Preface

Numerous physical and chemical stress factors, endogenous or exogenous, challenge living organisms. Metabolization processes generate highly reactive intermediates which can covalently bind to DNA, resulting in bulky addition products called "adducts." DNA adduct formation appears to be a general response of plants to organic chemical exposure, whether in controlled conditions or in the field. In particular, common carcinogenic pollutants and pesticides promote the formation of DNA adducts in plants. The authors of this book examine the development of DNA adducts, as well as the ways in which they can be eliminated due to DNA repair pathways. In this book, the data from in vivo transgenic assays is also examined, which can help to clarify specific pre-mutagenic adducts, DNA repair functions and mutational events that may be involved in the mutagenicity of human carcinogens. Other chapters in this book identify and discuss novel anthracyclines capable of forming DNA adducts, the role of DNA adducts as early biomarkers in the screening and development of marine anticancer drugs, the genotoxicity, such as DNA adduct formation, of air pollutants and its assessment by in vivo mutagenesis and a discussion of oxidative DNA damage, which can play an important role in the carcinogenic processes of PAHs and aromatic amines, in addition to bulky-DNA adducts formation.

Chapter I - Even small DNA-protein cross links (DPCs) can be more than 100 times the size of small base modifications. Most current strategies for the study of the elimination of DPCs focus on examination of massive numbers of protein adducts. Although DPCs represent a small fraction of total DNA lesions inflicted by damage such as radiation, these adducts are much more lethal to cells compared to most DNA modifications. Therefore, the elimination of DPCs is critical for cell survival, since the persistence of such lesions can lead to cell death mainly by posing a barrier to DNA and RNA synthesis. As DPCs are a minor overall component of most DNA damaging treatments, the study of their repair is complicated due to the low frequency at which they occur. To facilitate the investigation of DNA repair for this important DNA damage class, a few groups, including the authors, have used a different tactic to evaluate the repair of DPCs in oligodeoxyribonucleotides at defined sites, which implicates nucleotide excision repair in this process. To extend the *in vitro* results and obtain *in vivo* information on cellular mechanisms that maintain genomic integrity, the authors have also constructed plasmids modified with a single DPC. They will evaluate the implications of these data with respect to therapies using DNA cross-linking

agents. This chapter will summarize the results and understanding of DPC repair obtained from these studies.

Chapter II - Carcinogenesis is a complex, multistep process resulting from a series of mutations in critical genes controlling cell division, genome stability or cell death. It is well-established that most mutations result from interaction of chemical carcinogens with DNA to produce DNA adducts. Numerous genotoxicity assays have been conceived to measure the ability of a chemical to induce DNA lesions, mutations and chromosomal damage. Among these, *in vivo* studies of mutagenesis have been developed to account for the complexity of a whole-organism, providing valuable clues to the understanding of the mechanisms of mutation. The unique mutational spectrum produced by an agent in its target organ(s) is dictated mostly by the type and location of DNA adducts and by the repair framework triggered by the lesion, and thus can amplify the knowledge on the mechanisms of *in vivo* DNA repair, mutagenesis and carcinogenesis.

Using a *lacZ* plasmid-based transgenic mouse model, we have analyzed the frequency and spectrum of mutations induced by agents known to elicit different pre-mutagenic lesions in DNA. Additionally, we generated the *Parp1*$^{-/-}$ *lacZ* mouse model, with inactivation of the enzyme poly (ADP-ribose) polymerase (Parp1), and the authors tried to evaluate whether Parp1 deficiency influenced the *in vivo* mutagenic effect of the monofunctional alkylating agent N-Methyl-N-Nitrosurea (MNU).

Based on those studies we provided evidence that cisplatin, an anticancer drug, has mutagenic activity *in vivo* and produces a specific profile of base substitutions and small deletions/insertions in the liver of *lacZ* mice, in agreement with its preferential DNA binding sites. In a subsequent study, we showed that MNU increased the mutant frequency in *Parp1*$^{+/+}$ and *Parp1*$^{-/-}$ *lacZ* mice. Interestingly, our data evidenced a shift in the pattern of MNU-induced mutations in testis of *Parp1*$^{-/-}$ as compared to *Parp1*$^{+/+}$ *lacZ* mice, consisting of a higher frequency of deletions/insertions and a lower frequency of point mutations. In contrast, the MNU-induced point mutation spectra in testis were independent of the Parp1 status.

