CHEMICAL REVIEWS

REVIEW

pubs.acs.org/CR

Mechanisms of Chemical Carcinogenicity and Mutagenicity: A Review with Implications for Predictive Toxicology

Romualdo Benigni* and Cecilia Bossa

Istituto Superiore di Sanita', Environment and Health Department, Viale Regina Elena, 299 00161 Rome, Italy

CONTENTS

1. INTRODUCTION

Cancer and its prevention are among the most critical health issues. Cancer is the second cause of death in Western countries, just after the circulatory diseases. According to the World Health

Received: July 15, 2010
Published: January 25, 2011

dx.doi.org/10.1021/cr100222q | *Chem. Rev.* 2011, 111, 2507–2536

Organization (WHO), the worldwide cancer burden is set to increase by as much as 50% by the year 2020 unless further preventive measures are put into practice. Unfortunately, this trend is taking shape in recently developed countries as well.[1,2]

At odds with the long-standing assessment[3,4] that the environment plays only a little role in cancer causation, we view that the causes are largely environmental if one correctly includes lifestyle, diet, and work-place exposure as part of the environment. As noted below, different pieces of evidence converge to an estimated 80% environmental component of cancer.[5−7] In this respect, particularly cogent is the following evidence: (a) classical studies on the geographical distribution of tumors and changes in tumor profiles and incidence in migrant populations show that the migrants acquire the same tumor pattern of the new locations in one or two generations;[5] (b) the comparison of cancer concordance data of monozygotic twins against that of dizygotic twins shows that inherited genetic factors make a minor contribution to susceptibility to most types of neoplasms;[8] (c) different estimates of the relationship between genetic patterns (i.e., differential frequency of ABO blood groups) in the populations and tumor incidence agree in pointing only to 10−20% correlation;[9,10] whereas (d) tumor profiles of populations were demonstrated to correlate with cultural/historical patterns.[11]

Environment includes lifestyle, diet, smoking, pollution, indoor and workplace exposure, etc.: an essential component of all this is the chemicals.[12] A central role in understanding and preventing the chemical induction of cancer is played by the study of its mechanisms of action. These studies range from biochemical investigations (e.g., on the metabolism of chemicals, on their interaction with critical cellular macromolecules, etc.), to the use of assay systems (e.g., rodent bioassay, *in vitro* and *in vivo* short-term mutagenicity tests), to structure−activity relationship (SAR) studies. Whereas a rich and diverse body of literature on the mechanisms of action of carcinogens is available, this evidence is quite sparse with few attempts to put into perspective results obtained with the different approaches. In this paper, we try to summarize the different results. Because of the close relationship with, and the involvement of mutagenicity in carcinogenicity, we consider also the mutagenicity end point.

While the mechanistic knowledge deriving from experiments is the main focus of this review, we present also theoretical models on the relationship between chemical structure and the molecular mechanisms of toxic activity; however, this is limited only to those studies that contribute to a better mechanistic understanding. A short appraisal of how the mechanistic knowledge of chemical carcinogenicity contributes to predictive toxicology is provided as well.

Before illustrating the different chemical classes and mechanisms, a short background on the main results of the research on chemical carcinogenicity is presented.

2. SCIENTIFIC BACKGROUND

More than other toxicity end points, mutagenicity and carcinogenicity have been the subject of a long series of mechanistic investigations, and considerable scientific background is available. The electrophilic theory of chemical carcinogenesis developed by James and Elizabeth Miller[13−15] enabled the activity of the large majority of animal carcinogens known by the 1970s to be tentatively rationalized. Historically, the Millers first noted the electrophilicity of carcinogenic alkylating agents. Since then, a number of acylating agents were found to be carcinogenic, and these chemicals were also electrophilic as administered. In addition, the Millers were much impressed by the variety of chemical carcinogens of rather different structures for which metabolism to electrophilic reactants had been demonstrated. Overall, this evidence led them to suggest "that most, if not all, chemical carcinogens either are, or are converted *in vivo* to, reactive electrophilic derivatives which combine with nucleophilic groups in crucial tissue components, such as nucleic acids and proteins".[13]

The research on the mechanisms of chemical carcinogenicity interacted with and was cross-fertilized by the concomitant research on chemical mutagenicity to a large extent. This leads to the theory that electrophilic chemicals could be both mutagens and carcinogens, with the corollary that mutagenicity could be a step in the carcinogenicity process. It should be emphasized that at the beginning of their research James and Elizabeth Miller could only say that there were good theoretical reasons why mutagens should be carcinogens, but there was still insufficient data to support such a view in a compelling manner. The view that mutagens were carcinogens did not really happen until after Heinrich Malling showed that the S30 liver fraction can metabolically transform many nonmutagenic carcinogens into mutagens, and Bruce Ames used an S9 liver fraction systematically in a bacterial assay to create a large database of mutagens that were also carcinogens.[16]

The term mutagenicity indicates the ability to induce transmissible genetic damage, which includes gene mutations, chromosomal aberrations, and changes in the number of chromosomes. It should be noticed that gene mutations are provoked by interactions with DNA, whereas the other two types of mutations may derive from interactions both with DNA and with other cellular targets (e.g., proteins). Another term often used is genotoxicity, which is a broader category than mutagenicity. In addition to mutagenic (fixed and transmissible) effects, genotoxicity includes also effects on the genetic material that may ultimately give rise to mutations but are not transmitted as such to daughter cells (e.g., DNA damage).[16]

Whereas the main and definitive proof that a chemical is a human carcinogen derives from observations in humans collected through epidemiological studies, the large majority of carcinogens have been identified by studies in animals. Rats and mice have been preferred experimental models because of their relatively short life span, the limited cost of their maintenance with respect to animals with a larger size, their widespread use in pharmacological and toxicological studies, their susceptibility to tumor induction, and the availability of inbred or sufficiently characterized strains.[17−19] Thus animal experiments are the major source of information on chemical carcinogens. However, because the rodent bioassay is long and costly and involves the use of large numbers of animals, particularly important has been the research aimed at the creation of cheaper and shorter-term alternatives to the rodent bioassay. Many investigators gave contributions to this research.[16] Among others, Bruce Ames created a series of genetically engineered *Salmonella typhimurium* bacterial strains, each strain having some sensitivity to specific classes of chemical carcinogens (e.g., alkylating, intercalating, etc.). The *Salmonella* or Ames test is an *in vitro* model of chemical carcinogenicity, and it consists of a range of bacterial strains that together are sensitive to a large array of DNA damaging agents.[20−23] Because most of the carcinogens known at that time acted through genotoxic mechanisms, the activity of carcinogens as mutagens to *Salmonella* almost

always seemed plausible within the context of the Millers' hypothesis.[24]

The application of the Ames test to large numbers of chemicals has shown that this assay has a high positive predictivity for DNA-reactive chemical carcinogens: Ames-test mutagens have a high probability of being also carcinogenic, whereas a negative result has no discriminatory value (a chemical negative in *Salmonella* can be either a noncarcinogen or a nongenotoxic carcinogen with the same probability).[22,25] It should be emphasized that the excellent performance of the Ames test in the identification of carcinogens derives directly from its brilliant scientific design: it has been purposely constructed to detect a range of chemical classes of DNA-reactive agents. However, the correlation between the Ames test and carcinogenicity is valid only at the level of yes/no activity, whereas mutagenic and carcinogenic potencies are uncorrelated:[26] thus only the (first ?) rate-limiting step of the chemical−cell interaction is in common in the two systems.

The above evidence fits into the overall scheme of chemical carcinogenesis, as generally accepted today. According to this view, chemical carcinogenesis is a multistage, multifactorial process consisting of three operational stages: initiation, promotion, and progression. Initiation is thought to involve a mutational event that may include gene mutation, chromosome aberration, translocation, and instability. Promotion involves clonal expansion of initiated cells to reach a critical mass by, for example, cell proliferation, inhibition of programmed cell death, persistent chronic inflammation, inhibition of terminal differentiation, and loss of growth control. Progression may involve a second mutation event, the loss of tumor suppressor gene, impairment of immune surveillance, and acquisition of ability to metastasize.[27] Within the above scheme, the gene mutation-based Ames test detects chemicals potentially able to trigger the first stage of carcinogenesis, that is, initiation, but is not sensitive to factors that affect the subsequent stages. The disjunction between the pathway leading to the expression of mutations in *Salmonella* and that leading to the tumor promotion and progression in animals, after the first common step of DNA reaction, is responsible for the lack of correlation between toxic potencies in the two systems.

Another important contribution came from John Ashby, which resulted in the definition and compilation of structural alerts (SAs) following the electrophilicity theory of the Millers.[24,28] The SAs for carcinogenicity are defined as molecular functional groups or substructures that are mechanistically linked to the carcinogenic activity of the chemicals. Thus, the SAs identify chemical classes potentially able to cause cancer. Because the attack to and the modification of DNA is the main step in the mechanism of action of many carcinogens, the SAs relative to such classes of carcinogens are also valid for the *Salmonella* mutagenicity end point.

The brilliant results obtained with the *Salmonella* Ames test convinced the scientific community that the correlation between chemical carcinogenicity and mutation was a general one and that it was possible to increase the above correlation by considering also genetic events different from those at the basis of the Ames test (i.e., base substitutions and deletions/additions): thus a myriad of further genotoxicity assays, based on events such as structural chromosome aberrations (breaks and rearrangements) and numerical chromosome aberrations (loss or gain of chromosomes, defined as aneuploidy) were developed. In addition, since the Ames test uses bacteria, tests complementary to *Salmonella* were sought by extending the experimental systems to *in vitro* mammalian cells, as well as to *in vivo* assays.

However, even though several of the "additional" genotoxicity assays have gained great popularity and have been adopted in various regulatory settings, rigorous comparative studies have failed to demonstrate a correlation with and predictive ability with respect to rodent carcinogenicity. As a matter of fact, the correlation between cancer and mutation seems to be valid only for a limited area of the chemical space, that is, the DNA-reactive chemicals. It does not hold for the chemicals that are negative in *Salmonella* and do not have alerts for DNA reactivity, even though they are able to induce mutation in systems different from *Salmonella* (e.g., chromosomal aberrations, or aneuploidy).[25,29−33]

To conclude this section, it should be emphasized that the original hypothesis of the electrophilic reactivity of (many classes of) chemical carcinogens maintains its validity, and it has been incorporated into a more general theory on chemical carcinogens. From the point of view of their mechanism of action, carcinogens are commonly classified into the following categories: (a) Genotoxic carcinogens, for which mutations are supposed to be one of the first steps in the development of cancer.[34] As explained above, a more convincing correlation with carcinogenicity is that relative to DNA-reactive carcinogens only (to exclude mutations caused by different pathways). (b) Nongenotoxic or epigenetic carcinogens that do not bind covalently to DNA, do not directly cause DNA damage, and are usually negative in the standard mutagenicity assays.[35] Whereas the nongenotoxic carcinogens act through a large variety of different and specific mechanisms with no apparent unifying concept, the genotoxic carcinogens (to be more precise, the DNA-reactive carcinogens) have the unifying feature that they are either electrophiles *per se* or can be activated to electrophilic reactive intermediates, as originally postulated by the Millers.

3. MECHANISMS OF ACTION OF CHEMICAL CARCINOGENS AND MUTAGENS: A GENERAL PERSPECTIVE

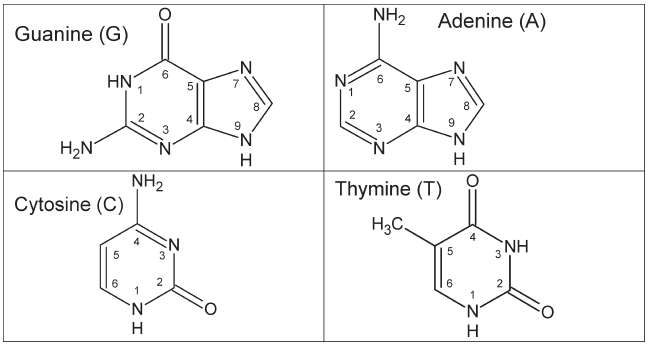
3.1. Genotoxic (DNA-Reactive) Carcinogens

A first, necessary step in both the carcinogenic and mutagenic activity of genotoxic carcinogens is considered to be their interaction with DNA, either directly or after metabolic activation. Following Miller's theory, sites that potentially could interact with electrophilic species are the DNA nucleophilic centers: nitrogens and oxygens of pyrimidine and purine bases and the phosphodiester backbone.

In addition to adduction to a single site on a nucleobase, bifunctional agents may react with another nucleophilic site in (i) the same nucleobase, forming a bicyclic or tricyclic system, (ii) a different nucleobase in the same or in the opposite DNA strand, forming inter- or intrastrand cross-links, respectively, or (iii) a protein, forming a DNA−protein cross-link.

In addition to the chemical nature of the reactive species, the specificity of the reactions at the different sites strongly depends on the nucleophilicity of the DNA centers and on steric factors (see Chart 1 for the numbering of atoms in the DNA bases). The most nucleophilic sites are endocyclic nitrogens such as N3 and N7 of guanine and adenine, while exocyclic base oxygens are less nucleophilic.[36] Guanine N7, exposed in the major groove of normal helical DNA, is more accessible and hence more able to react with electrophiles than the N3 position of adenine oriented to the minor groove. By similar reasoning, highly nucleophilic sites such as N1 of adenine and N3 of cytosine, do not react

Chart 1. Numbering of Atoms of the DNA Bases[a]



[a] In the text, superscripts indicate exocyclic oxygen or nitrogen (e.g., O^6), whereas regular format indicates endocyclic atoms (e.g., N7).

extensively because of steric hindrance.[37] In addition to adducts to nitrogens and oxygens, other adducts have been detected. For example, despite its weak nucleophilicity, adducts at the C8 position of guanine are frequently encountered as well (e.g., in the case of reaction with reactive metabolites of the aromatic amines). However, there is evidence suggesting that formation of these guanine adducts involves an initial attack at the N7 position, followed by rearrangement.[38,39]

Inside this general panorama, the chemical carcinogens are highly differentiated in terms of patterns of DNA adducts. Alkylating agents are chemicals capable, *per se* or after metabolic activation, of transferring alkyl residues (that may contain quite complex structures including an aromatic system not conjugated with the site of substitution) to DNA. The N7-position of guanine is frequently the site modified most extensively by alkylating agents. The preferences observed at other sites commonly have been rationalized in terms of hard−soft reactivity principles.[40−42] Hard alkylating agents (defined by small size, positive charge, and low polarizability) display increased reactivity with hard oxygen nucleophiles in DNA. On the other hand, soft (large, uncharged, and polarizable) alkylating agents favor reactions at the softer nitrogen. Notably, the exocyclic amino groups are not effectively targeted by the alkylating agents.

Another class of chemical carcinogens are the acylating agents.[13,15] The chemicals in this class react by transferring an acyl moiety to DNA nucleobases. Adduction to the O^6 position of guanine and to the exocyclic amino groups of cytosine, adenine, and guanine have been reported.[43,44]

Another pattern of substitution sites is displayed by the arylaminating agents (e.g., aromatic amines and amides, aminoazo dyes, and nitroaromatics): they are bioactivated to strongly electrophilic species that react with DNA to yield arylamino derivatives. The major class of DNA adducts formed by these compounds are at the N^2 and C8 atoms of guanine.[39]While N^2-guanyl adducts may be easily rationalized in terms of an electrophilic reaction of the activated nitrenium ion (or its carbenium equivalent), the mechanisms of the reaction at C8 are less clear. This position is in fact a favored site for certain radical addition reactions but does not react readily with other electrophilic species. A mechanism involving initial formation of an N7-guanyl adduct has been proposed.[38]

Another way by which chemicals can interact with DNA is intercalative insertion in the space between adjacent base pairs. Intercalation is a noncovalent association, whose affinity is governed by the strength of electrostatic (e.g., hydrogen bonding) and van der Waals interactions. Planar polycyclic aromatic molecules are classical intercalating agents, able to insert partially or completely between adjacent DNA base pairs.[45] In addition, DNA intercalative activity has been documented for diverse molecules with unfused multiring structures.[46]

Potential consequences of intercalation comprise local structural changes to the DNA, including unwinding of the double helix and lengthening of the damaged DNA strand, double strand breaks through topoisomerase poisoning,[47] and frameshift mutagenesis in bacteria (especially in repetitive DNA sequences).[48] However, pure DNA intercalation in the absence of addition or strong bonding does not necessarily give rise to a genotoxic event.[49,50]

A large number of polycyclic aromatic hydrocarbons (PAH) cause the formation of bulky DNA lesions by covalent binding after metabolic activation to highly reactive diol epoxides.[45] A crucial factor for the production of covalent DNA adducts by diol epoxides depends on their ability to form, in a first step, intercalative noncovalent complexes with DNA.[51] Differently from other agents, PAH diol epoxides react primarily with exocyclic amino groups of deoxyadenosine and deoxyguanosine. The relative extent of the two purine nucleoside adducts depends on the chemical structure of the PAH.[52]

The adducts generated by DNA-reactive chemicals are subjected to a variety of repair processes in the cells. Inefficient repair may result in mutational consequences during DNA replication. Different probabilities for causing miscoding have been associated with the different sites of adduct formation. Adducts at coding sites, such as O^6, N1, and N^2 of guanine, N1 and N^6 of adenine, O^2, N3, and N^4 of cytosine, and O^4 and N3 of thymine, may be classified as promutagenic lesions, because unless successfully repaired they can interfere with appropriate base pairing during DNA replication and cause a mutation.[37] In contrast, adduct formation at the N7 position of guanine and adenine and the N3 position of adenine generates less stable adducts that spontaneously depurinate, producing efficiently repaired apurinic sites.[53] An exception to this is represented by some large N7 lesions, such as those provoked by aflatoxins, that can have potent biological activities, including mutagenicity.[54]

Besides the adducts formed at coding sites, the adducts that alter DNA structure or prevent replication can also give rise to genotoxic effects. This is the case for several DNA adducts derived from bulky aromatic carcinogens that induce mutations by causing conformational changes in DNA.[55]

3.2. Nongenotoxic Carcinogens

As emphasized before, nongenotoxic carcinogens act by a variety of mechanisms with no apparent unifying concept. Below is an overview on the major classes of mechanisms of nongenotoxic carcinogenicity.

