C. J., and Rule, G. S. (1994) Crystal structure of human class mu glutathione transferase GSTM2-2. Effects of lattice packing on conformational heterogeneity. *J Mol Biol 238*, 815-832.

Ralston, S. L., Seidel, A., Luch, A., Platt, K. L., and Baird, W. M. (1995) Stereoselective activation of dibenzo[*a,l*]pyrene to (–)-*anti*-(11*R*,12*S*,13*S*,14*R*)- and (+)-*syn*-(11*S*,12*R*,13*S*,14*R*)-11,12-diol-13,14-epoxides which bind extensively to deoxyadenosine residues of DNA in the human mammary carcinoma cell line MCF-7. *Carcinogenesis 16*, 2899-2907.

Reinemer, P., Dirr, H. W., Ladenstein, R., Huber, R., Lo Bello, M., Federici, G., and Parker, M. W. (1992) Three-dimensional structure of class pi glutathione S-transferase from human placenta in complex with S-hexylglutathione at 2.8 A resolution. *J Mol Biol 227*, 214-226.

Riedl, T., Hanaoka, F., and Egly, J. M. (2003) The comings and goings of nucleotide excision repair factors on damaged DNA. *Embo J 22*, 5293-5303.

Robertson, I. G., Guthenberg, C., Mannervik, B., and Jernström, B. (1986) Differences in stereoselectivity and catalytic efficiency of three human glutathione transferases in the conjugation of glutathione with 7β,8α-dihydroxy-9α,10α-oxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene. *Cancer Res 46*, 2220-2224.

Robertson, I. G., and Jernström, B. (1986) The enzymatic conjugation of glutathione with bay-region diol-epoxides of benzo[*a*]pyrene, benz[*a*]anthracene and chrysene. *Carcinogenesis 7*, 1633-1636.

Robinson, A., Huttley, G. A., Booth, H. S., and Board, P. G. (2004) Modelling and bioinformatics studies of the human Kappa-class glutathione transferase predict a novel third glutathione transferase family with similarity to prokaryotic 2-hydroxychromene-2-carboxylate isomerases. *Biochem J 379*, 541-552.

Romert, L., Dock, L., Jenssen, D., and Jernström, B. (1989) Effects of glutathione transferase activity on benzo[*a*]pyrene 7,8-dihydrodiol metabolism and mutagenesis studied in a mammalian cell co-cultivation assay. *Carcinogenesis 10*, 1701-1707.

Rossjohn, J., McKinstry, W. J., Oakley, A. J., Verger, D., Flanagan, J., Chelvanayagam, G., Tan, K. L., Board, P. G., and Parker, M. W. (1998) Human theta class glutathione transferase: the crystal structure reveals a sulfate-binding pocket within a buried active site. *Structure 6*, 309-322.

Rowe, J. D., Nieves, E., and Listowsky, I. (1997) Subunit diversity and tissue distribution of human glutathione S-transferases: interpretations based on electrospray ionization-MS and peptide sequence-specific antisera. *Biochem J 325*, 481-486.

Ruan, Q., Kolbanovskiy, A., Zhuang, P., Chen, J., Krzeminski, J., Amin, S., and Geacintov, N. E. (2002) Synthesis and characterization of site-specific and stereoisomeric fjord dibenzo[*a,l*]pyrene diol epoxide-N(6)-adenine adducts: unusual thermal stabilization of modified DNA duplexes. *Chem Res Toxicol 15*, 249-261.

Sancar, A. (1996) DNA excision repair. *Annu Rev Biochem 65*, 43-81.

Sauvain, J. J., Vu Duc, T., and Huynh, C. K. (2001) Development of an analytical method for the simultaneous determination of 15 carcinogenic polycyclic aromatic hydrocarbons and polycyclic aromatic nitrogen heterocyclic compounds. application to diesel particulates. *Fresenius J Anal Chem 371*, 966-974.

Sayer, J. M., Yagi, H., Croisy-Delcey, M., and Jerina, D. M. (1981) Novel bay-region diol epoxides from benzo[*c*]phenanthrene. *J Am Chem Soc 103*, 4970-4972.