Those studies illustrate how the data from *in vivo* transgenic assays can help to clarify specific pre-mutagenic adducts, DNA repair functions and mutational events that may be involved in the mutagenicity of human carcinogens.

Chapter III - Numerous physical and chemical stress factors, endogenous or exogenous, challenge living organisms. Cells take up various xenobiotic compounds which are subsequently detoxified through metabolization, conjugation and elimination. However, metabolization processes generate highly reactive intermediates which can covalently bind to the DNA, resulting in bulky addition products called "adducts". In animal cells, when they escape elimination by the DNA repair systems, adducts perturb gene expression and induce mutations. Formation of DNA adducts has therefore been highly correlated with carcinogenesis. Originally considered only in the animal kingdom, these genotoxic processes also occur in plants and are likely to impair their gene expression. The literature in that domain is still limited, but DNA adduct formation appears to be a general response of plants to organic chemical exposure, whether in controlled conditions or in the field. In particular, common carcinogenic pollutants and pesticides promote the formation of DNA adducts in plants. The modified nucleotides generated from a given chemical can be similar or different

between plant and animal cells, reflecting similarities or differences in the metabolization pathways of the exogenous compounds. On the other hand, it seems possible to identify the origin of some DNA adducts occurring in field plants by co-migration with samples from plants treated with a defined chemical in controlled conditions. Experimental data indicate that the analysis of DNA adducts in test-plants can be a relevant and sensitive alternative to characterize oxidative and genotoxic stress from pesticides in crops or to evaluate the genotoxicity of the atmospheric environment. Cells can also eliminate DNA adducts thanks to DNA repair pathways. In human cells, the nucleotide excision repair (NER) pathway is known to eliminate bulky adducts. The NER pathway also exists in plants, but shows significant differences as compared to its mammalian counterpart.

Chapter IV - Most human cancers result from environmental exposures and gene-environment interactions. Diet is an important component of lifestyle and its role in the maintenance of good health and as a determinant of different kind of chronic diseases, including cancer, has been extensively analyzed. Diet can contribute to cancer risk through the consumption of food mutagens, including those generated by cooking. On the other hand, diet that emphasizes the consumption of whole grain foods, legumes, vegetables and fresh fruit and that limit animal fat has been associated with decreased cancer risk. Indeed, a general protective effect against cancers at different sites has been reported for a number of fresh fruit and vegetables. In particular, fresh fruit is a food rich of phytochemicals that can act against reactive oxygen species (ROS) formation and induce the expression of DNA repair systems.

Among life-style habits, tobacco cigarette smoking is an established cancer risk factor. In addition to be a relevant source of mutagens, tobacco smoke is also a major source of oxidative stress and ROS, capable to cause oxidative DNA damage. ROS can also induce lipid peroxidation (LPO), whose by-products can lead to endogenous DNA damage formation.

MDA is a natural product of LPO, also formed during prostaglandins (PGH_2) biosynthesis via cyclooxygenase (COX-1 and COX-2). MDA is an aldehyde capable to interact with cellular constituents including DNA, inducing the formation of M_1dG adducts. The reaction with deoxyguanosine (dG) origins the formation of 3-(2-deoxy-β-D-erythro-pentafuranosyl)pyrimido[1,2-α]purin-10(3H)-one, e.g. the M_1dG adducts. In addition to LPO and PGH_2 biosynthesis, the formation of M_1dG adducts can be induced by ROS through the production of base propenal intermediates.

M_1dG adducts have been shown to block the replication of DNA and induce base pair and frameshift mutations. Moreover, increased levels of M_1dG adducts have been found in number of cancer patients. Higher amount of M_1dG adducts have been also associated to worst surviving in lung cancer patients. Thus, the analysis of M_1dG adduct formation is considered a promising biomarker that can reflect the whole oxidant status of an organism.