Peroxisome proliferators (PPs) are a class of chemicals found to cause liver cancer when chronically administered to rats and mice.[56] These chemicals are considered nongenotoxic agents, given generally negative results in genotoxicity assays. Even if the mechanism by which these chemicals cause tumors is not fully understood, peroxisome proliferator-activated receptor alpha (PPAR α) is thought to mediate most of the PP effects in the rodent liver.[57]

Two hypotheses have been proposed to account for PP-induced hepatocarcinogenesis in rodents: (i) increase in DNA damage through induction of oxidative stress[58] and (ii) alteration of hepatocyte growth control by enhanced cell proliferation or decreased apoptosis.[59]

Although PPs are generally diverse in structure, three classes to which many of them belong may be identified: (1) phenoxy acid derivatives, (2) alkylcarboxylic acids and precursors, and (3) phthalate esters.[35]

It has been demonstrated that among PPs, perfluorinated fatty acids of specific chain lengths down-regulate gap junctional intercellular communication (GJIC).[60] Altered GJIC has been linked with abnormal cell growth and development and has been implicated in the mechanism of action of nongenotoxic carcinogens.[61]

Many nongenotoxic carcinogens exhibit an inhibition or reduction in GJICs. Among them are chlordane, DDT, pentachlorophenol, phenobarbital, polybrominated biphenyls, butylated hydroxyanisol, acetamide, phenytoin, mirex, and nickel and arsenic compounds.[62]

Cytotoxicity is another mechanism associated with nongenotoxic carcinogens. High doses of cytotoxic agents may induce a hyperproliferative state in the target tissue, characterized by cell death and compensatory regenerative cell proliferation. Persistent cell proliferation in turn may be responsible for the development of neoplasia. Cytolethality, with consequent inflammation, regenerative hyperplasia, or immune response modulation, is thought to be a necessary step to drive the carcinogenesis process by cytotoxic carcinogens.[63] As an example, chloroform-induced tumor development in mouse liver has been shown to be secondary to events associated with continual cytotoxicity and subsequent regenerative cell proliferation.[64]

A number of nongenotoxic chemicals acting through a cytotoxic mode of action have been found to generate bladder tumors or hyperplasia by formation of bladder stones, calculi, or microcrystals.[35] Examples are saccharin-induced urinary precipitates and microcrystals in the rat bladder[65] and rodent urinary calculi and bladder stones produced by uracil, melamine, and terephthalic acid.[66,67]

Binding to $\alpha_{2\mu}$-globulin has been reported as another cytotoxic mechanism by which many chemicals of diverse structures induce kidney tumors in male rats. Substances like unleaded gasoline, trimethylpentane, D-limonene, and penta- and hexachloroethane are able to bind directly or after metabolism to $\alpha_{2\mu}$-globulin protein, present in large amounts in male rats of certain species.[35] The resultant complex is not easily digested by the cell. Consequent accumulation of $\alpha_{2\mu}$-globulin has been associated with cytotoxicity, necrosis of the tubule epithelial cells with sustained regenerative cell proliferation, and ultimately induction of renal tubule tumors.[68]

Immunosuppression may be another crucial event in cancer induction by nongenotoxic carcinogens. Immunosuppressive therapies are often associated with higher incidence of development of malignant disease.[69] The role of immunosuppressant drugs during the first steps of tumorigenesis remains controversial. However, impaired immune surveillance of neoplastic cells and depressed antiviral immune activity are thought to be involved in the progression and dissemination of cancer.[70] A cell-autonomous mechanism independent of host immunity was reported for cancer promotion by the widely used immunosuppressant cyclosporine A.[71]

Another class of nongenotoxic carcinogen acts through induction of hormonal imbalance and subsequent overproduction of trophic hormones. Persistently increased hormonal levels stimulate cell proliferation and eventually tumors development.[35] Long time exposure to estrogenic hormones have been associated with increased cancer risk in hormone-sensitive tissues (breast, endometrium).[72] The major mechanism of their carcinogenic effect is considered to be the stimulation of cellular proliferation through nuclear estrogen receptor-mediated signaling.[73] Estrogen genotoxic effects, through cytochrome P450-mediated metabolic activation, are thought to be an important complementary pathway in their carcinogenicity. Reactive electrophilic metabolites (estrogen *o*-quinones) are generated, which are able to directly form adducts with DNA or induce oxidative damage through redox cycling processes and reactive oxygen species (ROS) production.[72,74]

Drug-induced hormonal disturbances have been demonstrated to cause tumorigenic effects on thyroid gland in rodents. Different nongenotoxic mechanisms may be involved: disruption in biosynthesis (e.g., perchlorate, amitrole, thiourea) or secretion (e.g., lithium) of thyroid hormones; increased peripheral metabolism of thyroid hormones through induction of hepatic microsomal enzymes (e.g., phenobarbital, chlordane, DDT, TCDD, PCB); increase of thyroid-stimulating hormone (TSH) secretion via inhibition of the enzyme 5′-monodeiodinase (FD&C Red No. 3, diphenylthiohydantoin).[35,75] Associated with hormonal imbalance, agents that cause thyroid tumor development have been found to induce chronic TSH hypersecretion leading to progression of follicular cell hypertrophy, hyperplasia, and eventually neoplasia.[76] Thyroid carcinogens are generally diverse in chemical structure. Nonetheless, the presence of a thionamide structural element was reported to increase the likelihood of thyroid carcinogenic potential, though not sufficient by itself to generate such activity.[35]

Oxidative stress is another possible mechanism by which nongenotoxic carcinogens may function. It derives from an imbalance between ROS production and the antioxidant capacity of the target cell. ROS may interact with critical cellular components such as protein, lipids, and DNA.[77] Furthermore, it has been demonstrated that ROS interfere with signal transduction pathways and regulation of gene expression (through a modulation of a host of signaling pathways).[78,79] These epigenetic effects are thought to be important mainly in the tumor promotion stage of carcinogenesis, whereas oxidative DNA damage contributes predominantly to the initiation process.

The carcinogenesis process has frequently been associated with an altered DNA methylation profile.[80] DNA methylation, that is, the 5-methylcytosine content of DNA, is an epigenetic mechanism involved in transcriptional regulation. Altered patterns of methylation are thought to interfere with gene expression and have been observed in tumor cells and tumor tissue.[81] In particular, DNA hypermethylation or hypomethylation of promoter regions, leading, respectively, to suppression of tumor suppressors or overexpression of oncogenes, is thought to play a role in carcinogenesis.[27,82,83] Furthermore, increased DNA methylation increases the probability of C to T point mutations, due to spontaneous deamination of 5-methylcytosine to thymine.[83] Numerous studies have been carried out in order to explore altered DNA methylation patterns induced by chemical carcinogens.[35]

4. INDIVIDUAL CLASSES OF CHEMICAL CARCINOGENS AND THEIR MECHANISMS OF ACTION

After the above overview on the mechanisms of chemical carcinogenicity (and mutagenicity), individual chemical classes will be detailed. To organize the presentation, the recent work aimed at updating existing lists of structural alerts will be exploited. The SAs are the distillation of and code for the action mechanisms with the widest and most scientifically solid

Table 1. Structural Alerts for Carcinogenicity and Mechanisms of Action[a]

	Acylating, Direct Acting Agents
SA_1	acyl halides
SA_15	isocyanate and isothiocyanate groups
SA_6	β-lactones (and γ-sultones)
	Alkylating, Direct Acting Agents
SA_2	alkyl (C < 5) or benzyl ester of sulfuric, sulfonic, phosphoric, or phosphonic acid
SA_3	*N*-methylol derivatives
SA_5	S or N mustard
SA_6	β-lactones and γ-sultones
SA_7	epoxides and aziridines
SA_8	aliphatic halogens
SA_9	alkyl nitrite
SA_10	α,β-unsaturated carbonyls
SA_11	simple aldehyde
SA_12	quinones
	Alkylating, Indirect Acting Agents
SA_4	monohaloalkene
SA_13	hydrazine
SA_14	aliphatic azo and azoxy
SA_16	alkyl carbamate and thiocarbamate
SA_21	alkyl and aryl *N*-nitroso groups
SA_22	azide and triazene groups
SA_23	aliphatic *N*-nitro group
SA_24	α,β-unsaturated aliphatic alkoxy group
	Intercalating and DNA Adduct Forming, Indirect Acting Agents
SA_18	polycyclic aromatic hydrocarbons
SA_19	heterocyclic polycyclic aromatic hydrocarbons
SA_30	coumarins and furocoumarins
	Aminoaryl DNA Adducts Forming, Indirect Acting Agents
SA_25	aromatic nitroso group
SA_26	aromatic ring *N*-oxide
SA_27	nitro-aromatic
SA_28	primary aromatic amine, hydroxyl amine, and its derived esters
SA_28bis	aromatic mono- and dialkylamine
SA_28ter	aromatic *N*-acyl amine
SA_29	aromatic diazo
	Nongenotoxic Carcinogens
SA_20	(poly) halogenated cycloalkanes
SA_17	thiocarbonyl
SA_31a	halogenated benzene
SA_31b	halogenated PAH
SA_31c	halogenated dibenzodioxins

[a] The table reports the compilation of chemical classes with recognized mechanistic link to carcinogenicity and mutagenicity, coded as structural alerts in the expert system Toxtree 2.1.0 (with Toxtree codes). The classes are grouped under broad mechanisms of action (see text). Toxtree 2.1.0 is an open-source, freely available software application that places chemicals into categories and predicts various kinds of toxic effect by applying various decision tree approaches. Toxtree was developed by IdeaConsult Ltd. (Sofia, Bulgaria) under the terms of an ECB contract (Worth, A. P. et al. *SAR QSAR Environ. Res.* **2007**, *18*, 111). Download site: http://ecb.jrc.ec.europa.eu/qsar/qsar-tools/index.php?c=TOXTREE.

knowledge basis. Following the SA compilation by Ashby, other authors tried to update the list of chemical classes whose ability to induce cancer (and mutations) is recognized.[23,84] These more recent compilations incorporate also a number of SAs for nongenotoxic mechanisms of carcinogenesis.

In this review, the compilation of SAs recently implemented into the expert system Toxtree 2.1.0[23,85] will be used as a basis for our presentation. Table 1 reports the SAs grouped according to their mechanisms of action. The genotoxic SAs provoke primary DNA damage by acylation, alkylation, intercalation, or formation of aminoaryl DNA adducts. A further distinction is between those capable of direct interaction with DNA and those that become reactive after metabolic transformation (indirect acting). Another class is that of nongenotoxic SAs. Figure 1 displays the structural features of each of the chemical classes.

4.1. Acylating, Direct Acting Agents

4.1.1. Acyl Halides. Chemicals that contain a carbonyl group bound to a halogen atom are potential direct-acting acylating agents toward DNA. In these substances, inductive electron withdrawal by the halogen atom increases the electrophilic character of the carbonyl carbon.

A classical example of this chemical class is dimethylcarbamoyl chloride,[86] which is classified in group 2A as "Probably Carcinogenic" to humans by the International Agency of Research on Cancer (IARC).[87] Dimethylcarbamoyl chloride is used primarily as a chemical intermediate in the production of dyes, pharmaceuticals, and pesticides.[87] Dimethylcarbamoyl chloride and diethylcarbamoyl chloride were reported to react *in vitro* with deoxyguanosine, forming O^6 acyl derivatives (6-dimethylcarbamyloxy-2′-deoxyguanosine and 6-diethylcarbamyloxy-2′-deoxyguanosine, respectively, see Figure 2).[44]

4.1.2. Isocyanate and Isothiocyanate Groups. Isocyanates are highly reactive compounds that have a variety of commercial applications. In particular, diisocyanates and their oligomers are used for manufacturing polyurethane foam, elastomers, paints, adhesive, coatings, insecticides, and many other products. Monoarylisocyanates are important intermediates in the manufacturing of pharmaceuticals and pesticides.[88] They have been shown to cause severe irritation to the mucous membranes of the eyes and respiratory tract on inhalation exposure.[43]

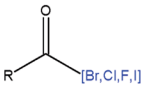
Isocyanates are direct-acting acylating agents, which do not require metabolic activation. Isocyanate adducts may result from electrophilic reaction of the −N═C═O group with the nucleophilic atoms of DNA and protein. Aromatic isocyanates may also undergo hydrolysis to the carbamic acid and subsequent decarboxylation to the corresponding aromatic amine, giving further arylamine adducts with DNA.[89]

The chemical structure of potential isocyanate adducts present *in vivo* is not known. DNA adducts of isocyanates have been synthesized with methyl,[90,91] phenyl,[91,92] 2-naphthyl,[93] 4-chlorophenyl, and 4-methylphenyl isocyanate.[43] In these adducts, carbamoylation at exocyclic amino groups of cytosine, adenine, and guanine have been observed (Figure 3 displays the case of 4-chlorophenyl and 4-methylphenyl isocyanate). No adduct was detected with thymine.

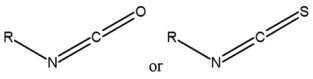
Isothiocyanates are highly biologically active compounds formed upon enzymatic hydrolysis of glucosinolates, which are naturally occurring thioglycosides contained in a variety of cruciferous vegetables. Some of these compounds are effective protective agents against the carcinogenic effect of different compounds, including nitrosamines, heterocyclic aromatic

Acylating, direct acting agents

SA_1: Acyl halides

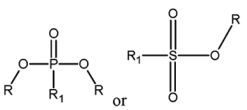


SA_15: isocyanate and isothiocyanate groups

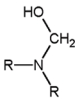


Alkylating, direct acting agents

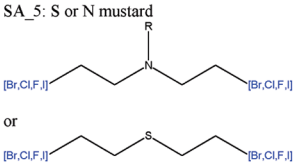
SA_2: alkyl (C<5) or benzyl ester of sulphonic or phosphonic acid



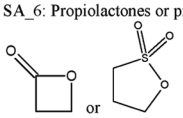
SA_3: N-methylol derivatives



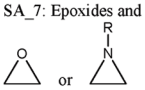
SA_5: S or N mustard



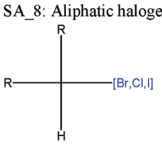
SA_6: Propiolactones or propiosultones



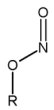
SA_7: Epoxides and aziridines



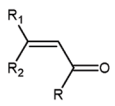
SA_8: Aliphatic halogens



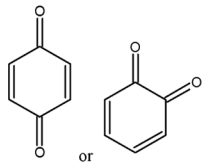
SA_9: Alkyl nitrite



SA_10: α, β unsaturated carbonyls



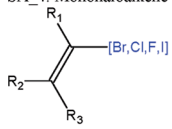
SA_12: Quinones



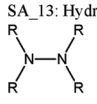
SA_11: Simple aldehyde

Alkylating, indirect acting agents

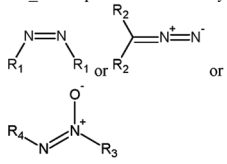
SA_4: Monohaloalkene



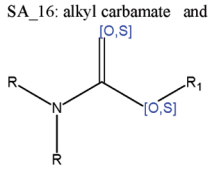
SA_13: Hydrazine



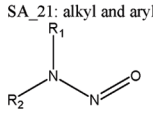
SA_14: Aliphatic azo and azoxy



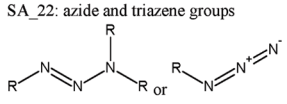
SA_16: alkyl carbamate and thiocarbamate



SA_21: alkyl and aryl N-nitroso groups

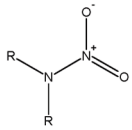


SA_22: azide and triazene groups

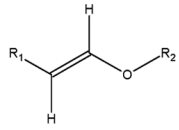


Alkylating, indirect acting agents

SA_23: aliphatic N-nitro group



SA_24: α, β unsaturated aliphatic alkoxy group



Intercalating and DNA-adducts forming, indirect acting agents

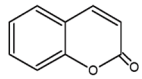
SA_18: Polycyclic Aromatic Hydrocarbons

Three or more fused rings, not heteroaromatic

SA_19: Heterocyclic Polycyclic Aromatic Hydrocarbons

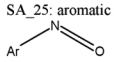
Three or more fused rings, heteroaromatic

SA_30: Coumarins and Furocoumarins

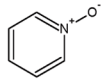


Aminoaryl DNA-adducts forming, indirect acting agents

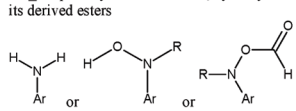
SA_25: aromatic nitroso group



SA_26: aromatic ring N-oxide

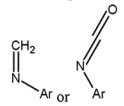


SA_28: primary aromatic amine, hydroxyl amine and its derived esters



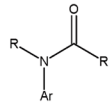
SA_27: Nitro-aromatic

or amine generating group:



SA_28bis: Aromatic mono- and dialkylamine

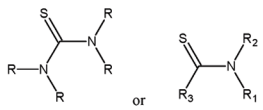
SA_28ter: aromatic N-acyl amine



SA_29: Aromatic diazo

Nongenotoxic carcinogens

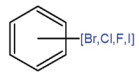
SA_17: Thiocarbonyl (nongenotoxic)



SA_20: (Poly) Halogenated Cycloalkanes (nongenotoxic)

Any cycloalkane skeleton with three or more halogens directly bound to the same ring

SA_31a: Halogenated benzene (nogenotoxic)



SA_31b: Halogenated PAH (nogenotoxic)

Ar = naphthalene, biphenyl, diphenyl

SA_31c: Halogenated dibenzodioxins (nogenotoxic)

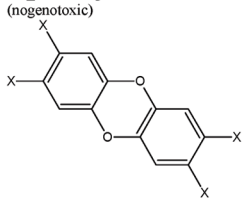


Figure 1. Structural features of the chemical classes listed in Table 1.

dx.doi.org/10.1021/cr100222q |*Chem. Rev.* 2011, 111, 2507–2536

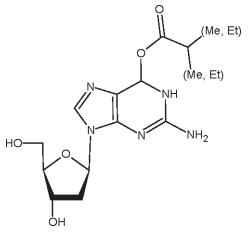


Figure 2. DNA adducts of dimethylcarbamoyl chloride and diethylcarbamoyl chloride.

amines, and polycyclic aromatic hydrocarbons.[94] Isothiocyanates are believed to possess multiple anticarcinogenic mechanisms (e.g., inhibition of carcinogen-activating enzymes such as cytochrome P450 isoforms, induction of carcinogen-detoxifying enzymes, increase of apoptosis, inhibition of cell proliferation, modulation of oxidative stress, arrest of cell cycle progression).[94]

Despite their chemopreventive effect, DNA damage have been observed for a number of isothiocyanates.[95−98] Indeed in the case of allyl isothiocyanate it has also been found that it causes bladder tumors in rats,[99] while for benzyl isothiocyanate and phenethyl isothiocyanate, tumor-promoting activities has been reported.[100] The general mechanism through which the above isothiocyanates exert their genotoxic effects is unknown. However, several investigations suggest that ROS generation may be involved in the genotoxicity of these compounds. In addition, it has been reported that the N═C═S group of isothiocyanates can undergo spontaneous hydrolysis and lead to the formation of superoxide and hydrogen peroxide, inducing oxidative DNA damage.[100] Moreover, ROS production may also be mediated by cytochrome P450 enzymes.[101] However, it should be remarked that the ability of the isothiocyanates as a class to cause genotoxic effects is still under investigation.