Seidel, A., Frank, H., Behnke, A., Schneider, D., and Jacob, J. (2004) Determination of dibenzo[*a,l*]pyrene and other fjord-region PAH isomers with MW 302 in environmental samples. *Polycycl Arom Compd 24*, 759-771.

Sengupta, S., and Harris, C. C. (2005) p53: traffic cop at the crossroads of DNA repair and recombination. *Nat Rev Mol Cell Biol 6*, 44-55.

Shellard, S. A., Fichtinger-Schepman, A. M., Lazo, J. S., and Hill, B. T. (1993) Evidence of differential cisplatin-DNA adduct formation, removal and tolerance of DNA damage in three human lung carcinoma cell lines. *Anticancer Drugs 4*, 491-500.

Shen, X., Sayer, J. M., Kroth, H., Ponten, I., O'Donnell, M., Woodgate, R., Jerina, D. M., and Goodman, M. F. (2002) Efficiency and accuracy of SOS-induced DNA polymerases replicating benzo[*a*]pyrene-7,8-diol 9,10-epoxide A and G adducts. *J Biol Chem 277*, 5265-5274.

Shimada, T., and Fujii-Kuriyama, Y. (2004) Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and 1B1. *Cancer Sci 95*, 1-6.

Shinohara, K., and Cerutti, P. A. (1977) Excision repair of benzo[*a*]pyrene-deoxyguanosine adducts in baby hamster kidney 21/C13 cells and in secondary mouse embryo fibroblasts C57BL/6J. *Proc Natl Acad Sci USA 74*, 979-983.

Sims, P., Grover, P. L., Swaisland, A., Pal, K., and Hewer, A. (1974) Metabolic activation of benzo(*a*)pyrene proceeds by a diol-epoxide. *Nature 252*, 326-328.

Singh, S. V., Varma, V., Zimniak, P., Srivastava, S. K., Marynowski, S. W., Desai, D., Amin, S., and Ji, X. (2004) Structural basis for catalytic differences between alpha class human glutathione transferases hGSTA1-1 and hGSTA2-2 for glutathione conjugation of environmental carcinogen benzo[*a*]pyrene-7,8-diol-9,10-epoxide. *Biochemistry 43*, 9708-9715.

Sinning, I., Kleywegt, G. J., Cowan, S. W., Reinemer, P., Dirr, H. W., Huber, R., Gilliland, G. L., Armstrong, R. N., Ji, X., Board, P. G., et al. (1993) Structure determination and refinement of human alpha class glutathione transferase A1-1, and a comparison with the Mu and Pi class enzymes. *J Mol Biol 232*, 192-212.

Srivastava, S. K., Hu, X., Xia, H., Bleicher, R. J., Zaren, H. A., Orchard, J. L., Awasthi, S., and Singh, S. V. (1998) ATP-dependent transport of glutathione conjugate of 7β,8α-dihydroxy-9α,10α-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene in murine hepatic canalicular plasma membrane vesicles. *Biochem J 332*, 799-805.

Srivastava, S. K., Hu, X., Xia, H., Awasthi, S., Amin, S., and Singh, S. V. (1999) Metabolic fate of glutathione conjugate of benzo[*a*]pyrene-(7*R*,8*S*)-diol (9*S*,10*R*)-epoxide in human liver. *Arch Biochem Biophys 371*, 340-344.

Srivastava, S. K., Watkins, S. C., Schuetz, E., and Singh, S. V. (2002) Role of glutathione conjugate efflux in cellular protection against benzo[*a*]pyrene-7,8-diol 9,10-epoxide-induced DNA damage. *Mol Carcinog 33*, 156-162.

Suh, M., Ariese, F., Small, G. J., Jankowiak, R., Hewer, A., and Phillips, D. H. (1995) Formation and persistence of benzo[*a*]pyrene-DNA adducts in mouse epidermis in vivo: importance of adduct conformation. *Carcinogenesis 16*, 2561-2569.