In the present study, the authors have investigated the relationship between tobacco smoking, the consumption of fresh fruit and the formation of M_1dG adducts in a group of people resident in Map Ta Phut (MTP), Rayong province, Thailand, including 53 residents in the Map Ta Phut Industrial Estate (MIE) area, and 46 individuals living in a control district of the same province without industrial exposures. The DNA adduct analysis was performed using the ^{32}P-DNA postlabelling assay (Munnia et al., 2006). The potential involvement of

the polymorphism of glutathione S-transferase Mu-1 (GSTM1) in the formation of endogenous DNA adducts has been also evaluated.

Our findings indicate a statistically significant effect of tobacco smoke on M_1dG formation ($p < 0.05$). The increased intake of fresh fruit was inversely associated to the M_1dG adduct production ($p < 0.05$). Then, the GSTM1 polymorphism was not associated with the formation of M_1dG adducts.

Our data indicate that tobacco smoking habits can induce an enhanced formation of M_1dG adducts, possibly through the production of ROS and LPO. Conversely, the consumption of fresh fruits was associated to a decreased formation of DNA damage. The increased formation of M_1dG adducts in current smokers can be related to an excess of LPO induced from tobacco smoke constituents, as well from ROS produced during carcinogen metabolism. The protective effects of fresh fruit on M_1dG adduct production may act on different biological mechanisms, for example by the induction of enzymes involved in DNA repair and by antioxidant effects. The GSTM1 polymorphism could be not relevant for the frequency of M_1dG adducts.

Chapter V - The anthracyclines (doxorubicin, epirubicin, idarubicin and daunorubicin) are among the most widely-used anticancer chemotherapeutic agents. They are generally classified as topoisomerase II inhibitors but also have a variety of other targets in cells. It has been known for some years that the anthracyclines (particularly doxorubicin) are capable of forming DNA adducts, but the relevance and extent of these adducts in cells and their role in causing cell death has remained obscure. When the structure of adducts was solved, it became clear that formaldehyde was an absolute requirement for adduct formation. Adducts form primarily at GC sequences and involve an N-C-N aminal linkage between the drug and the N-2 of guanine, with the central carbon being derived from formaldehyde. The adducts have been extensively characterised *in vitro* by 2D-NMR, X-ray crystallography and mass spectrometry, and the aminal linkage has been shown to be reversible, with a half-life of up to 40 h. In cells, the DNA lesions produced appear to be dependent on two competing cellular pathways that can be switched from primarily topisomerase II lesions to primarily drug-DNA adducts by the use of formaldehyde-releasing prodrugs such as AN-9. The anthracyclines-DNA adducts are able to overcome the major forms of resistance to doxorubicin, are more cytotoxic than topoisomerase II lesions, and invoke conventional apoptotic pathways that are independent of topoisomerase II. Significant levels of adducts have now been detected in breast cancer cells (approximately 1,300 adducts per cell) following a 4 h treatment with clinically relevant levels of doxorubicin (25 nM) as a single agent, and this is elevated some 100-fold by co-treatment with formaldehyde-releasing prodrugs. The synthetic anthracendione anticancer agent, mitoxantrone, can also form adducts with DNA when activated by formaldehyde. These adducts exhibit specificity for CG and CA sequences but are rather unstable. More stable adducts have been detected with pixantrone, a second-generation mitoxantrone analogue that contains primary amino groups on the side-chain. However, the need to identify novel anthracenediones with enhanced anthracenedione-DNA adduct stability remains if these adducts ultimately are to be useful in the clinic.

Chapter VI - Despite several marine anticancer compounds to be DNA-interactive, causing from reductive DNA cleavage to DNA adduct formation, not much attention has been given to the DNA adduct as early biomarker in the screening and development of