4.1.3. *β*-Lactones. The high chemical reactivity of *β*-lactones is due to the strained four-membered ring, which reacts rapidly with nucleophilic centers, as both (i) alkylating and (ii) acylating agents. For a more detailed explanation, see section 4.2.4 below.

4.2. Alkylating, Direct-Acting Agents

4.2.1. Alkyl (C < 5) or Benzyl Esters of Sulfuric, Sulfonic, Phosphoric, or Phosphonic Acid. Alkylic or benzylic esters of phosphonic and phosphoric acid are potentially genotoxic carcinogens. In the common structural element P−O−C, both P and C are electrophilic sites. As a consequence, two general mechanisms of interaction with nucleic acids are possible: (a) mechanism 1, alkylation may occur as a result of nucleophilic attack on the carbon with subsequent cleavage of the C−O bond; (b) mechanism 2, alternatively, a nucleophile can preferentially attack the electrophilic phosphorus and undergo phosphorylation[102] (see Figure 4).

The first mechanism is generally believed to be the major contributor to the genotoxicity of organophosphorus compounds. As the size of the alkyl group increases, the alkylation activity decreases; however halogenation on the alkyl group can be expected to increase the electrophilic reactivity.[103] Thiophosphorus compounds are less effective in alkylation, but they can undergo metabolic oxidation to the corresponding organophosphorus compounds.

Organophosphorous pesticides, which are agrochemicals that are used worldwide, pertain to this class of compounds. They are

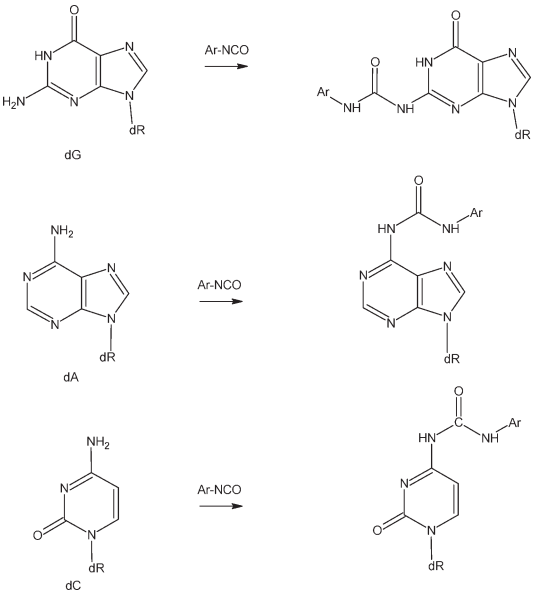


Figure 3. DNA adducts of aryl isocyanates (Ar = 4-chlorophenyl or 4-methylphenyl): dR = deoxyribose; dG = deoxyguanosine; dA = deoxyadenosine; dC = deoxycytidine.

mainly known by their neurotoxic effects due to the inhibition, through phosphorylation, of acetylcholinesterase. In addition, genotoxic effects have been associated with these substances,[104,105] and different mechanisms have been hypothesized.

Guanine N7 alkylation has been observed for various organophosphorus insecticides.[106−108] Furthermore, phosphorylating properties have been associated with their genotoxicity,[109] and it has been reported recently that these substances may induce oxidative stress in humans and animals.[110]

Similar to organophosphorus compounds, also sulfonates and dialkylsulfates (with small alkylic groups or benzylic substituents) are reactive molecules. Their genotoxic action is carried out via a direct alkylating mechanism. Nucleophiles attack the methylene carbon atom α to the ester oxygen atom, displacing the sulfonic acid anion (Figure 5). The reaction may occur either by an S_N1 mechanism, with electrophilic carbocation intermediates, or by an S_N2 route involving concerted displacement.[111]

As an example, methyl methanesulfonate, which is used extensively as a direct-acting methylating agent, is generally accepted to undergo an S_N2-type reaction, resulting in 7-methylguanine as predominant adduct in double-stranded DNA,[112,113] while isopropyl methanesulfonate and 2-butyl methanesulfonate show high S_N1 reactivities and preferential O^6 alkylation of guanine.[111]

Busulfan (1,4-butanediol dimethanesulfonate), an antineoplastic agent, is another classic alkylating agent from this class of ompounds. This drug is a bifunctional agent, able to produce intrastrand cross-links in DNA through a guanine−guanine bridge.[114,115]

4.2.2. N-Methylol Derivatives. The potential toxicity of this class of compounds is probably mediated through the generation of formaldehyde via hydrolysis,[116,117] which is a highly reactive genotoxic agent (see Simple Aldehydes, section 4.2.9, below). Figure 6 shows the generation of formaldehyde from *N*-methylol compounds.

4.2.3. S or N Mustards. Nitrogen and sulfur mustards are potential alkylating agents. The scheme of the alkylation mechanism

Figure 4. Alkylation mechanisms by esters of phosphonic acid (see details in the text). Nu= Nucleophile.

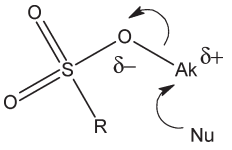


Figure 5. Alkylation mechanism of esters of sulfonic acid: R = remaining part of the molecule; Nu = nucleophile; Ak = alkyl group.

is described in Figure 7. The departure of a halogen generates a carbonium ion, which can be partially stabilized by cyclization to an aziridinium/episulfonium ion (1). This reactive intermediate reacts with guanine bases in DNA to form an N7-alkylated guanine derivative (2). The guanine monoadduct can form another reactive aziridinium/episulfonium intermediate (3), which can react either with water to form a 2-hydroxyethyl monoadduct (4) or with a second guanine residue to form an interstrand cross-link (5).[118]

Chemicals such as mustard gas (dichlorethyl sulfide) and mechlorethamine have been used extensively as warfare agents.[119] Afterward, their cytotoxic potential was unveiled, and their usage as archetypal chemotherapeutic agents was promoted.[120] The high degree of cytotoxicity of this class of compounds is attributed to their ability to inhibit replication by induction of DNA interstrand cross-links.[121]

4.2.4. β-Lactones and γ-Sultones. The high chemical reactivity of β-lactones is due to the strained four-membered ring, which reacts rapidly with nucleophilic centers as both (i) an alkylating and (ii) an acylating agent (Figure 8).[122]

The first reported carcinogen of this class was β-propiolactone, which acts directly as an alkylating agent by undergoing ring-opening. The *in vitro* reaction with DNA takes place mainly at N7 of guanine and N1 of adenine to form carboxyethyl derivatives.[102,123] Adducts with N3 of cytosine and thymine have been also reported.[87,123−125]

A series of eight β-lactones was tested for carcinogenic activity by one or more routes of administration; substitution on carbon atoms 2 or 3 resulted in a decrease or loss in activity.[126] Diketene, for example, which bears a methylene function on carbon atom 3, was inactive as a carcinogen despite its high chemical activity with nucleophilic and electrophilic reagents. This compound hydrolyzes in water to its acid; this factor, combined with its chemical reactivity, may preclude its reaction at *in vivo* sites important for carcinogenesis.[126]

Similar to lactones, sultones are monofunctional alkylating agents, which react spontaneously with a nucleophile along the pathway in Figure 9.[102]

The class progenitor, propane sultone, is a relatively potent alkylating agent[127] and has been found to be mutagenic in several

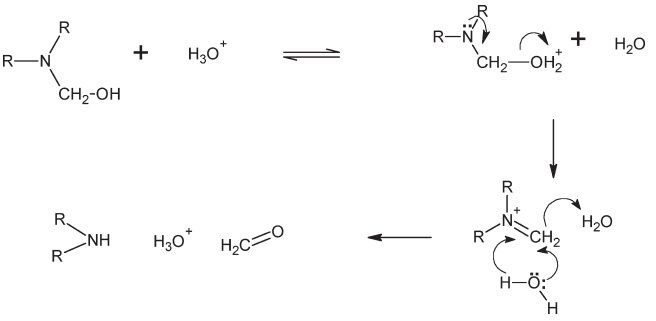


Figure 6. Generation of formaldehyde from *N*-methylol compounds. R = remaining part of the molecule.

systems. It is also carcinogenic to experimental animals when tested by several routes of administration. It has been shown that propane sultone reacts with DNA to produce 7-(3-propanesulfonic acid) guanine. Two minor adducts were also detected: N1- and O^6-alkylguanosines.[128]

4.2.5. Epoxides and Aziridines. These agents are extensively used as intermediates in the chemical industry, especially in polymer production. Moreover, epoxides may be produced endogenously by the enzymatic oxidation of other chemicals, many of which are common environmental pollutants (such as PAH and alkenes). Consequently, considerable human exposure, both direct and indirect, may arise.

Due to the large ring strain associated with the three-membered ring, epoxides are highly reactive molecules. The two electrophilic carbons of epoxides may react with nucleophilic centers of DNA molecules, giving rise to alkylated products (Figure 10).

Substituted epoxides react primarily by the less substituted and more accessible carbon, due to steric hindrance. However, in case of substituents that increase the positive charge at the adjacent carbon (such as aromatic or vinyl group), both carbons could react.[129]

The site of alkylation of the DNA constituents is mainly determined by the ionic character of the epoxide.[129,130] Simple alkyl epoxides react predominantly at endocyclic base nitrogens, following a bimolecular displacement. These chemicals give rise preferentially to β-hydroxyethyl derivatives of cytosine N3, adenine N1 and N3, and guanine N7. 3-Hydroxyalkyl cytosine adducts may undergo deamination, producing 3-hydroxyalkyl uracil derivatives that are potentially mutagenic lesions.

The N1-hydroxyalkyl adenine adduct may be spontaneously converted to N^6-substituted adenine by Dimroth rearrangement. Both substitutions are at coding sites and therefore are potentially mutagenic.[129,131] Small N7G and N3A hydroxyalkyl adducts are unstable and usually eliminated spontaneously, leaving behind the depurinated sites that can result in mutations only if not repaired before DNA replication occurs.[132]

Molecules that are more efficient in stabilizing an ionic charge may also modify exocyclic groups; examples are styrene oxide, butadiene monoepoxide, and PAH.[129,130]

In addition to covalent binding to nucleobases, ethylene oxide and propylene oxide have been reported to alkylate phosphate groups in the DNA backbone, thus increasing the frequency of DNA strand breaks.[133]

Aziridines are extremely reactive alkylating agents that may react by ring-opening reactions similar to those of epoxides. There are several classes of aziridine-containing natural products that exhibit potent biological activity. Among them are the

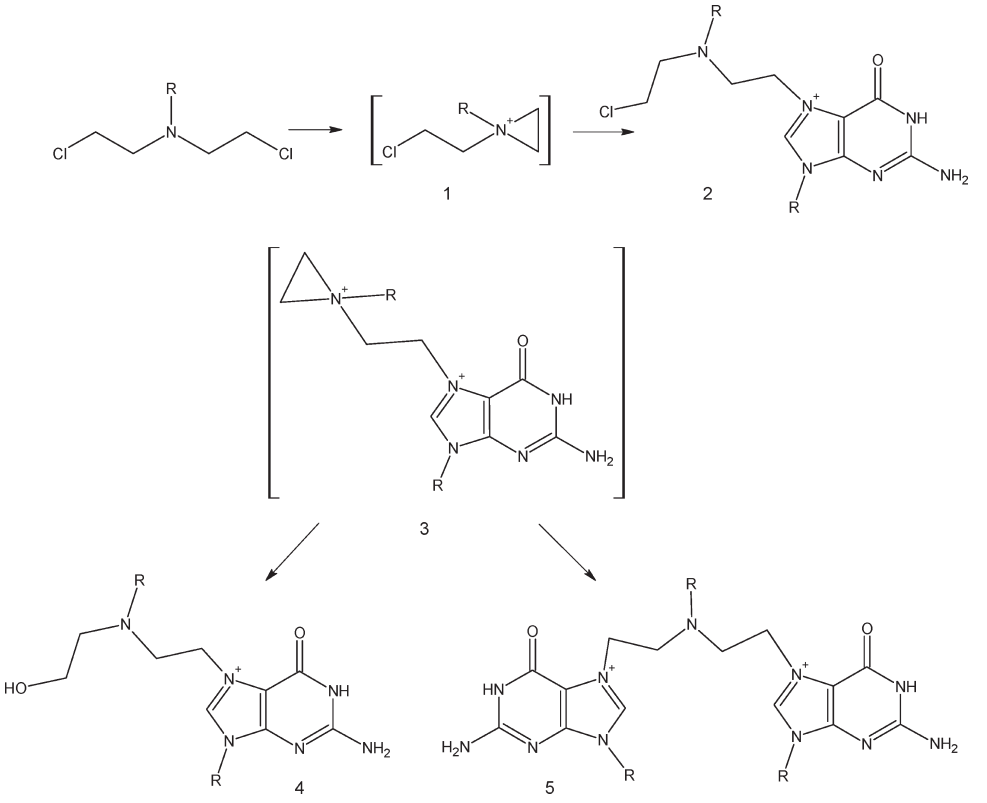


Figure 7. Alkylation mechanism of nitrogen and sulfur mustards. R = remaining part of the molecule.

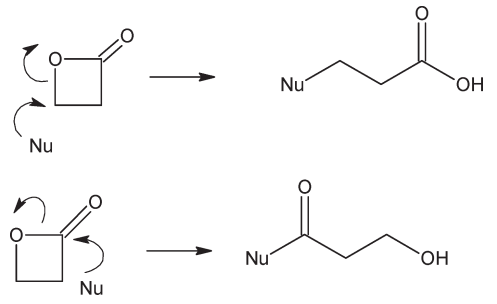


Figure 8. Alkylating (up) and acylating (down) schemes of β-lactones. Nu = nucleophile.

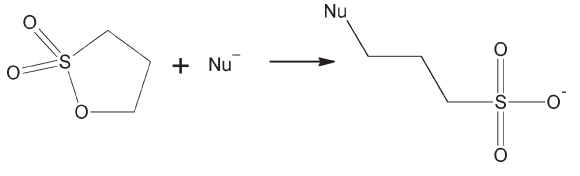


Figure 9. Alkylation scheme of sultones. Nu = nucleophile.

mitomycins, which exhibit both antitumor and antibiotic activity,[134] the aziridine ring being identified as essential for such antitumor activity. Mitomycin C is used clinically as a cancer chemotherapeutic agent against a variety of solid tumors. This agent forms covalent cross-links with duplex DNA by alkylating deoxyguanosine at the N^2-amino group.[135] Mitomycins require initial bioreduction from the native quinone form to the hydroquinone to generate DNA-reactive species.

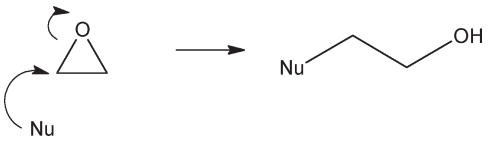


Figure 10. Alkylation scheme of epoxides. Nu = nucleophile.

Another class of naturally occurring aziridine derivatives with potent cytotoxic and antitumor activities is the azinomycin family. The activity of these compounds depends on their ability to act as DNA cross-linking agents, via nucleophilic ring-opening of the aziridine and epoxide moieties by N7 positions of purines.[136] It is not clear at present which ring-opening reaction takes precedence in the cross-linking event.

The PBI (pyrrolo[1,2-*a*]benzimidazole) class of natural products represents another type of DNA-alkylating species containing an aziridine moiety. In these compounds, the aziridine undergoes ring-opening by nucleophilic attack of the DNA phosphate backbone, resulting in formation of a hydrolytically labile phosphotriester, which may eventually cause DNA cleavage.[137]

4.2.6. Aliphatic Halogens. Halogenated alkanes are used widely in industry as solvents, components of refrigerants, aerosol propellants, and fire extinguishers, in medicine as anesthetic agents, and in agriculture. Numerous haloalkanes have been tested for carcinogenic and mutagenic activities. In general, the genotoxic potential is dependent on the nature, number, and position of halogen(s) and the molecular size of the compound.[138]

Methyl halides (methyl chloride, methyl bromide, methyl iodide) are used widely as industrial methylating agents.

dx.doi.org/10.1021/cr100222q |*Chem. Rev.* 2011, 111, 2507–2536

Excluding fluoride, which is believed to be biologically inert, they show pronounced acute and chronic toxicity.[139] Their mechanism of toxicity is unclear. Methyl bromide and methyl iodide have been shown to directly alkylate critical macromolecules such as proteins and DNA.[139]

Biotransformation may also play an important role in their toxicity. The first step in the hepatic metabolism of monohalomethanes is conjugation with glutathione (GSH), resulting in the formation of *S*-methylglutathione. This substance may ultimately be converted to methanethiol, which has been proposed to be the toxic metabolite. Alternatively, production of formaldehyde may result in cellular damage. Formaldehyde may originate either from direct oxidation of the parent compound by cytochromes P450 (see Figure 11) or from metabolism of methanethiol.[140,141]

Regarding monohaloethanes, National Toxicology Program (NTP) bioassays have been performed and reported for ethyl chloride and ethyl bromide.[142,143] Both compounds showed acute toxicities much lower than those of their methyl counterparts. Similar to halomethanes, metabolism of haloethanes has been reported to involve oxidation by cytochrome P450 (producing acetaldehyde, Figure 11) and conjugation with GSH via glutathione-*S*-transferase.[144,145] Even though acetaldehyde has been characterized as a carcinogen, the oxidative metabolites of haloethanes do not seem to be directly involved in the carcinogenicity process. Conjugation of haloethanes with GSH and subsequent depletion of tissue GSH or formation of toxic GSH-derived metabolite(s) are the hypothesized mode of carcinogenic action.[144,145]

Most dihaloalkanes can be activated either through oxidation by cytochrome P450 or by GSH conjugation. Concerning 1,2-bifunctional alkanes, it is hypothesized that most of them are capable of causing genotoxic damage by the GSH-dependent pathway, having the leaving group a major influence on the extent of binding and biological activities.[146] In particular, for vic-dihaloethanes the GSH conjugation pathway involves a half-mustard formation and a postulated reactive episulfonium ion, which finally produces the N7-guanyl adduct as the major DNA adduct (Figure 12).[147]

Polyhalogenated (>2) alkanes may be activated by cytochrome P450. For example, chloroform is hydroxylated to a gem-haloydrin that spontaneously dehydrohalogenates to phosgene, a highly reactive electrophilic intermediate.[148]

Fully halogenated haloalkanes tend to act by free radical or nongenotoxic mechanisms.[138] In the case of CCl_4, cytochrome P450 reduces CCl_4 to the trichloromethyl radical, which can bind to cellular molecules (nucleic acid, protein, lipid), impairing crucial cellular processes. Adduct formation between $CCl_3^{\bullet}$ and DNA is thought to function as the initiator event of hepatic cancer. This radical can also react with oxygen to form highly reactive species, including the trichloromethylperoxy radical $CCl_3OO^{\bullet}$, which may initiate the chain reaction of lipid peroxidation and ultimately generate phosgene[148] (see Figure 13.).