Sundberg, K., Widersten, M., Seidel, A., Mannervik, B., and Jernström, B. (1997) Glutathione conjugation of bay- and fjord-region diol epoxides of polycyclic aromatic hydrocarbons by glutathione transferases M1-1 and P1-1. *Chem Res Toxicol 10*, 1221-1227.

Sundberg, K., Johansson, A. S., Stenberg, G., Widersten, M., Seidel, A., Mannervik, B., and Jernström, B. (1998a) Differences in the catalytic efficiencies of allelic variants of glutathione transferase P1-1 towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons. *Carcinogenesis 19*, 433-436.

Sundberg, K., Seidel, A., Mannervik, B., and Jernström, B. (1998b) Detoxication of carcinogenic fjord-region diol epoxides of polycyclic aromatic hydrocarbons by glutathione transferase P1-1 variants and glutathione. *FEBS Lett 438*, 206-210.

Sundberg, K., Seidel, A., Mannervik, B., and Jernström, B. (1999) Catalytic efficiency of glutathione transferase P1-1 variants towards bay- and fjord-region diol epoxides of polycyclic aromatic hydrocarbons. *Polycycl Arom Compd 17*, 43-51.

Sundberg, K., Townsend, A. J., Seidel, A., and Jernström, B. (2000) Glutathione conjugation and DNA adduct formation of diol epoxides in V79 cells expressing human glutathione transferase P1-1. *Polycycl Arom Compd 21*, 123-133.

Swedmark, S., Jernström, B., and Jenssen, D. (1996) Comparison of the mRNA sequences for Pi class glutathione transferases in different hamster species and the corresponding enzyme activities with *anti*-benzo[*a*]pyrene-7,8-dihydrodiol 9,10-epoxide. *Biochem J 318*, 533-538.

Szeliga, J., and Dipple, A. (1998) DNA adduct formation by polycyclic aromatic hydrocarbon dihydrodiol epoxides. *Chem Res Toxicol 11*, 1-11.

Tan, W. G., Carnelley, T. J., Murphy, P., Wang, H., Lee, J., Barker, S., Weinfeld, M., and Le, X. C. (2001) Detection of DNA adducts of benzo[*a*]pyrene using immunoelectrophoresis with laser-induced fluorescence. Analysis of A549 cells. *J Chromatogr A 924*, 377-386.

Thakker, D. R., Yagi, H., Levin, W., Wood, A. W., Conney, A. H., and Jerina, D. M. (1985) Polycyclic aromatic hydrocarbons: Metabolic activation to ultimate carcinogens. In *Bioactivation of Foreign Compounds* (Anders, M. W., Ed.) pp 177-242, Academic Press, London.

van Hoffen, A., Balajee, A. S., van Zeeland, A. A., and Mullenders, L. H. (2003) Nucleotide excision repair and its interplay with transcription. *Toxicology 193*, 79-90.

van Lieshout, E. M., Roelofs, H. M., Dekker, S., Mulder, C. J., Wobbes, T., Jansen, J. B., and Peters, W. H. (1999) Polymorphic expression of the glutathione S-transferase P1 gene and its susceptibility to Barrett's esophagus and esophageal carcinoma. *Cancer Res 59*, 586-589.

Wang, R. W., Newton, D. J., Huskey, S. E., McKeever, B. M., Pickett, C. B., and Lu, A. Y. (1992) Site-directed mutagenesis of glutathione S-transferase YaYa. Important roles of tyrosine 9 and aspartic acid 101 in catalysis. *J Biol Chem 267*, 19866-19871.

Warholm, M., Guthenberg, C., Mannervik, B., von Bahr, C., and Glaumann, H. (1980) Identification of a new glutathione S-transferase in human liver. *Acta Chem Scand B 34*, 607-621.

Weisburger, J. H., and Williams, G. M. (1983) The distinct health risk analyses required for genotoxic carcinogens and promoting agents. *Environ Health Perspect 50*, 233-245.