marine anticancer drugs. Most of these compounds have been tested *in vitro* by high-throughput cost-effective screening assays for their anticancer potential. In the present study, chemically diverse marine compounds were screened using benzo[*a*]pyrene (BP)-derived DNA adduct formation in MCF-7 cells. Briefly, MCF-7 cells were incubated with the marine compounds, namely manzamine A, sarcophine, aaptamine, verongiaquinol, curcuphenol, and curcudiol (10, 50 and 100 μM) for 24 h followed by treatment with BP (0.5 μM). After 24 h re-incubation, cellular DNA was isolated and analyzed for BP-derived DNA adducts by ^{32}P-postlabeling technique. Interestingly, at 50 μM dose, only manzamine A, a probable antitumor compound, increased the BP-DNA adducts by 3-folds while curcuphenol and curcudiol lowered the BP-DNA adduction by up to 50%. Other compounds insignificantly modulated the BP-DNA adduction. At 10 μM, all the marine compounds were ineffective except aaptamine, which induced BP-DNA adduction by 2-fold. Further at 100 μM dose, all the compounds except manzamine A and verongiaquinol increased the BP-DNA adducts by up to 500%. In addition, verongiaquinol (50 μM) substantially down-regulated the levels of p53, p21, and p16, while manzamine A (50 μM), significantly inhibited the expression of p53. Aaptamine (50 μM) induced the p53 expression by 40%, however, other compounds were ineffective. All other marine compounds resulted in a differential response related to p21 and p16 expression. The residual DNA repair ability was almost completely abolished by manzamine A and verongiaquinol (50 μM) while other compounds seemed ineffective. Based on the preliminary results, it seems that these marine compounds *per se* may not be genotoxic but have potential of differentially modulating the carcinogen-derived DNA adducts enhancing the

Chapter VII - Various mutagenic polycyclic aromatic hydrocarbons (PAHs) including nitro-PAHs, associated with suspended particulate matter (SPM), are possibly deposited in the lungs and other organs of residents in urban areas. To assess the mutagenicity of air pollution and its carcinogenic risk in the lung and other organs, it is necessary to assess the genotoxicity *in vivo* of the various PAHs and nitro-PAHs in urban air. In this chapter, the authors review the genotoxicity, such as DNA adduct formation, of air pollutants and its assessment by *in vivo* mutagenesis.

Chapter VIII - Aflatoxin B1 (AFB1), resulting in the formation of AFB1-DNA adducts, is a known human carcinogen. AFB1-exposed individuals with inherited susceptible carcinogen-metabolizing or repairing genotypes may experience an increased risk of genotoxicity. This study was designed to investigate whether the polymorphisms of two genes, namely, one metabolic gene Glutathione S-transferase M1 (GSTM1) and one DNA repair gene x-ray repair cross-complementing group 3 (XRCC3) codon 241, affected the levels of AFB1-DNA adducts in a Guangxi population (n = 966), from an AFB1-exposure area. AFB1-DNA adducts were measured by ELISA, and GSTM1 and XRCC3 codon 241 genotypes were identified by PCR-RFLP. The GSTM1-null genotype (adjusted odds ratio [OR] = 2.09; 95% confidence interval [CI] = 1.61–2.71) and XRCC3 genotypes with 241 Met alleles (namely, XRCC3-TM and –MM; adjusted ORs [95% CI] were 1.43 [1.08–1.89] and 2.42 [1.13–5.22], respectively) was significantly associated with higher levels of AFB1-DNA adducts. Compared with those individuals who did not express any putative risk genotypes as reference (OR = 1), individuals featuring all of the putative risk genotypes did experience significantly higher DNA-adduct levels (adjusted ORs were 2.87 for GSTM1-null

and XRCC3-TM; 5.83 for GSTM1-null and XRCC3-MM). Additionally, there was a positive joint effect between XRCC3 genotypes and long-year AFB1 exposure in the formation of AFB1-DNA adducts. These results suggest that individuals with susceptible genotypes GSTM1-null, XRCC3-TM, or XRCC3-MM may experience an increased risk of DNA damage elicited by AFB1 exposure.

Chapter IX - Adduct formation has been considered a major factor of DNA damage by carcinogenic polycyclic aromatic hydrocarbons (PAHs) and aromatic amines with metabolic activation. Benzo[a]pyrene (BP), contained in tobacco smoke and air pollution, is one of the most investigated compounds as a carcinogenic PAH. It is considered to express genotoxicity by forming DNA adducts of its metabolite, BP-7,8-diol-9,10-epoxide (BPDE). In addition, BP may induce oxidative DNA damage. A metabolite of BP, benzo[a]pyrene-7,8-dione (BP-7,8-dione) causes oxidative DNA damage, including 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) formation.