4.2.7. Alkyl Nitrites. Genotoxic activity of various alkyl nitrites has been documented.[149,150] Although not conclusive, evidence in favor of a nitrosating mechanism of alkyl nitrites was reported.[149] According to this mechanism, genotoxicity would arise by nitrosation of DNA or by the production of alkylating agents through the nitrosation of amines. It has been also suggested that the nitrite moiety enhances the reactivity of the compound by increasing the electrophilicity of its nearest carbon atom together with its ability to act as a leaving group.[151]

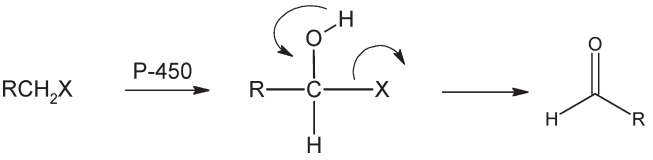


Figure 11. Generation of formaldehyde (R = H) or acetaldehyde (R = CH_3) by metabolic transformation of aliphatic halogens.

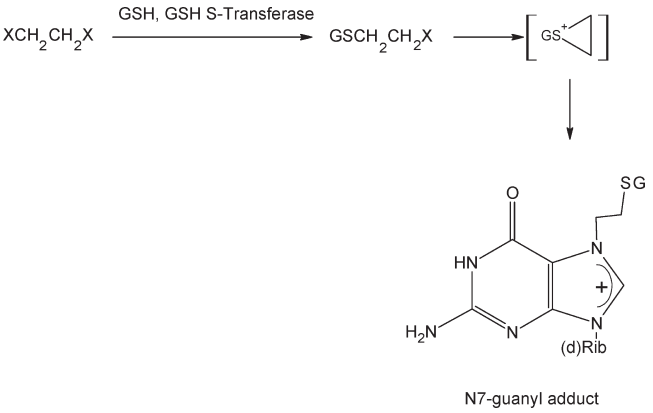


Figure 12. Metabolic transformation pathway of dihaloethanes. X = halogen.

4.2.8. α,β-Unsaturated Carbonyls. Compounds with an α,β-unsaturated carbonyl are bis-electrophiles that may interact with electron-rich biological macromolecules. In addition to the carbon in the carbonylic functionality, the β-carbon is positively polarized because of conjugation with the carbonyl group and becomes the preferred site of nucleophilic attack, as in a classic Michael-type addition.[152]

Despite a common structural feature, α,β-unsaturated carbonyl compounds can undergo different interactions with DNA, which lead to different genotoxic and mutagenic responses. The following genotoxic mechanisms are conceivable: formation of cyclic adducts, frameshift mutations, strand breaks, and cross-linking. In addition to direct interactions, other metabolic activation pathways are possible, such as metabolic epoxidation and formation of radicals.[153]

The predominant interaction of α,β-unsaturated carbonyl compounds with DNA components is the formation of cyclic 1,N^2-deoxyguanosine adducts (Figure 14). This reaction occurs through an initial Michael addition to the exocyclic nitrogen of deoxyguanosine (1), followed by ring closure and formation of the 8-hydroxypropano adduct (2). Further reactions are also possible, including formation of cross-links with proteins and nucleic acids. For example, adduct 1 may react with the exocyclic amino group of another guanine to form carbinolamine (3), which could dehydrate to imine (4). The latter might ultimately cyclize to pyrimidopurinone (5).[154]

Other α,β-unsaturated carbonyl compounds may act by different mechanisms. For example, epoxidation of the double bond of α,β-unsaturated ketones has been reported.[153]

4.2.9. Simple Aldehydes. All compounds carrying an aldehydic group can potentially undergo Schiff base formation with a primary amine. They are potentially genotoxic, as demonstrated by their *in vivo* ability to react with nucleobases without metabolic activation, giving rise to DNA adducts, interbase cross-links

dx.doi.org/10.1021/cr100222q |*Chem. Rev.* 2011, 111, 2507–2536

(both intra and interstrand), and DNA−protein cross-links. The length of the carbon chain for aliphatic aldehydes and in general molecular size can strongly modulate the formation of every type of cross-link and even the accessibility of the DNA nucleobases (Romano Zito, personal communication).[155]

The simplest aldehyde, formaldehyde, is a highly reactive chemical, widely used in the production of resins, as an intermediate in the manufacture of industrial chemicals, and as a disinfectant and preservative in many applications.[156] Formaldehyde induces cytotoxic and genotoxic effects, and there is sufficient evidence in experimental animals and in humans for its carcinogenicity.[156] DNA−protein cross-links have been reported as the primary DNA damage induced by formaldehyde.[157] Besides DNA−protein cross-links, formaldehyde also induces hydroxymethyl adducts in DNA, but the relevance of these DNA modifications for formaldehyde-induced mutagenicity is unclear.[158]

In the case of acetaldehyde (Figure 15), the reaction with DNA involves formation of an unstable Schiff base with the exocyclic amino group of deoxyguanosine (1). This intermediate (1) could be stabilized by reduction, producing N^2-ethyl-deoxyguanosine (2), or alternatively may react with a second molecule of acetaldehyde forming a new aldehyde adduct (3) that can ultimately cyclize in an 8-hydroxypropano adduct (4). This adduct, which is also a product of metabolic transformation of crotonaldehyde (see α,β-unsaturated carbonyls), has been reported to induce miscoding sequences and inhibit DNA synthesis.[159] Moreover, adduct 4 exists in equilibrium with its ring-opened aldehyde form (4) and may undergo condensation with another guanine to form imine-linked bis-nucleoside (5), which in turn cyclizes to pyrimidopurinone (6).[160] The genotoxicity of this ubiquitous aldehyde may thus be caused by both monoadducts (4) and interstrand cross-links (6). In addition, the ring-opened form (4) can also react with peptides, leading to the formation of DNA−protein cross-links.[161] Some aldehydes may also induce hydroxyalkyl adducts in DNA, but the relevance of these DNA modifications for mutagenicity is unclear.[158]

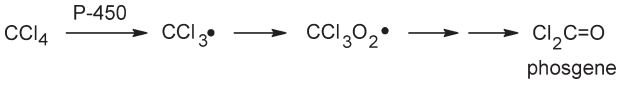


Figure 13. Metabolic transformation of tetrachloromethane.

4.2.10. Quinones. Quinones are electrophiles and oxidants. The striking feature of quinone chemistry is the ease of reduction, the driving force being the formation of a fully aromatic system. The toxicology of quinones is thus modulated by the presence of substituents that effectively determine the relative participation of their oxidant and electrophilic properties. Two major mechanisms of quinone toxicity have been proposed: alkylation of cellular nucleophiles and stimulation of oxidative stress.

Because they belong to the class of Michael acceptors, quinones can exert adverse effects through alkylation of crucial cellular proteins or DNA. They react readily with sulfur nucleophiles, such as GSH or cysteine residues on proteins, leading to depletion of cellular GSH levels or protein alkylation.[162]

As an example of DNA alkylation, quinone metabolites of benzene, hydroquinone, and *p*-benzoquinone have been reported to form four major DNA adducts (Figure 16). These are hypothesized to be involved in the toxic and carcinogenic effects of benzene.[163,164]

In addition, quinones are potent redox-active compounds. They can undergo enzymatic (i.e., cytochrome P450/P450 reductase) and nonenzymatic redox cycling with their corresponding semiquinone radical, leading to formation of ROS, including superoxide, hydrogen peroxide, and ultimately the hydroxyl radical (Figure 17).[165] Production of ROS can cause severe oxidative stress within cells through the formation of oxidized cellular macromolecules, including lipids, proteins, and DNA. Formation of oxidatively damaged bases such as 8-oxodeoxyguanosine has been associated with aging and carcinogenesis.[166]

4.3. Alkylating, Indirect-Acting Agents

4.3.1. Monohaloalkenes. Haloalkenes are high-volume chemicals used in industrial, synthetic, and pharmaceutical applications and are common environmental pollutants. These

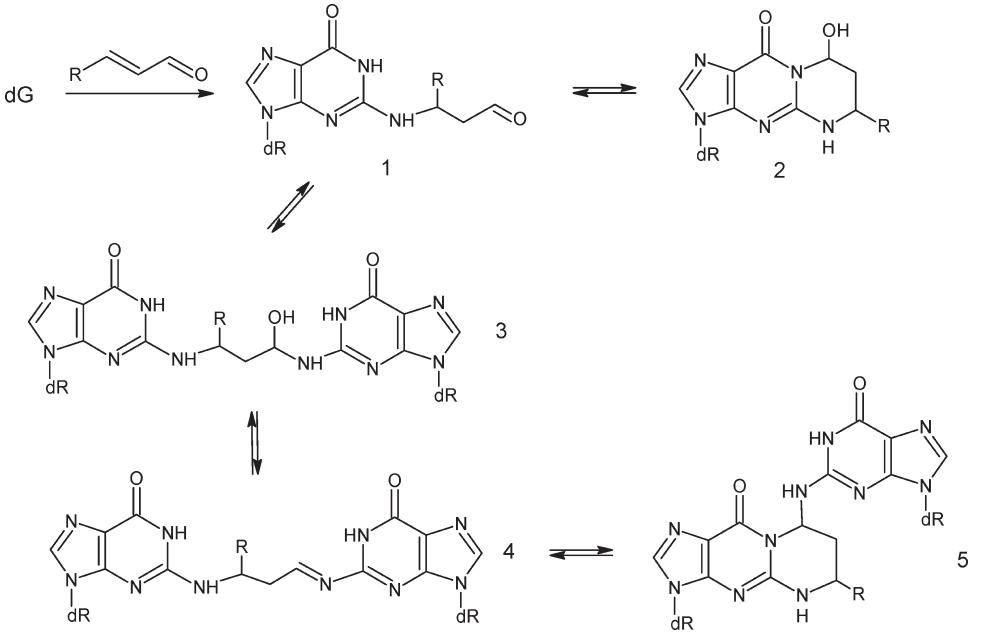


Figure 14. Generation of DNA-adducts by α,β-unsaturated carbonyl compounds. R = remaining part of the molecule.

dx.doi.org/10.1021/cr100222q |*Chem. Rev.* 2011, 111, 2507–2536

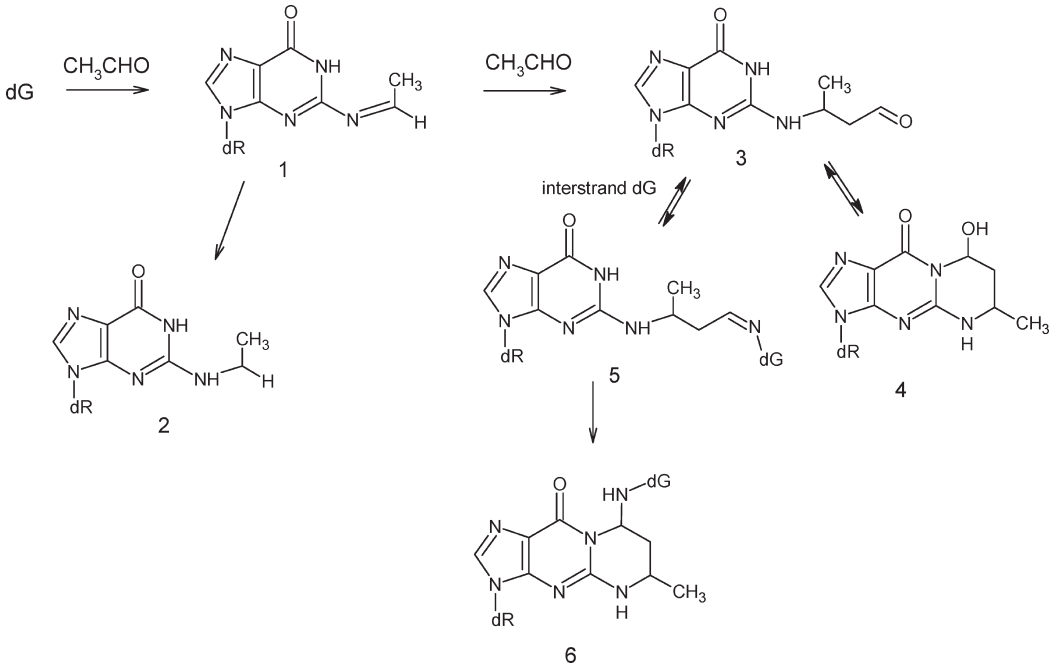


Figure 15. Generation of DNA adducts by acetaldehyde. dR = deoxyribose; dG = deoxyguanosine.

agents may induce DNA adduct formation following cytochrome P450-mediated biotransformation into epoxide metabolites (Figure 18).[167]

Some of the simpler vinyl halides are oxidized to simple halooxiranes that directly alkylate DNA (see section 5.1.2, Epoxides). A typical example is vinyl chloride (VC), recognized as a human and rodent carcinogen.[168] The reactive metabolite of VC is chloroethylene oxide. For this compound, DNA adduct formation is dominated by the attack of ring nitrogens on the unsubstituted methylene of 2-halooxirane.

The formation of epoxides and their reactions are more complex with the more highly halogenated olefins. These substances are metabolized by cytochrome P450 to a carbocationic intermediate, which may rearrange to halogenated oxiranes or to different acyl halides.[167] For example, the scheme below (Figure 19) shows how the metabolism of trichloroethylene (1) by liver cytochromes P450 produces the electrophilic metabolites dichloroacetyl chloride (2), trichloroethylene oxide (3), and chloral (4); all the above metabolites can eventually bind to key macromolecules.[169]

Another bioactivation pathway by which haloalkenes can exert carcinogenicity involves conjugation with L-glutathione in the mammalian liver and subsequent processing of the substances in the kidney into nephrotoxic and mutagenic species via the cysteine conjugate β-lyase pathway.[170] Generation of DNA-reactive halothioketenes ($X_2C{=}C{=}S$) has been documented for different polyahalogenated vinyls.[171]

4.3.2. Hydrazines. Toxicity of hydrazine derivatives has been ascribed to generation of reactive species (catalyzed by enzyme systems such as the cytochrome P450-dependent mixed-function oxidases and the flavin monooxygenases), namely, carbocations and carbon-centered radicals and also to reactive oxygen species.[172] For these reactive intermediates, DNA alkylation, as well as other DNA lesions, has been reported.[172,173]

Hydrazine derivatives constitute an important class of compounds to which the human population is often exposed, because they are natural components of mushrooms and tobacco and they are constituents of herbicides, rocket fuel, and drugs. Several hydrazines display pharmacological activities. Phenelzine is an antidepressant; hydralazine is an antihypertensive agent; procarbazine is part of a chemotherapeutic cocktail used in the treatment of Hodgkin's disease, melanoma, and bronchogenic carcinoma; and isoniazide is one of the basic antibiotics employed in the treatment of tuberculosis.[172] However, despite their pharmacological applications as therapeutic agents in important pathologies, their use is limited by toxic side effects, such as hepatotoxicity, induction of systemic lupus erythematosus, and carcinogenicity.[173,174]

Alkyl or aryl monosubstituted derivatives have been shown to be metabolically oxidized to carbon-centered or aryl radicals by several enzymatic systems. In the metabolism of disubstituted hydrazines (Figure 20), there is evidence of the formation of carbonium ions and alkyl radicals by distinct pathways after oxidation of azo compounds.[172,175] Hydrazines can be activated nonenzymatically by endogenous substances such as metal ions, resulting in active radical species causing DNA damage.[176]

4.3.3. Aliphatic Azo and Azoxy. Aliphatic azo and azoxy species are intermediates in the activation pathways of 1,2-dialkylhydrazines (see above).

4.3.4. Alkyl Carbamates and Thiocarbamates. In this class of compounds, the most studied and representative molecule is urethane (ethyl carbamate). Urethane is a known multisite carcinogen capable of inducing tumors in various organs and animal species.[177] In the past, urethane was administered to man as a hypnotic, as well as for the treatment of a host of illnesses including chronic leukemia, multiple myeloma, and varicose veins. Commercially urethane has been utilized as a cosolvent for pesticides, fumigants, and cosmetics.[177] Urethane is also formed as a byproduct of fermentation processes and is found in tobacco leaves and smoke. Currently, the greatest risk of human exposure to urethane is through consumption of alcoholic beverages and fermented foods.[178] It is accepted that urethane metabolism

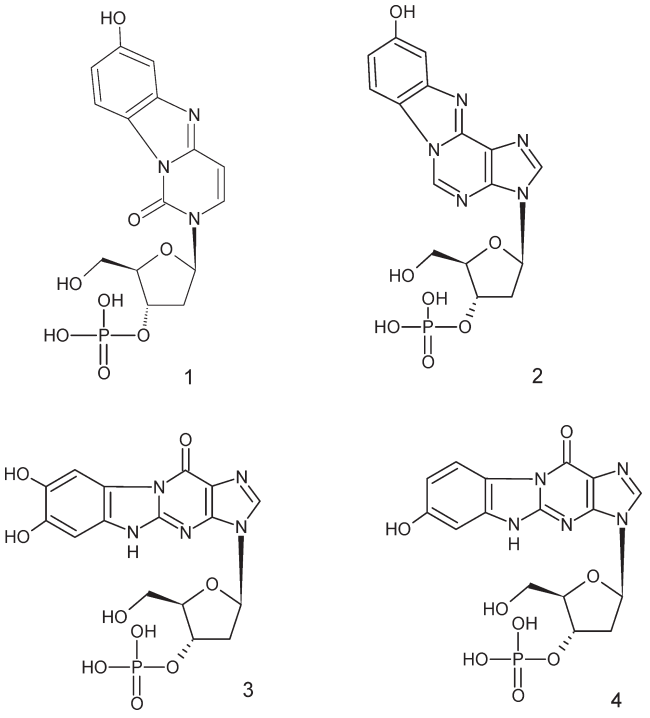


Figure 16. DNA adducts of hydroquinone and *p*-benzoquinone: (1) (3′′-hydroxy)-3,N^4-benzetheno-2′-deoxycytidine 3′-monophosphate; (2) (3′′-hydroxy)-1,N^6-benzetheno-2′-deoxyadenosine 3′-monophosphate; (3) (3′′,4′′-dihydroxy)-1,N^2-benzetheno-2′-deoxyguanosine 3′-monophosphate; (4) (3′′-hydroxy)-1,N^2-benzetheno-2′-deoxyguanosine 3′-monophosphate.