Wood, A. W., Chang, R. L., Levin, W., Thakker, D. R., Yagi, H., Sayer, J. M., Jerina, D. M., and Conney, A. H. (1984) Mutagenicity of the enantiomers of the diastereomeric bay-region benzo(*c*)phenanthrene 3,4-diol-1,2-epoxides in bacterial and mammalian cells. *Cancer Res 44*, 2320-2324.

Wood, R. D. (1996) DNA repair in eukaryotes. *Annu Rev Biochem 65*, 135-167.

Wood, R. D. (1999) DNA damage recognition during nucleotide excision repair in mammalian cells. *Biochimie 81*, 39-44.

Wu, J., Zhu, B. B., Yu, J., Zhu, H., Qiu, L., Kindy, M. S., Gu, L., Seidel, A., and Li, G. M. (2003) In vitro and in vivo modulations of benzo[*c*]phenanthrene-DNA adducts by DNA mismatch repair system. *Nucleic Acids Res 31*, 6428-6434.

Wu, M., Yan, S., Patel, D. J., Geacintov, N. E., and Broyde, S. (2002) Relating repair susceptibility of carcinogen-damaged DNA with structural distortion and thermodynamic stability. *Nucleic Acids Res 30*, 3422-3432.

Xia, H., Pan, S. S., Hu, X., Srivastava, S. K., Pal, A., and Singh, S. V. (1998) Cloning, expression, and biochemical characterization of a functionally novel alpha class glutathione S-transferase with exceptional activity in the glutathione conjugation of (+)-*anti*-7,8-dihydroxy-9,10-oxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene. *Arch Biochem Biophys 353*, 337-348.

Yagi, H., Hernandez, O., and Jerina, D. M. (1975) Letter: Synthesis of (+/–)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene, a potential metabolite of the carcinogen benzo(*a*)pyrene with stereochemistry related to the antileukemic triptolides. *J Am Chem Soc 97*, 6881-6883.

Yang, L. L., Maher, V. M., and McCormick, J. J. (1980) Error-free excision of the cytotoxic,mutagenic N2-deoxyguanosine DNA adduct formed in human fibroblasts by (+/–)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene. *Proc Natl Acad Sci USA 77*, 5933-5937.

Yang, S. K., McCourt, D. W., Roller, P. P., and Gelboin, H. V. (1976) Enzymatic conversion of benzo(*a*)pyrene leading predominantly to the diol-epoxide *r*-7,*t*-8-dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene through a single enantiomer of *r*-7,*t*-8-dihydroxy-7,8-dihydrobenzo(*a*)pyrene. *Proc Natl Acad Sci USA 73*, 2594-2598.

Yoon, J. H., Besaratinia, A., Feng, Z., Tang, M. S., Amin, S., Luch, A., and Pfeifer, G. P. (2004) DNA damage, repair, and mutation induction by (+)-*syn* and (–)-*anti*-dibenzo[*a,l*]pyrene-11,12-diol-13,14-epoxides in mouse cells. *Cancer Res 64*, 7321-7328.

Yu, S., and Campiglia, A. D. (2005) Direct determination of dibenzo[*a,l*]pyrene and its four dibenzopyrene isomers in water samples by solid-liquid extraction and laser-excited time-resolved Shpol'skii spectrometry. *Anal Chem 77*, 1440-1447.

Zhang, Y., Wu, X., Guo, D., Rechkoblit, O., Geacintov, N. E., and Wang, Z. (2002) Two-step error-prone bypass of the (+)- and (–)-*trans*-*anti*-BPDE-N2-dG adducts by human DNA polymerases eta and kappa. *Mutat Res 510*, 23-35.

Zimniak, P., Nanduri, B., Pikula, S., Bandorowicz-Pikula, J., Singhal, S. S., Srivastava, S. K., Awasthi, S., and Awasthi, Y. C. (1994) Naturally occurring human glutathione S-transferase GSTP1-1 isoforms with isoleucine and valine in position 104 differ in enzymic properties. *Eur J Biochem 224*, 893-899.