Considered carcinogenic aromatic amines contained in cigarette smoke, 2-naphthylamine (2-NA) and 4-aminobiphenyl (4-ABP), have been known to form DNA adducts after metabolic activations. Nitroso derivatives of 2-NA and *N*-hydroxy derivatives of 4-ABP, which are metabolites of each aromatic amine, cause oxidative DNA damage with redox reaction.

Mononuclear compounds, such as benzene and *o*-toluidine, seem to express carcinogenicity by causing oxidative DNA damage rather than DNA adduct formation after metabolic activation. Oxidative DNA damage can play an important role in the carcinogenic processes of PAHs and aromatic amines, in addition to bulky-DNA adduct formation.

Chapter X - Epidemiological evidence suggests the existence of a relationship between high environmental levels of Particulate Matter (PM) and increased incidence of both morbidity and mortality due to respiratory and/or cardiovascular diseases. The consistency among the findings of epidemiological studies argues for a causal association, but it is still difficult to attribute acute health effects to concentration levels in light of the current knowledge. The mechanisms underlying these adverse effects are also not well understood, and major questions still remain concerning the specific size fraction, chemical composition and causative toxicological mechanisms leading to the observed health effects. Hence, to improve the knowledge of air pollution PM-induced toxicity in human lungs, with a particular interest in the crucial role played by coated organic compounds, the authors focused our attention on the metabolic activation of Polycyclic Aromatic Hydrocarbon (PAH)-coated onto air pollution PM and, thereafter, the formation of PAH-DNA adducts in three in vitro cell lung models: A549 cell line, L132 cell line and human Alveolar Macrophages (AM). PAH, PolyChlorinated Dibenzo-p-Dioxins and -Furans (PCDD/F), Dioxin-Like PolyChlorinated Biphenyls (DLPCB) and marker PolyChlorinated Biphenyls (PCB) coated onto collected PM were determined (i.e., GC/MS and HRGC/HRMS, respectively). Cells were exposed to Dunkerque City's $PM_{2.5}$ at its lethal concentrations at 10% and 50% (i.e., A549: LC_{10} = 6.33µg/cm²; and LC_{50} = 31.63µg/cm²; L132: LC_{10} = 5.02µg PM/cm²; and LC_{50} = 20.10µg PM/cm²; AM: LC_{10} = 5.97µg PM/cm²; and LC_{50} = 29.85µg PM/cm²). Cytochrome P450 (CYP) 1A1 gene expression (i.e., RT-PCR) and enzymatic activity (i.e., EROD activity) and the formation of PAH-DNA adducts (i.e., ^{32}P-postlabelling) were investigated after 24, 48 and/or 72h. Particular (i.e., desorbed PM, dPM) and PAH-

positive (i.e., Benzo[*a*]Pyrene, B[*a*]P; 1μM) controls were included in the experimental design. A statistically significant increase in *CYP1A1* gene expression was observed in the three cell lung models under study, 24, 48 and/or 72h after their exposure to PM or B[*a*]P. Similarly, *CYP1A1* gene expression was highly induced 24, 48 and/or 72h after lung cell exposure to dPM, suggesting thereby that the employed outgassing method was not efficient enough to remove total PAH.

Accordingly, EROD activity was significantly increased only in A549 cells and AM, 24, 48 and/or 72h after their exposure to dPM, PM or B[*a*]P. Even if B[*a*]P exposure caused important bulky DNA adduct formation, only very low levels of PAH-DNA adducts, which could not be reliably quantified, were reported 72h after A549 cells and AM exposure to PM and dPM. The relatively low levels of PAH, together with the presence of PCDD/F, DLPCB and PCB coated onto Dunkerque City's $PM_{2.5}$ could contribute to explain this borderline detection of PAH-DNA adducts. Neither significant EROD activity nor PAH-DNA adduct formation could be measured in PM- or in B[*a*]P-exposed L132 cells. In contrast, A549 cells and AM seem to present higher sensitivity to coated organic chemical-induced damage. The authors also concluded that, in the three lung cellular models we used and in the experimental conditions we chose, bulky DNA adduct formation only partly contribute to the lung toxicity arising from the exposure to Dunkerque City's $PM_{2.5}$.