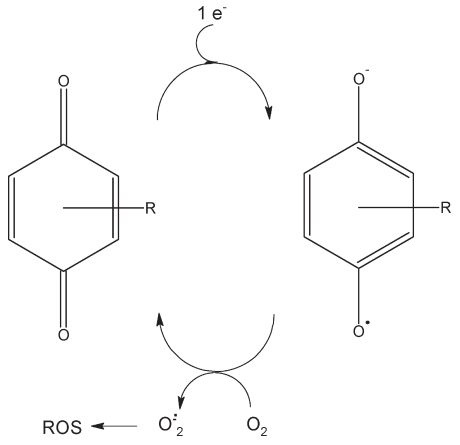


Figure 17. Quinone redox cycling. R = remaining part of the molecule.

occurs via two major pathways (Figure 21).[179] One is a detoxification pathway, which is thought to be catalyzed by esterase, leading to the formation of ethanol, ammonia, and carbon dioxide.[180] The second, a bioactivation pathway, entails oxidative metabolism of urethane catalyzed by cytochromes P450, leading to the formation of vinyl carbamate by dehydrogenation and subsequently to the vinyl carbamate epoxide. Vinyl carbamate epoxide is considered to be the ultimate carcinogen metabolite of urethane, able to bind to DNA and produce promutagenic etheno-type adducts.[178]

A group of chemicals sharing this reactive functional group is the carbamate pesticides. Within the class, there are three

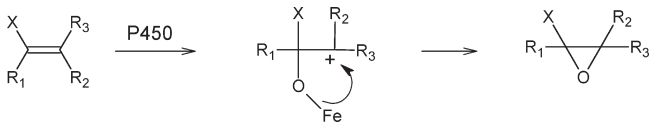


Figure 18. Metabolic transformation of monohaloalkenes. $R_{1,2,3}$ = remaining part of the molecule.

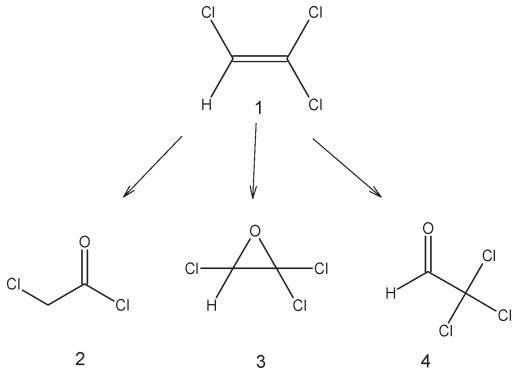


Figure 19. Metabolic transformation of trichloroethylene.

distinct subgroups: *N*-methyl carbamates, thiocarbamates, and dithiocarbamates.

N-Methyl carbamates are an important group of insecticides (e.g., Aldicarb, Bendiocarb, Carbaryl, Carbofuran, Fenobucarb, Propoxur, Methomyl). They affect the nervous system by inhibiting the enzyme acetylcholinesterase, causing its reversible carbamylation.[181] The carbamyl−acetylcholinesterase combination dissociates more readily than the phosphoryl−acetylcholinesterase complex produced by organophosphate compounds, conferring a lower acute toxicity to *N*-methyl carbamate insecticides. For this reason, they are preferred in the control of insects, especially in household areas where contamination of the human environment can easily occur.

Genotoxicity tests of *N*-methyl carbamate insecticides have given contradictory results.[181] *N*-Methyl carbamates are converted to *N*-nitroso metabolites by N-nitrosation in the stomach and in the presence of nitrites or nitrogen oxides.[182] *N*-Nitroso methyl carbamates are not cholinesterase inhibitors and are less toxic to mammals than the parent *N*-methyl carbamates. They are, nonetheless, quite carcinogenic and mutagenic.[182] It has been reported that *N*-methyl carbamate insecticides and their *N*-nitroso derivatives have the potential to act through epigenetic and genotoxic mechanisms, respectively.[181]

Thiocarbamates (e.g., *S*-ethyl-*N*-*N*-dipropylthiocarbamate (EPTC), Molinate, Pebulate, Triallate, Butylate, Cycloate, Thiobencarb, Vernolate) are a major class of herbicides used extensively in the agricultural industry. Thiocarbamate herbicides are generally thought to be of low to moderate toxicity to mammals. However some evidence, though contradictory, is available on major herbicides having genotoxic properties, which may be expected to produce mutagenic or carcinogenic effects.[183]

Thiocarbamates have been shown to undergo metabolic bioactivation via S-oxidation[184] to form reactive sulfoxide and sulfone intermediates, which are potent carbamoylating agents. Recent animal studies found that EPTC sulfoxide can form DNA adducts in rat hepatocytes[184] and induces DNA damage in human lymphocytes.[185] Furthermore, a positive association between ETPC exposure and both colon cancer and leukemia has been observed.[186]

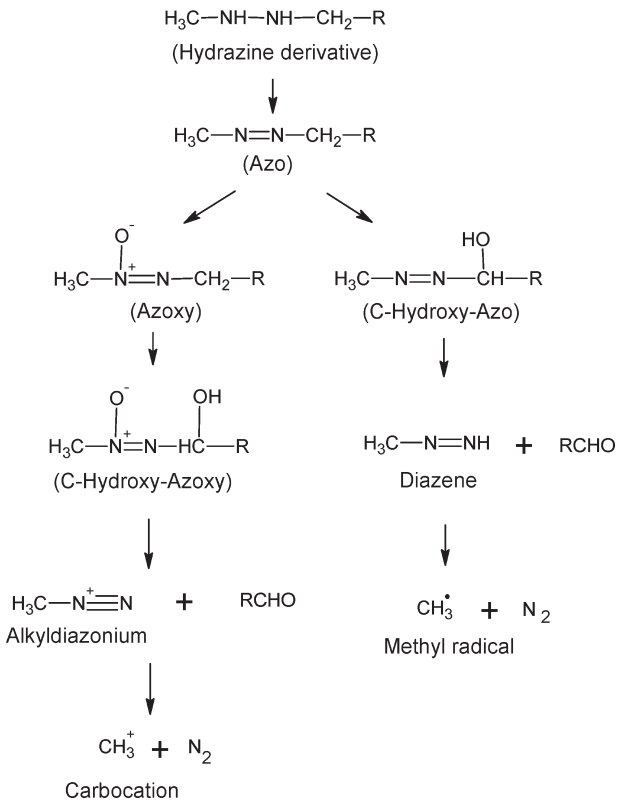


Figure 20. Metabolic transformation of alkyl hydrazines. R = remaining part of the molecule.

The subgroup of dithiocarbamates is composed mainly of fungicides, like Mancozeb, Maneb, Metiram (Zineb), Ziram, Ferbam, Thiram, Metam Sodium. For these chemicals, toxic effects, including *in vitro* and *in vivo* genotoxicity, have been reported. However, the mechanisms by which they elicit these toxic actions are not fully elucidated.[187−189]

A number of metal ethylenebisdithiocarbamate pesticides (i.e., Maneb, Mancozeb, and Metiram) have been shown to possess carcinogenic activity in rats,[190,191] possibly related to the generation of the main metabolite ethylenethiourea (ETU), which has been reported to have carcinogenic, teratogenic, and goitrogenic effects.[191] Studies on the genotoxicity of the three insecticides have been performed,[190−192] but at present there is no clear concordance. Although ETU exhibits a weak genotoxic activity in chronic treatments,[190] this chemical is classified by IARC as nongenotoxic.[193] One possible alternative mechanism to explain the chronical toxicity of these metal−EBDC pesticides has been associated with their ability to act as a prooxidant agent.

4.3.5. Alkyl and Aryl *N*-Nitroso Groups. *N*-Nitroso compounds (*N*-nitrosamines and *N*-nitrosamides) represent a well-established class of chemical carcinogens.[194,195] Several *N*-nitrosamines are known to be carcinogenic in various animal species. Human exposure to preformed *N*-nitrosamines occurs through the diet, in certain occupational settings (e.g., in rubber industry), and through the use of tobacco products, cosmetics, pharmaceuticals, and agricultural chemicals. In addition, *N*-nitrosamines are generated in the body by nitrosation of amines (via acid or bacterially catalyzed reaction with nitrite) or by reaction with products of nitric oxide generated during inflammation or infection.[196]

In particular, alkylnitrosamides can be activated nonenzymatically by reaction with water or sulfhydryl or amino groups to form an alkyldiazonium ion.[13] These reactive electrophiles are known to react with nucleophiles in cellular macromolecules such as proteins and nucleic acids, resulting in formation of a variety of alkylated products. This latter interaction has been studied as a probable mechanism by which the alkylnitrosamides exert their biological effects, especially mutagenicity and carcinogenicity.[182]

N-Nitrosamines require metabolic activation to form DNA adducts that are critical for their mutagenic and carcinogenic activity. The well-established major pathway is α-hydroxylation (adjacent to the *N*-nitroso group), catalyzed by cytochrome P450 enzymes.[197] As shown in Figure 22 for *N*-nitroso methylamines, the cytochrome P450-mediated α-hydroxylation (at methyl or methylene carbon) produces alkyldiazonium ions, the same electrophilic alkylating agents formed directly from the corresponding nitrosamides. Additional DNA damage could arise through the generation of formaldehyde via methyl hydroxylation of *N*-nitrosomethyl compounds.[197]

4.3.6. Azide and triazene groups. The biological action of triazenes is a consequence of their capacity to alkylate DNA. Figure 23 shows the case of 1-aryl-3,3-dimethyltriazenes (1). These compounds undergo metabolic oxidation by cytochrome P450 enzymes to give hydroxymethyltriazenes (2), which, by loss of formaldehyde, generate the monomethyltriazenes (3). These are known alkylating agents, capable of methylating DNA and RNA via the generation of a methyldiazonium ion (4).[198]

In the presence of a carbonyl group bound to the triazene moiety, a nonenzymatic activation is possible by spontaneous hydroxylation to the reactive monomethyltriazene (3) (see, as an example, the activation mechanism of the anticancer drug temozolomide[199]).

4.3.7. Aliphatic *N*-Nitro Group. The mechanism of action of these chemicals has not been clearly understood. Some authors reported that hydroxylation and nitroreduction are required to activate dimethylnitramine into an alkylating and mutagenic agent.[200] A nonalkylating mechanism has been proposed as well, with formaldehyde as the suggested intermediate responsible for mutagenicity, after oxidation of *N*-nitrodimethylamine at the methyl group.[201,202]

4.3.8. α,β-Unsaturated Aliphatic Alkoxy Group. This chemical feature has been associated with the mutagenic activity of various chemicals.[203] In particular, it is contained in the bisfuranoid substructure of most mutagenic aflatoxins and is considered a key element in the bioactivation of these compounds. As an example, aflatoxin B1, a potent environmental mutagen and carcinogen, requires metabolic activation, primarily by cytochrome P450, to the *exo*-8,9-epoxide (Figure 24), which forms covalent adducts with DNA.[204]

4.4. Intercalating and DNA-Adduct-Forming, Indirect-Acting Agents

4.4.1. Polycyclic Aromatic Hydrocarbons. For PAHs (including heterocyclic aromatic compounds) with relatively planar highly conjugated aromatic structures, metabolic activation is required to exert their mutagenic/carcinogenic effects. The most generally accepted mechanism for activation of PAHs involves the formation of diol-epoxides (Figure 25). This activation pathway requires three steps: initial epoxidation by the cytochrome P450 monooxygenases, followed by epoxide hydrase (EH) enzyme-mediated hydrolysis to the trans diol, and a second epoxidation at the adjacent double bond.[205] This metabolic process yields a diol epoxide that can interact with tissue nucleophiles, giving rise to the alkylation of DNA.

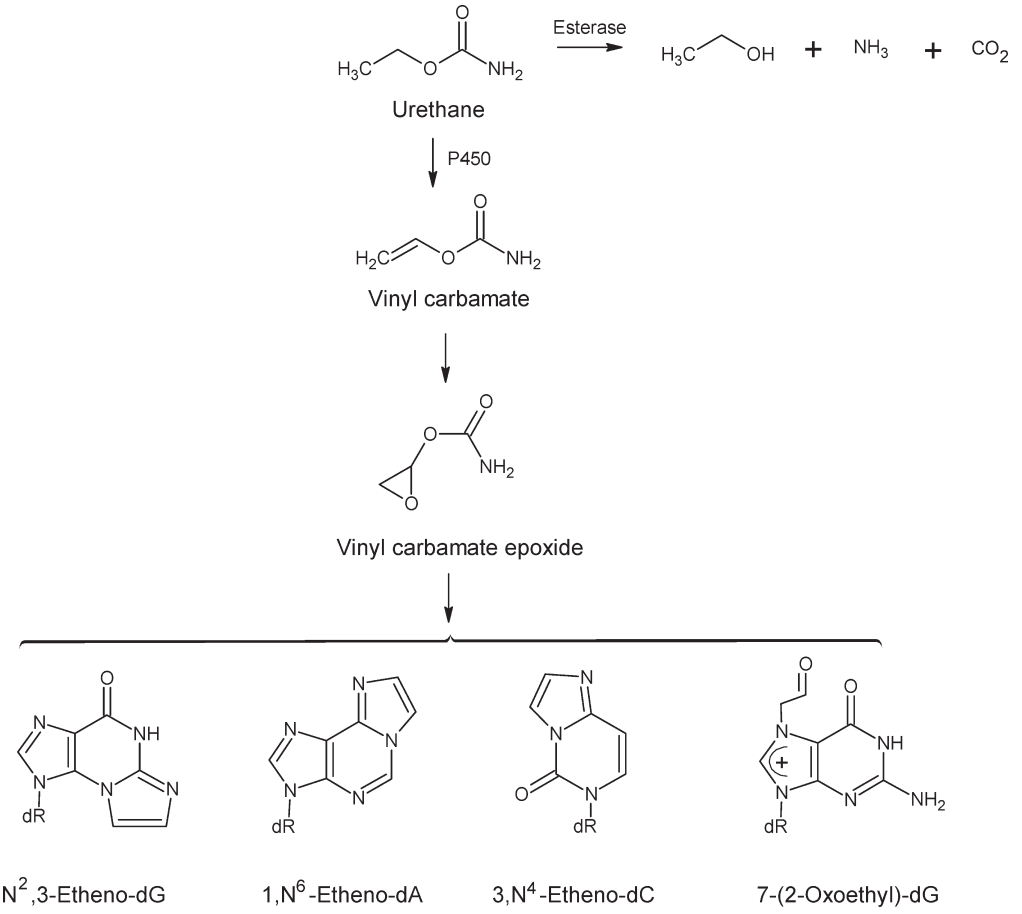


Figure 21. Metabolic transformation of urethane. dR = deoxyribose; dG = deoxyguanosine; dA = deoxyadenosine; dC = deoxycytidine.

The ability of PAH diol epoxides to form harmful DNA adducts is extremely dependent on the position of the epoxy group. Epoxidation of the terminal ring adjacent to a "bay" or "fjord" region (Figure 26) produces highly mutagenic and tumorigenic compounds.[206] For bay region diol-epoxide of PAH, the major adducts have been found to result from bonding between the benzylic carbon of the epoxide and the exocyclic amino groups of deoxyguanosine and deoxyadenosine residues in the DNA.[52]

Another relevant mechanism involved in the metabolic activation of PAHs to initiate cancer is the cytochrome P450 one-electron oxidation to produce radical cations. As illustrated below for unsubstituted and methyl-substituted PAH (Figure 27), removal of an electron from the π-system generates a radical cation in which the positive charge can be localized mainly at an unsubstituted carbon atom (path 1) or adjacent to the methyl group (path 2). Nucleophilic substitution then specifically occurs at the position of highest charge density.[207]

DNA adducts formed through PAH radical cation have been reported to be unstable,[208] leading to spontaneous depurination in addition to some stable adducts. These unstable DNA adducts arise primarily from binding at the N7 position of guanine bases and the N7 or N3 positions of adenine bases, where the glycosidic bond is cleaved to lose the deoxyribose moiety of DNA, resulting in depurination. Spontaneous depurination of adducts would result in formation of apurinic sites as the major type of DNA damage.

The role in carcinogenicity of stable versus unstable covalent DNA adducts remains in discussion.[209]

A third pathway of PAH activation that involves the formation of reactive and redox active *o*-quinones catalyzed by dihydrodiol dehydrogenases (DDs) has been postulated[210,211] (Figure 28). DDs successfully compete with cytochromes P450 to oxidize *trans*-dihydrodiol proximate carcinogens (1). These diols undergo $NADP^+$-dependent oxidation to form a ketol that spontaneously rearranges to form a catechol (2). The catechol is unstable and undergoes autoxidation in air. The first one-electron oxidation results in the formation of an *o*-semiquinone anion radical (3) and hydrogen peroxide. The second one-electron oxidation produces the fully oxidized *o*-quinone (4) and superoxide anion ($O_2^{\bullet -}$). The redox-active PAH−*o*-quinone can be reduced to reform catechol by a nonenzymatic two-electron reduction or to reform the semiquinone anion radical via a one-electron enzymatic reduction. These events establish futile redox cycles in which the formation of ROS (hydrogen peroxide, hydroxyl radical $OH^{\bullet}$, and $O_2^{\bullet -}$) are amplified.[52,212] Furthermore, *o*-quinones are highly reactive Michael acceptors that can form both stable and depurinating DNA adducts.

4.4.2. Heterocyclic Polycyclic Aromatic Hydrocarbons. Heterocyclic polycyclic aromatic hydrocarbons (heteroPAHs) are expected to behave, in many aspects, in a similar manner to their homocyclic analogs (see PAHs). Like PAHs, most heteroPAHs require metabolic activation.[194,195]

A number of nitrogen heterocyclic aromatic compounds (acridines) were reported to be activated via the bay region diol-epoxide pathway.[52]

dx.doi.org/10.1021/cr100222q |*Chem. Rev.* 2011, 111, 2507–2536

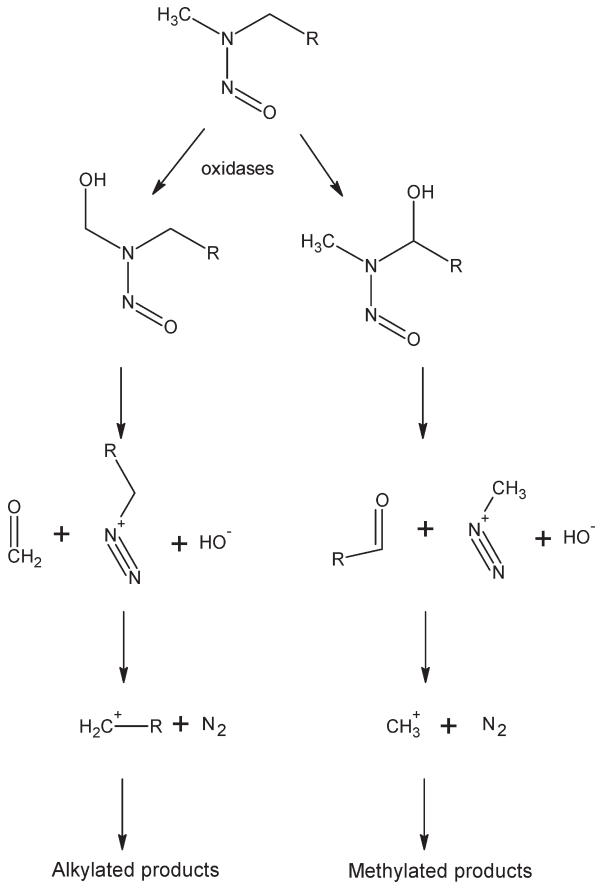


Figure 22. Metabolic transformation of *N*-nitroso methylamines. R = remaining part of the molecule.

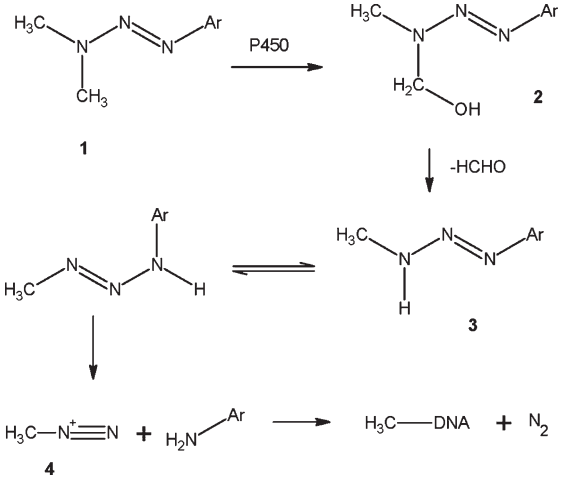


Figure 23. Metabolic transformation of 1-aryl-3,3-dimethyltriazenes. Ar = aryl.

Concerning the radical cation pathway, the presence of the heteroatom may influence the ionization potential of the molecule and thus the probability to form a relatively stable radical cation.[52]

In addition, nitrogen heterocyclic aromatic compounds may undergo N-oxidation.[194,195]

4.4.3. Coumarins and Furocoumarins. The mechanism(s) of carcinogenic activity of coumarins is not completely understood.[194,195]

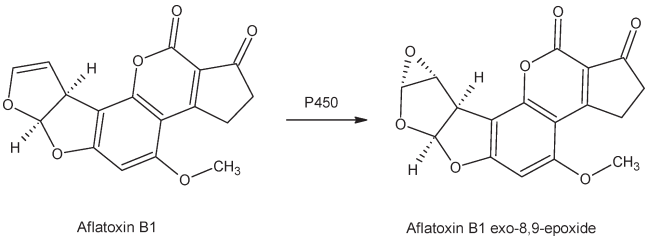


Figure 24. Metabolic transformation of aflatoxin B1.

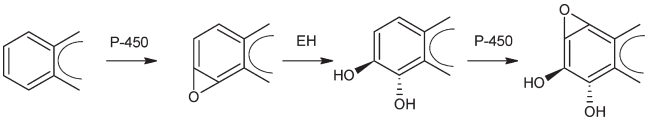


Figure 25. Metabolic activation of PAHs through the generation of diol-epoxides. EH = epoxide hydrase.

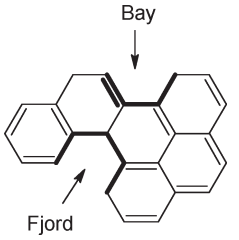


Figure 26. "Bay" and "fjord" regions of PAHs.

In coumarin metabolism, marked species differences have been reported. Coumarin 7-hydroxylation and 3,4-epoxidation are generally recognized as the predominant metabolic pathways in humans and rodent (Figure 29). In humans coumarin is metabolized mostly by hepatic cytochrome P450 to 7-hydroxycoumarin (5), which is excreted in the urine as glucuronide and sulfate conjugates. The major pathway of coumarin metabolism in rodent is via coumarin 3,4-epoxide (1) resulting in the formation of ring-opened metabolites including *o*-hydroxyphenylacetaldehyde (2), *o*-hydroxyphenylethanol (3), and *o*-hydroxyphenylacetic acid (4).[213]

It has been postulated that the coumarin 3,4 epoxide pathway could lead to DNA damage through covalent binding.[194,195] Nevertheless, studies on genotoxic potential of coumarin and its metabolites gave contradictory results, and some authors suggest a nongenotoxic mechanism of action.[213,214]

Concerning furocoumarins, they are potential cross-linking agents. Psoralens, a family of natural products belonging to the class of furocoumarins, interact with double-stranded DNA via intercalation. Psoralen molecules contain two photoreactive double bonds, a 3−4 double bond on the pyrone side and a 4′−5′ bond on the furan side that can form DNA interstrand cross-links when activated by irradiation with long-wavelength UV light (Figure 30).[118]

4.5. Aminoaryl DNA-Adduct-Forming, Indirect-Acting Agents

4.5.1. Aromatic Nitroso Group. Mechanism of activation of compounds containing aromatic amine, nitro, nitroso, or hydroxylamine moieties can be explained by partially overlapping metabolic pathways. A common intermediate is an aromatic

dx.doi.org/10.1021/cr100222q |*Chem. Rev.* 2011, 111, 2507–2536

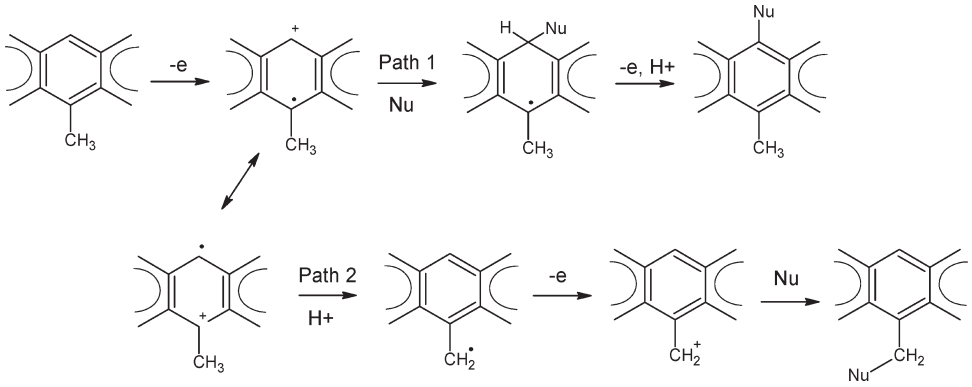


Figure 27. Metabolic activation of PAHs through the generation of radical cations. Nu = nucleophile.

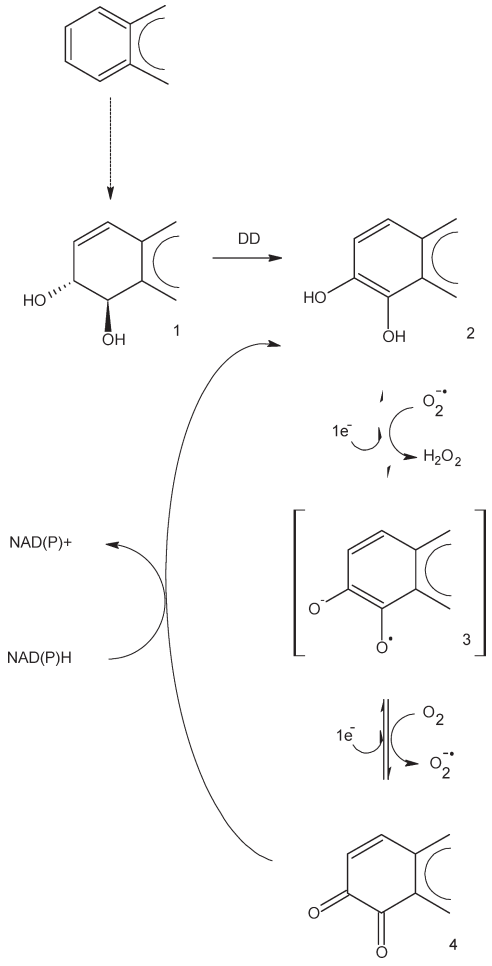


Figure 28. Metabolic activation of PAHs through the generation of active quinones. DD = dihydrodiol dehydrogenases.

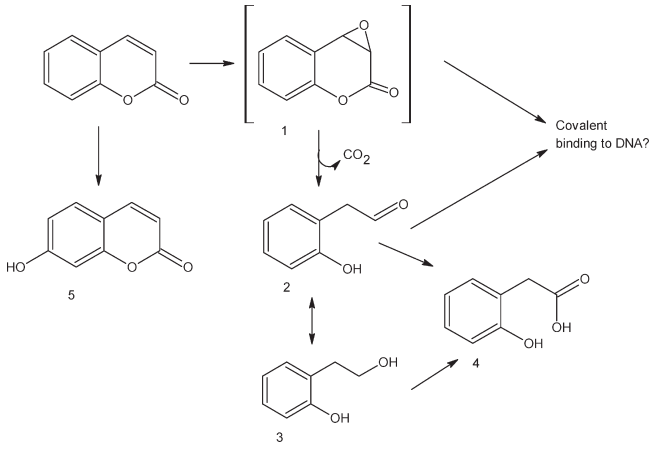


Figure 29. Metabolic transformation of coumarins.

hydroxylamine that can be further activated through enzymatic esterification to finally produce electrophilic species (nitrenium ions) that react with bionucleophiles to form covalent adducts. For nitrosoarenes (Figure 31), a nonenzymatic reduction to the correspondent hydroxylamine has been suggested.[215]

4.5.2. Aromatic Ring *N*-Oxides. Although the mechanism of action is not completely clear, the aromatic *N*-oxide moiety was found to be an essential component of various carcinogens, such as 4-nitroquinoline-*N*-oxide and purine *N*-oxides (guanine, xanthine, and adenine).[216] The latter have been shown to require enzymatic esterification to attain their ultimate carcinogenic reactivity.[217]

4.5.3. Nitroarenes. Metabolic activation of nitroaromatic compounds to toxic metabolites involves initial one-electron reduction of the nitro group to yield a resonance-stabilized nitro-anion radical. Under aerobic conditions, the radical can reduce molecular oxygen to form the superoxide anion, which can generate various toxic and DNA-reactive oxygen species. In a more anaerobic environment, the nitro-anion radical can be reduced further to nitroso, hydroxylamine, and amine (Figure 32).[218] Further activation of hydroxylamine produces the reactive nitrenium ions.

4.5.4. Aromatic Amines. Aromatic amines have to be metabolized to reactive electrophiles in order to exert their carcinogenic potential. For aromatic amines and amides, this typically involves an initial N-oxidation to *N*-hydroxyarylamines and *N*-hydroxyarylamides, which is mediated by cytochrome P450. Upon further activation by enzymatic esterification, nitrenium ions are formed. These highly reactive intermediates bind covalently to biomolecules, generating aminoaryl derivatives (Figure 33).[219,220]

In addition to the reactions of nitrogen (main activation pathway), certain aromatic amines are converted into electrophilic derivatives through ring oxidation pathways. Ring hydroxylation and subsequent enzymatic or spontaneous

dx.doi.org/10.1021/cr100222q |*Chem. Rev.* 2011, 111, 2507–2536

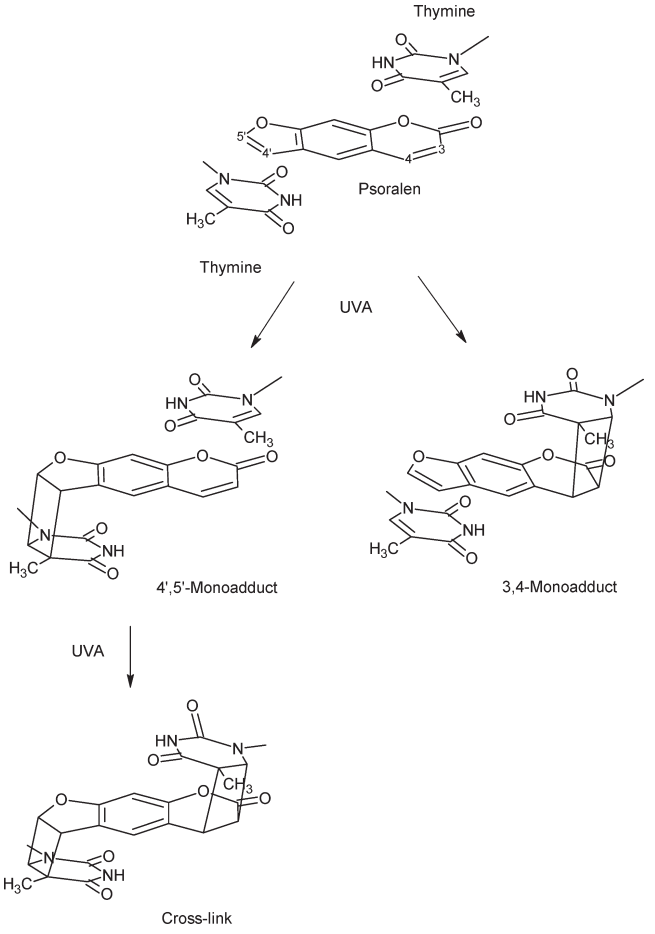


Figure 30. Generation of DNA interstrand cross-links by furocoumarins.

dehydrogenation can result in the formation of iminoquinones, which are directly electrophilic metabolites (Romano Zito, personal communication).

4.5.5. Aromatic Diazo. Azo compounds are biologically active through their metabolites. The reduction of the azo bonds, via the enzymes azoreductases in the intestinal microflora produces the release of aromatic amines (Figure 34).[221]

4.6. Nongenotoxic Carcinogens

4.6.1. Thiocarbonyls. The mechanisms of carcinogenesis of this class of compounds is not completely understood. It is generally believed that they are not genotoxic. The tumorigenicity of thiourea (TU), for example, has been attributed to its strong "antithyroid" activity, which leads to a disruption of the pituitary-thyroid hormonal regulatory system. However, tumor formation has been documented also in organs where hormonal disregulation mechanisms cannot be involved, and TU activity in various genotoxicity test systems has been reported.[222]

Ethylenethiourea (ETU) has been reported to have carcinogenic, teratogenic, and goitrogenic effects,[193] generally thought to be exerted mostly through thyroid hormone imbalance. The genotoxic activity of ETU has been analyzed in a wide variety of short-term tests. Due to the generally negative results of short-term genotoxicity tests, this chemical has been classified as nongenotoxic by IARC. However, there is evidence that ETU has weak genotoxic activity.[190,223]

4.6.2. (Poly)Halogenated Cycloalkanes. The mechanisms of carcinogenic action of this class of compouds is not fully

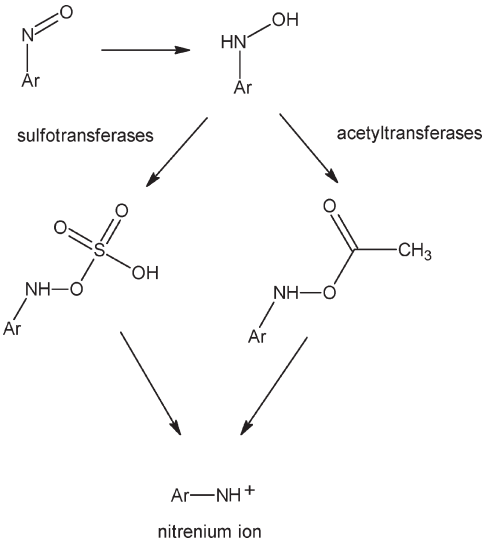


Figure 31. Metabolic activation of nitrosoarenes. Ar = aryl.

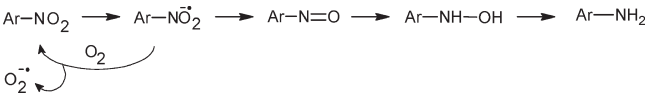


Figure 32. Metabolic activation pathways of nitroarenes. Ar = aryl.

understood. Several possible epigenetic mechanisms have been proposed that include (i) inhibition of intercellular communication, (ii) degranulation of the rough endoplasmic reticulum, and (iii) hormonal imbalance.[194,195]

Production of reactive oxygen species by organochlorine pesticides also has been implicated in the toxicity and carcinogenicity of these compounds; however, the mechanism by which these agents stimulate the production of oxygen radicals is unknown.[224] Among them, Dieldrin, an organochlorine insecticide, has also been demonstrated to be genotoxic as well, and evidence on the relationships between genotoxicity and oxidative stress has been reported.[225]

4.6.3. Halogenated Benzenes, PAHs, and Dibenzodioxins. The mechanism of carcinogenicity of halogenated aromatic compounds is not completely understood. However they are considered to exert their carcinogenic action via nongenotoxic mechanisms rather than by direct action on DNA.

The toxicological effects of dioxins, a large class of organohalogens, including several polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and a few polychlorinated biphenyls (PCBs), are thought to depend on binding to the aryl hydrocarbon (Ah) receptor. This receptor binds 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and its planar congeners in a saturable manner and with high affinity and is expressed in virtually every tissue of adult rodents and humans. After the binding of dioxins, the activated Ah receptor forms a heterodimer with another transcription factor (Ah receptor nuclear translocator, Arnt). This ternary complex binds to regulatory sequences on DNA and specifically activates transcription of a battery of dioxin-inducible genes. Primary target genes identified so far are a number of genes encoding drug-metabolizing enzymes such as cytochrome P450 1A1 and gluthathione-*S*-transferase Ya subunit.[226]

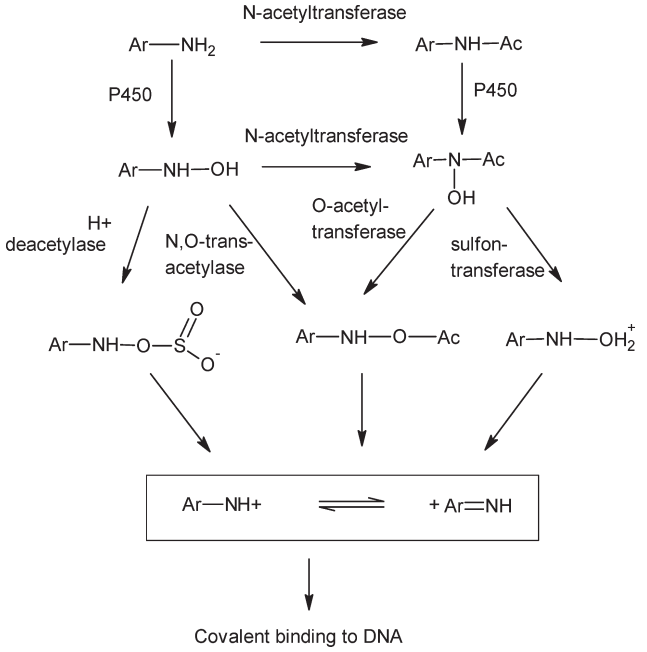


Figure 33. Metabolic activation pathways of aromatic amines. Ar = aryl; Ac = acyl.

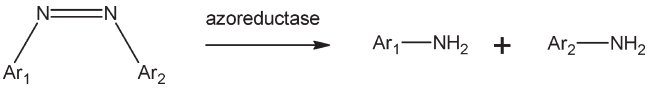


Figure 34. Metabolic transformation of diazo compounds into aromatic amines. Ar = aryl.

It has been suggested that the majority of biochemical and toxicological effects of dioxins may occur through Ah receptor-mediated changes in transcription of other target genes, by a mechanism similar to cytochrome P450 1A1.[227]

On the other hand, other halogenated biphenyls, naphthalenes, and benzenes that induce the cytochrome P450 2B family have been postulated to act via inhibition of intercellular communication.[194,195]

5. A LOOK AT ACTION MECHANISMS THROUGH THE QSAR PERSPECTIVE

The core of this review is definitely the illustration of the evidence on the chemical carcinogenicity and mutagenicity mechanisms, as substantiated by a large array of experimental results (see previous sections). This section provides an additional look at the action mechanisms through the lens of theoretical studies focusing specifically on the relationship between chemical structure and biological activity, namely, the quantitative structure−activity relationship (QSAR) methods. However, this section is aimed at giving an account neither of the whole QSAR field and approaches nor of all the applications to mutagenicity and carcinogenicity: we consider only the QSAR studies relevant to confirm and to better understand the mechanisms of action presented in the previous sections.

QSAR is a widely developed branch of chemical research. Its foundation came about 40 years ago, when Corwin Hansch found the way to bring together two areas of science that had seemed far apart for many years: physical organic chemistry and the study of chemical−life interaction.[228,229] A cornerstone of physical organic chemistry is the Hammett equation:

$$\log k = \rho\sigma + \text{constant}$$

The Hammett equation models the reaction mechanisms of organic chemicals: k is a rate or equilibrium constant; ρ is a measure of the sensitivity of the reaction to substituents changes; σ is a parameter characteristic of each chemical. The Hammett equation was subsequently extended by Taft who considered also steric factors. Next, Hansch showed that this type of model can also be used for chemico-biological reactions by introducing a hydrophobic parameter:

$$\log k = f(\text{electronic, steric, hydrophobic})$$

This approach was developed from, and meant to be applied to, sets of congeneric chemicals, that is, chemicals structurally similar and acting through the same mechanism of action (better, the same rate-limiting step): for optimal applications, QSAR analysis requires a very clear definition of the applicability domain of the model (i.e., the type of chemicals to which it applies). Finally, the model is derived from the statistical analysis of a (training) set of chemicals; thus its character is fundamentally empirical. This model worked for an enormous number of biological problems, and its success is demonstrated clearly by its widespread diffusion.

In the years subsequent to the 1960s, the need to solve new problems, together with the contributions of many other investigators, generated hundreds of variations of the Hansch approach, as well as approaches that are formally completely new. For example, new descriptors generated through direct mathematical modeling of chemical structures were introduced,[230−234] and the range of mathematical models used to link chemistry and biology has expanded accordingly.[235−239] However, the QSAR science still maintains a fundamental unity, founded on the systematic use of mathematical models and on the multivariate point of view. At present, the QSAR science is one of the basic tools of modern drug and pesticide design and has an increasing role in environmental sciences.[240−246] A great aspect of the QSARs, especially when applied within individual chemical classes, is that they point to the chemical determinants of the biological activity of the compounds; thus they can contribute to the rationalization of the biological activity mechanisms.[229]

QSARs have been generated for a number of individual chemical classes of chemical carcinogens and mutagens (including aromatic amines, nitroarenes, quinolines, triazenes, polycyclic aromatic hydrocarbons, lactones, aldehydes).[247,248] The majority of these QSARs are relative to *in vitro* mutagenicity; however, a number of QSAR models for animal carcinogenicity exist as well. In this section, we provide an overview of existing QSARs for the individual chemical classes, with specific emphasis on their contribution to mechanistic understanding.

5.1. Direct-Acting Alkylating Agents

5.1.1. Lactones. Lactones are reactive chemicals widely used as intermediates in chemical synthesis, solvents, constituents of paint removers, and antibacterial agents. They are mutagenic in test systems without metabolic activation. The mutagenicity of lactones has been modeled.[249,250] Electrophilicity (expressed as energy of the lowest unoccupied molecular orbital (LUMO)) appears to be correlated with the mutagenic activity; no hydrophobic term appears in the model. The lack of the hydrophobic

term is rather characteristic of direct-acting mutagens (that do not require metabolic activation).[240]

5.1.2. Epoxides. Aliphatic epoxides have found widespread use as valuable industrial and laboratory alkylating agents, which has led to much interest in their potential genotoxicity. The mutagenicity of different sets of epoxides has been studied: in all studies, QSAR models based only or primarily on electronic reactivity parameters were found.[251−253] QSARs based on topological descriptors have described the modulating effects of alkyl linear chain substitutents.[254,255]

5.1.3. Simple and αβ-Unsaturated Aldehydes. Aldehydes are widely distributed in the environment; they have multiple uses, for example, in the synthesis of chemicals and pharmaceuticals, along with being used as solvents, food additives, and disinfectants. Models for both the mutagenic potency and activity have been established.[155,256] Mutagenicity depends on LUMO, molar refractivity (MR), which describes steric/polarizability properties of the molecules, and hydrophobicity (log *P*): high electrophilicity, low steric hindrance, and high lipophilicity favor the activity. This result is concordant with the scientific evidence on the mechanisms of action of these chemicals, which react as direct electrophiles to form adducts with DNA and proteins (the β and carbonyl carbons being the points of attack). A substructural analysis pointed to the activating effect of halogen atoms on the double bond.[257]

5.2. Indirect-Acting Alkylating Agents

5.2.1. *N*-Nitroso Compounds. The mutagenicity of some *N*-nitrosoamines has been modeled.[240,258] Overall, the QSAR models indicate that hydrophobic, electron-releasing substituents on the ring of *N*-nitroso-*N*-benzylmethylamines increase mutagenicity; this is in agreement with the evidence that hydrophobicity is a parameter of importance in mutagenicity, when direct alkylation of DNA is not involved.

The carcinogenicity of *N*-nitroso compounds has been studied.[259,260] These studies, while emphasizing the importance of transport properties of the compounds in their carcinogenic activity, did not produce mechanistic results as clear-cut as the above studies on mutagenicity. In a substructural analysis, hydrophobicity and polarity appear to exert a positive contribution on carcinogenicity. The balance between hydrophobicity and polarity may reflect transport properties, in agreement with the above study.[261]

5.2.2. Halogenated Aliphatics. Halogenated aliphatic hydrocarbons are used widely as industrial and household solvents, as intermediates in chemical synthesis, and for a variety of other uses. It is recognized that they may act by several toxicity mechanisms of action (see above for details). Briefly, short-chain monohalogenated (excluding fluorine) alkanes and alkenes are potential direct-acting alkylating agents. Dihalogenated alkanes are also potential alkylating or cross-linking agents (either directly or after GSH conjugation). Fully halogenated haloalkanes tend to act by free radical or nongenotoxic mechanisms or undergo reductive dehalogenation to yield haloalkenes that in turn could be activated to epoxides.[262]

The toxic and aneuploidizing activities of a large set of halogenated aliphatic hydrocarbons in *Aspergillus nidulans* have been studied.[263] Aneuploidy is a change in the correct number of chromosomes. A resulting QSAR model pointed to the importance of LUMO, MR, and size factors. The lack of log *P* correlation seems to exclude that this mutational event is driven by a specific disturbance at the cell membranes and suggests the involvement of reductive metabolism and the generation of free radicals that would damage critical targets of the mitotic apparatus.

5.3. Indirect-Acting, Intercalating and Large DNA-Adduct-Forming Agents

5.3.1. Polycyclic Aromatic Hydrocarbons. PAHs constitute a large class of ubiquitous environmental pollutants formed from the incomplete combustion of fossil fuels, tobacco products, food, and virtually any organic matter. The PAHs have been the subject of several QSAR analyses. Whereas some preliminary analyses were reported on mutagenicity,[264−266] the most informative analyses focus on carcinogenicity.[267−272]

The theories of K-region (e.g., 9,10-bond of phenanthrene) activation and bay (or L-) region (e.g., region between the 4 and 5 positions of phenanthrene) activation as responsible for carcinogenicity were found to be able to rationalize the QSAR analyis results performed by Zhang and co-workers.[267] The analysis pointed to the importance of physical chemical (log *P*, HOMO) and structural parameters related to the activating metabolic steps.

5.4. Indirect-Acting, Aminoaryl DNA-Adduct-Forming Agents

5.4.1. Aromatic Amines and Nitroarenes. Due to their environmental and industrial importance, the aromatic amines are the single chemical class most studied for their ability to induce mutations and cancer. They have a wide variety of uses in many industries, that is, the manufacture of polymers, rubber, agricultural chemicals, dyes and pigments, pharmaceuticals, and photographic chemicals. Many aromatic amines have been reported to be powerful carcinogens and mutagens or hemotoxicants. The concern for the mutagenicity and carcinogenicity shown by many nitroaromatic compounds derives from the fact that they have long been of importance as intermediates in the synthesis of several kinds of industrial chemicals, as well as present in matrices of environmental importance (e.g., automobile exhaust fumes). Nitroaromatics are also used for their antibacterial activity and in chemotherapy.

The aromatic amines and the nitroarenes appear to induce mutations and cancer through a similar mechanism. Both types of compounds are believed to be biochemically transformed to a common hydroxylamine intermediate, which is then activated to give an electrophilic nitrogen species. The difference is that the amines are oxidized by cytochrome P450 enzymes, whereas the nitroarenes are reduced to the critical hydroxylamine by cytosolic reductases.[273,274]

In parallel with the large number of aromatic amines and nitroarenes tested experimentally, several QSARs, based on a variety of approaches, have been published for these classes. In particular, the gradation of the mutagenic potency in *Salmonella* has been the object of a number of studies.[275−286]

Further studies on the mutagenicity of aromatic amines (and nitroarenes) showed that the QSAR models for the gradation of potency of the active compounds were not adequate to discriminate between active and inactive chemicals,[287] so specific models for the activity (+ versus −) were necessary in addition to those for the toxic potency. QSARs for discriminating between mutagenic and nonmutagenic aromatic amines have been reported,[288] along with other studies.[285,286]

Regarding the carcinogenicity of the aromatic amines, there are two pioneering studies of note.[289,290] Subsequently, the carcinogenicity of nonheterocyclic aromatic amines has been

studied more extensively in two related papers: (a) in the first analysis only the carcinogenic chemicals were considered, and the structural factors that influence the gradation of carcinogenic potency in rodents were investigated;[291] (b) the second analysis established models to discriminate between carcinogenic and noncarcinogenic amines;[292] further results are reported.[293]

In the most mechanistically oriented QSAR analyses, the toxic activity of the amines was demonstrated to correlate with the ease of formation of the *N*-hydroxylamine,[290] with the stability of the nitrenium ion,[276,281] and with the ease of formation of epoxides on the aromatic ring.[290] Loew et al. also found that the ease of formation of phenols (a detoxifying pathway) is negatively correlated with the carcinogenic activity.[290]

Various studies[275,277,291] pointed to the central role of hydrophobicity in the modulation of the mutagenic and carcinogenic potency of the aromatic amines.

Regarding the reactivity (electronic descriptors), the energies of the highest occupied molecular orbital (HOMO, which describes the tendency of molecules to be easily oxidized) and of the LUMO were found to have a role both for the mutagenicity in *S. typhimurium*[277,281,294−296] and for the carcinogenicity in rodents.[291,292] The role of HOMO energy can be rationalized in terms of propensity of the toxic amines to form the intermediate metabolite hydroxylamine. The role of the LUMO energy is puzzling. The two terms LUMO and HOMO may be linked together through the concept of "hardness" (η = (LUMO − HOMO)/2) as a measure of chemical reactivity, or LUMO energy may account for the reduction of the nitro group present, together with the amino group, in a number of amines.[277]

Regarding the steric effects, bulky substituents at the nitrogen of the amino group generally inhibit bioactivation.[291,292] A general finding is that the activity decreases with steric bulk *ortho* to the amino function. This is consistent with the decrease in mutagenic potency found by Trieff et al.,[275] the decrease in carcinogenic potency in mouse,[291] the decreased probability of the amines of being carcinogenic in rat,[292] and the decreased probability of the subclass of diphenyl methanes of being mutagenic in *S. typhimurium*.[294] These findings are in line with the observation that bulky alkyl substituents *ortho* to the amino group decrease the mutagenicity of the arylamines.[297] The mechanistic rationale for these observations is that steric bulk prevents enzymatic access to the nitrogen and formation of the reactive intermediate.

Several authors used topological or substructural parameters, as well as indicator variables. A finding common to many of them is the correlation between activity or potency and number of (fused) aromatic rings.[277,280,281,283,284,289,292,296] This can be interpreted in different ways: (a) indicator for the planar systems apt to induce frameshift mutations in TA98 *S. typhimurium* strain; (b) indicator for the hydrophobicity of polycyclic and condensed aromatic rings; (c) indicator for the presence of extended conjugated systems that favor the formation of reactive intermediates. Debnath et al.[277] showed that, beside log *P*, an additional contribution to the mutagenic potency in TA98 was given by the presence of three or more fused rings. This effect was absent in strain TA100 and was related to the specificity of TA98 for frameshift mutations.

Carcinogenic potency also depends on the type of the ring system: aminobiphenyls (and, in the case of the rat, also fluorenamines) are intrinsically more active than anilines or naphthylamines. A bridge between the rings of the biphenyls decreases carcinogenic potency,[291] as well as the probability of being carcinogenic.[292]

The related class of nitroarenes was also analyzed by QSAR approaches. Several studies considered the gradation of mutagenic potency in the *Salmonella* assay (some studies also considered the response to DNA damage (SOS inducing potential, or SOSIP) in the SOS chromotest using tester strain *Escherichia coli* PQ37), namely refs 298−309.

Overall, the QSAR studies on the mutagenic potency of the nitroaromatics provide a coherent picture. A common pattern is the presence of hydrophobicity and LUMO energy in most equations. The LUMO energy term indicates that the lower the energy of the lowest unoccupied molecular orbital (i.e., the more readily it can accept electrons), the more potent the mutagen. This suggests that the electronic effect is associated with the reduction step commonly accepted as crucial in the biotransformation of these compounds by the nitroreductases.

The steric effects were also subjected to extended investigations. Generally speaking, a major difference between the TA98 and TA100 strains of *S. typhimurium* was that steric effects were more effective for TA98. It also appeared that the SOS *Escherichia coli* system resembles the TA100 and not the TA98 system.[306] As discussed in ref 287, this difference can be related to the influence that the size of mutagens have in the induction of frameshift mutations (specific of TA98, and not of TA100).

A set of aromatic and aliphatic nitro compounds, including molecules with both aromatic amino and nitro groups, was studied with a substructural approach to model rodent carcinogenicity. Higher reactivity of the aromatic amino group with respect to the aromatic nitro group emerged from the study, with primary amines contributing more than tertiary to carcinogenic activity. The carcinogenicity of nitrocompounds containing aromatic amines increases with the aromaticity of the aromatic ring attached to the amine group. As expected, aliphatic nitro compounds showed a minor contribution to carcinogenic activity, with respect to aromatic, with an increase in the case of *N*-nitro aliphatic fragment.[310]

6. MECHANISMS OF ACTION AND STRUCTURE−ACTIVITY RELATIONSHIPS AS A BASIS FOR PREDICTIVE TOXICOLOGY

Since the birth of modern chemistry, the investigators have always been eager to understand the structural and physical chemical basis of the biological activity of chemicals, one of the main aims being that of "domesticating" them. A brilliant illustration of how the concepts and practice of structure−activity relationships had a strong acceleration in toxicology in the mid-1980s is provided by E. J. Ariens.[311] One approach is the qualitative one, which takes into account the significance of particular groups in the molecule for particular aspects, part processes, in the biological action. Examples are groups described as pharmacophores or toxicophores or structural alerts. The other approach is the quantitative one, that is, the formalized QSAR approach briefly described in the previous section.

Even though the QSAR approach was experiencing dramatic development in the 1980s, with an exponential increase in methods and computerized technologies proposed, the knowledge of the structural alerts, as recognition and classification of the molecular substructures and reactive groups responsible for the toxic effects, still plays a primary role in the mechanistic science of toxicology and provides powerful means of intervention

to "domesticate" the chemicals. This is even more so in the field of carcinogenicity and mutagenicity, both for historical reasons and because the availability of QSARs is still limited. As a matter of fact, the knowledge of the action mechanisms as exemplified by the SAs is routinely used in the regulatory context (see, for example, the mechanistically based reasoning as in ref 138) or in the prioritization of chemicals to be tested in the animal bioassay.[312] In addition, the SAs are at the basis of popular commercial (e.g., DEREK by Lhasa Ltd.[313]) and noncommercial software systems (e.g., Oncologic,[314,315] Toxtree[23,85]).

A vast body of literature is available on the subject of predictive toxicology (a few examples, out of many, are refs 84, 246, and 316−328), and we will not present it in detail. We will focus on the crucial issue of the predictive ability of the qualitative and quantitative information on the structure−activity relationships.

Even though in general it is difficult to assess the contribution that qualitative, mechanistically based SAR information has given to risk assessment and to the domestication of chemistry, various evidence is however available and indicates that the contribution is important. A brilliant case is represented by the priority setting process at the U.S. National Toxicology Program in selecting chemicals to be bioassayed. The analysis was performed when around 400 chemicals had been bioassayed for their carcinogenic activity. It appears that two-thirds were selected for the bioassay because they were "suspect", mainly on the basis of structural considerations. One third was selected because of production or exposure considerations. The analysis showed that the structural criteria adopted to short-list suspect chemicals were able to enrich the target up to ten times. In fact, 70% of the chemicals bioassayed as suspect carcinogens were carcinogens, whereas 7% of the chemicals bioassayed only on production or exposure considerations were carcinogens.[312]

Additional evidence on the positive influence of the mechanistic information is that the rate of drugs and pesticides with known SAs or with positive *Salmonella* results put into the market in recent times has considerably decreased, as shown by the personal experience of one of the authors (R.B.) in his regulatory work. This is confirmed by a comparison of the presence of known SAs among pharmaceutical drugs approved by the U.S. Food and Drug Administration and the historical database of chemicals tested for carcinogenicity.[329] This indicates that the mechanistic information on chemical carcinogenicity has become shared knowledge among the synthetic chemists and allows them to synthesize safer chemicals.

After the definition and compilation of structural alerts (SA) following the electrophilicity theory of the Millers by John Ashby,[24,28] in more recent times the mechanistic knowledge on chemical carcinogens has been implemented into computerized expert systems that permit faster and more flexible assessment of chemicals, notably Oncologic[194] and Toxtree[85] among the systems in the public domain.

The compilation of SAs implemented into the expert system Toxtree 2.1.0 has been subjected to validation studies: it appears that the SAs have both high sensitivity and specificity for the Ames test (overall accuracy = 0.79), while having a lower agreement with carcinogenicity (overall accuracy = 0.70). The lower agreement with carcinogenicity depends on the fact that the available SAs for nongenotoxic carcinogens is still limited. On the other hand, it should be emphasized that the SAs predictive ability for *Salmonella* mutagenicity (accuracy = 0.79) is of the same order of magnitude as the experimental variability of the test itself (interlaboratory reproducibility reported to be 80−85%[330]). This implies that the assessment of chemical mutagenicity through the Ames test and through the SAs have similar reliability.[32]

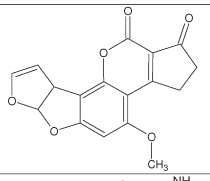
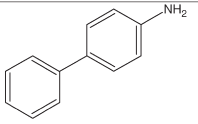
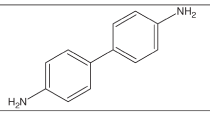
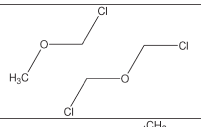
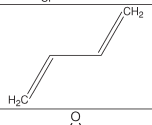
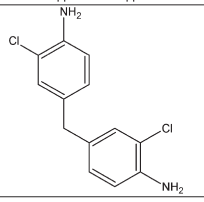
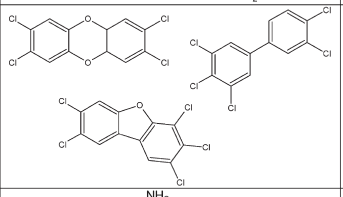
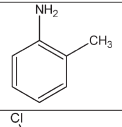
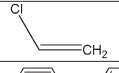
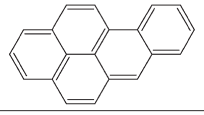
Another way of looking at the predictive ability of the SAs is provided in Table 2. Table 2 reports the positive predictivity for

Table 2. Structural Alerts for Carcinogenicity: Positive Predictivity[a]

	chemicals with SA	actives	positive predictivity, %
Acylating, Direct-Acting Agents			
SA_1	1	1	100
SA_15	5	3	60
Alkylating, Direct-Acting Agents			
SA_2	**13**	**12**	**92**
SA_3	2	2	100
SA_5	10	10	100
SA_6	4	4	100
SA_7	28	20	71
SA_8	75	51	68
SA_9	1	1	100
SA_10	55	37	67
SA_11	9	8	89
SA_12	**15**	**13**	**87**
Alkylating, Indirect-Acting Agents			
SA_4	6	6	100
SA_13	**68**	**57**	**84**
SA_14	8	8	100
SA_16	8	7	88
SA_21	**120**	**105**	**88**
SA_22	5	3	60
SA_23	5	5	100
SA_24	3	3	100
Intercalating and DNA-Adduct-Forming, Indirect-Acting Agents			
SA_18	14	11	79
SA_19	**14**	**12**	**86**
SA_30	6	5	83
Aminoaryl DNA-Adduct-Forming, Indirect-Acting Agents			
SA_25	3	3	100
SA_26	3	2	67
SA_27	88	63	72
SA_28	**107**	**87**	**81**
SA_28bis	15	10	67
SA_28ter	17	13	76
SA_29	26	20	77
Nongenotoxic Agents			
SA_17	24	13	54
SA_20	18	14	78
SA_31a	16	5	31
SA_31b	10	8	80
SA_31c	4	2	50

[a] For the individual structural alerts in Table 1, this table reports the positive predictivity for carcinogenicity. The SAs were applied to the ISSCAN v3a database on chemical carcinogens (see details in the text). The table reports the number of chemicals with each individual SA, and the number of actual carcinogens, with their percentage. In bold are classes with very high positive predictivity.

Table 3. Structural Alerts in the Recognized Human Carcinogens[a]

Human carcinogen	Structure	Structural Alert
Aflatoxins (Aflatoxin B1 showed)		SA_24
4-Aminobiphenyl		SA_28
Benzene		/
Benzidine		SA_28
Dyes metabolized to benzidine		SA_29
Bis(chloromethyl)ether and chloromethyl methyl ether		SA_8
1,3-Butadiene		/
Ethylene oxide		SA_7
Formaldehyde		SA_11
4,4′-methylenebis(2-chloroaniline)		SA_28
2,3,7,8-TCDD, 2,3,4,7,8-PeCDF, PCB 126		SA_31c, SA_19, SA_31b
o-Toluidine		SA_28
Vinyl chloride		SA_4
Benzo[*a*]pyrene		SA_18

[a] The table displays the recognized human carcinogens (pure substances), together with the SAs present in each of them.

the individual Toxtree SAs calculated from the carcinogenicity data contained in the ISSCAN v3a database. Positive predictivity is the probability that a chemical is carcinogenic when it contains a given SA. It is calculated as the ratio of the number of positive chemicals with a given SA to the number of all chemicals with the same SA. ISSCAN is a curated database on chemical carcinogens[331] freely available at http://www.iss.it/ampp/dati/cont.php?id=233&lang=1&tipo=7.

It should be remarked that the average positive predictivity of the SAs is 80%, which corresponds to a level of uncertainty similar to (or lower than) that of good experimental biological tests. An additional observation is that the SAs with high positive predictivity for carcinogenicity do not belong to one specific area of toxic mechanism or pathway but span the different categories and include both direct- and indirect-acting agents. In particular, the very high positive predictivity of (a) the alkylating agents *N*-nitroso compounds (SA_21), quinones (SA_12), hydrazines (SA_13), and esters of sulfonic or phosphonic acid (SA_2), (b) the heterocyclic PAH intercalating agents (SA_19), and (c) the arylamino DNA-adduct-forming aromatic amines (SA_28) should be noticed.

Table 3 adds another important piece of evidence. It shows that almost all the recognized human carcinogens (pure substances from the list in ref 332) contain SAs; thus the mechanistic knowledge collected into the SAs is a suitable basis to identify human carcinogens.

The predictivity of the formalized QSAR models for individual chemical classes of congeners has been assessed as well. A survey on the QSARs for mutagens and carcinogens in the public domain has been performed in a collaborative effort between the Istituto Superiore di Sanita' and the former European Chemicals Bureau. The details of the study are published in ref 328 and are put into a wider perspective in ref 333. The predictivity of the QSARs was checked with real external test sets. The analysis considered six QSARs that described the gradation of potency of toxic chemicals and five QSARs aimed at discriminating between active and inactive chemicals. The biological activities included *Salmonella* mutagenicity and rodent carcinogenicity for the classes of aromatic amines, nitroarenes, and aliphatic aldehydes. The QSARs for potency (applicable only to toxic chemicals) generated predictions 30−70% correct, whereas the QSARs for discriminating between active and inactive chemicals were 70−100% correct in their external predictions. To fully appreciate this result, the external predictivity of activity models (70−100% accuracy) should be compared with the variability range of the experimental tests. As reported above, the interlaboratory repeatability of a very good test like the Ames test has been estimated to be 80−85%.[330] Thus the level of uncertainty of "good" QSARs is comparable to that of "good" experimental assays.

7. CONCLUSIONS

The research on the mechanisms of chemical carcinogenesis has a long history now. Starting from the first attempts to identify and rationalize the chemical carcinogens discovered through early epidemiological obervations (e.g., the causation of scrotum cancer in chimney sweeps by soot, hypothesized 200 years ago by Percivall Pott[334]), this research has developed gradually for decades and centuries through the incorporation of new scientific perspectives and techniques. The cross-fertilization of different ideas, methods, and sciences in this field should be remarked, with special emphasis on the central role that chemistry has played in all its important achievements

(for example, by permitting the rationalization of the mechanisms by which the DNA-reactive chemicals cause mutations and cancer).

It is worthwhile to try and put the contribution of the knowledge on carcinogenicity mechanisms into the larger perspective of the risk assessment process. This process, as internationally accepted, is based on the integration of different tools, most of them being experimental (namely, the rodent bioassay and various short-term mutagenicity tests).[335,336] As explained in section 2., the Ames test is able to identify the DNA-reactive carcinogens with sufficient reliability, whereas the indications of the other mutagenicity STTs are blurred by the excess of positive results that do not correspond to carcinogens.[32] The knowledge on mechanisms has rationalized the area of DNA-reactive carcinogenicity and has been distilled into tools (e.g., the SAs) for predictive toxicology. The remaining area of the carcinogenicity chemical space, outside the DNA-reactivity domain, is populated by carcinogens acting by a variety of mechanisms, mostly nongenotoxic, and seems to lack adequate surveillance tools, except the time- and resource-consuming rodent bioassay. However, some promising alternative approaches, to be validated on larger databases of chemicals, seem to come across.

Recently, interest in the cell transformation assays has come to the limelight again.[337] These assays detect phenotypic alterations that are characteristic of tumorigenic cells and can be produced via a plethora of different molecular mechanisms. According to an analysis performed in our laboratory (our unpublished results, manuscript in preparation), the Syrian hamster embryo cell transformation (SHE) assay can detect both DNA-reactive and non-DNA-reactive carcinogens with a high efficiency (around 85% accuracy in a data set of 141 organic chemicals reported in ref 337). We performed a further exercise on testing strategies and found that if the Ames test or the SAs are applied in a first phase, and then SHE is applied to those chemicals that are negative in the first phase, only 8% of the original sample of carcinogens gets undetected. This remarkable enrichment in safe chemicals indicates that an efficient strategy to identify carcinogens without animal tests can be sketched, where the mechanistic knowledge (e.g., in the form of computerized SAs) plays a central role. We can expect that this strategy can be refined through two lines of research: (a) The first is efforts to set up new *in vitro* assays based on the modern omics technology, with the potential to trace molecular perturbations related to specific biochemical pathways.[338,339] This research is in progress, and its advancements need to be validated by building large databases of chemicals tested.[340] (b) The second is continued efforts to understand the mechanisms of nongenotoxic carcinogenicity from all the available *in vitro* and *in vivo* information and their translation into computerized codes (i.e., SAs and QSARs).

Overall, the research on the mechanisms of chemical carcinogenesis can be called one of the success story of biomedical research. Together with the intellectual beauty of descriptions that range from systems biology to molecular events, this research has provided practical tools to improve the safety of chemicals by, for example, avoiding the inclusion of some reactive moieties recognized as potentially toxic (i.e., SAs) in the synthesis of new products. The history of this research is not finished yet, but we can safely state that the route gone up to now is surely one paved of remarkable achievements.

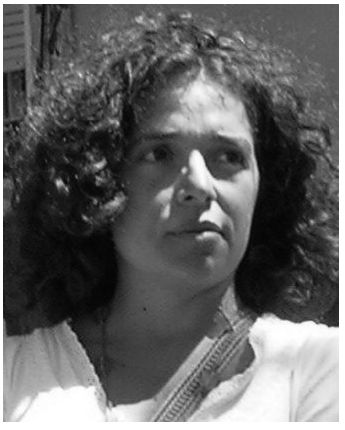
AUTHOR INFORMATION

Corresponding Author

*E-mail: romualdo.benigni@iss.it. Tel: 39 06 49902579.

BIOGRAPHIES

Romualdo Benigni received his education in Chemistry at the University of Rome "La Sapienza". He then joined the Istituto Superiore di Sanita' (Italian National Institute of Health), where he got a permanent position in 1977 and where he remained except for two sabbaticals, at the New York University in 1988 and at the Jawaharlal Nehru University in New Delhi in 2000. He worked experimentally in the field of molecular biology and environmental chemical mutagenesis. In the 1980s, he turned his attention to the statistical analysis and modeling of toxicological data and to the study of the relationships between the structure of organic compounds and their toxicological properties (mainly mutagenesis and carcinogenesis). Dr. Benigni has published over 160 journal articles and book chapters, applying a wide variety of quantitative analysis techniques, including QSAR, to the examination of chemical toxicity information.



Cecilia Bossa received her education in Chemistry at the University of Rome "La Sapienza", as well as a Ph.D. in Biophysics. She is an expert in the mechanisms of action of chemical mutagens and carcinogens and employs computational chemistry and chemoinformatics to translate such knowledge into QSARs, as well into computerized structure-based rules to predict toxicity. She has also experience in computational modeling of protein structure and activity.

ACKNOWLEDGMENT

This paper is dedicated to the memory of our friend and colleague Prof. Romano Zito.

LIST OF ABBREVIATIONS

DDT	dichlorodiphenyltrichloroethane
DNA	deoxyribonucleic acid
GSH	glutathione
heteroPAH	heterocyclic polycyclic aromatic hydrocarbon
HOMO	highest occupied molecular orbital
IARC	International Agency of Research on Cancer
log *P*	logarithm of the partition coefficient in the system *n*-octanol/water
LUMO	lowest unoccupied molecular orbital
MR	molar refractivity
NADP	nicotinamide adenine dinucleotide phosphate
NTP	National Toxicology Program
P450	cytochrome P450
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PeCDF	pentachlorodibenzofuran
(Q)SAR	(quantitative) structure−activity relationships
RNA	ribonucleic acid
ROS	reactive oxygen species
SA	structural alert
S_N1/S_N2	unimolecular/bimolecular nucleophilic substitution
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin, dioxin

REFERENCES

(1) Frankish, H. *Lancet* **2003**, *361*, 1278.
(2) Belpomme, D.; Irigaray, P.; Sasco, A. J.; Newby, J. A.; Howard, V.; Clapp, R.; Hardell, L. *Int. J. Oncol.* **2007**, *30*, 1037.
(3) Doll, R.; Peto, R. *J. Natl. Cancer Inst.* **1981**, *66*, 1191.
(4) Boffetta, P. *Mutat. Res.* **2006**, *608*, 157.
(5) Tomatis, L.; Huff, J.; Hertz-Picciotto, I.; Sandler, D. P.; Bucher, J.; Boffetta, P.; Axelson, O.; Blair, A.; Taylor, J.; Stayner, L.; Barrett, J. C. *Carcinogenesis* **1997**, *18*, 97.
(6) Minamoto, T.; Mai, M.; Ronai, Z. *Carcinogenesis* **1999**, *20*, 519.
(7) Albreht, T.; McKee, M.; Alexe, D. M.; Coleman, M. P.; Martin-Moreno, J. M. *Eur. J. Cancer* **2008**, *44*, 1451.
(8) Liechtenstein, P.; Holm, N. V.; Verkasalo, P. K.; Iliadou, A.; Kaprio, J.; Koskenvuo, M.; Pukkala, E.; Skytthe, A.; Hemminki, k. *N. Engl. J. Med.* **2000**, *343*, 78.
(9) Sokal, R. R.; Oden, N. L.; Rosenberg, M. S.; Thomson, B. A. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6067.
(10) Benigni, R.; Giuliani, A. *J. Environ. Sci. Health, Part C: Environ. Carcinog. Ecotoxicol. Rev.* **2000**, *C18*, 15.
(11) Benigni, R. *Environ. Res.* **2007**, *104*, 128.
(12) Belpomme, D.; Irigaray, P.; Hardell, L.; Clapp, R.; Montagnier, L.; Epstein, S.; Sasco, A. J. *Environ. Res.* **2007**, *105*, 414.
(13) Miller, E. C.; Miller, J. A. *Cancer* **1981**, *47*, 2327.
(14) Miller, J. A.; Miller, E. C. *Origins of Human Cancer*; Cold Spring Harbor Laboratory: Cold Spring Harbor, 1977; p 605.
(15) Miller, E. C.; Miller, J. A. *Cancer* **1981**, *47*, 1055.
(16) Zeiger, E. *Environ. Health Perspect.* **2004**, *44*, 363.
(17) Huff, J. E.; Haseman, J. K.; Rall, D. P. *Annu. Rev. Pharmacol. Toxicol.* **1991**, *31*, 621.
(18) Fung, V. A.; Barrett, J. C.; Huff, J. *Environ. Health Perspect.* **1995**, *103*, 680.
(19) Huff, J. *Ann. N.Y. Acad. Sci.* **1999**, *895*, 56.
(20) Ames, B. N. *Cancer* **1984**, *53*, 2030.
(21) Maron, D. M.; Ames, B. N. *Mutat. Res.* **1983**, *113*, 173.
(22) Zeiger, E. *Cancer Res.* **1987**, *47*, 1287.
(23) Benigni, R.; Bossa, C. *Mutat. Res. Rev.* **2008**, *659*, 248.
(24) Ashby, J.; Tennant, R. W. *Mutat. Res.* **1988**, *204*, 17.
(25) Zeiger, E.; Haseman, J. K.; Shelby, M. D.; Margolin, B. H.; Tennant, R. W. *Environ. Mol. Mutagen.* **1990**, *16*, 1.
(26) Fetterman, B. A.; Soo Kim, B.; Margolin, B. H.; Schidcrout, J. S.; Smith, M. G.; Wagner, S. M.; Zeiger, E. *Environ. Mol. Mutagen.* **1997**, *29*, 312.
(27) Woo, Y. T.; Lai, D. Y. *Cancer Risk Assessment: Chemical Carcinogenesis, Hazard Evaluation, and Risk Quantification*; Wiley: New York, 2010; Chapter 20, p 517.
(28) Ashby, J. *Environ. Mutagen.* **1985**, *7*, 919.
(29) Benigni, R. *Mutagenesis* **1992**, *7*, 335.
(30) Zeiger, E. *Regulat. Pharmacol. Toxicol.* **1998**, *28*, 85.
(31) Zeiger, E. *Mutat. Res.* **2001**, *492*, 29.
(32) Benigni, R.; Bossa, C.; Tcheremenskaia, O.; Giuliani, A. *Exp. Opin. Drug Metab. Toxicol.* **2010**, *6*, 1.
(33) Benigni, R.; Bossa, C.; Worth, A. P. *Mutagenesis* **2010**, *25*, 335.
(34) Arcos, J. C.; Argus, M. F. *Chemical Induction of Cancer. Modulation and Combination Effects*; Birkhauser: Boston, 1995; p 1.
(35) Woo, Y. T. *Quantitative Structure-Activity Relationship (QSAR) Models of Mutagens and Carcinogens*; CRC Press: Boca Raton, FL, 2003; Chapter 2, p 41.
(36) Pullman, B.; Pullman, A. *Carcinogenesis: Fundamental Mechanisms and Environmental Effects*; D. Reidel: Dordrecht, the Netherlands, 1980; p 55.
(37) La, D. K.; Swenberg, J. A. *Mutat. Res.* **1996**, *365*, 129.
(38) Guengerich, F. P. *Drug Metab. Rev.* **2002**, *34*, 607.
(39) Humphreys, W. G.; Kadlubar, F. F.; Guengerich, F. P. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 8278.
(40) LoPachin, R. M.; DeCaprio, A. P. *Toxicol. Sci.* **2005**, *86*, 214.
(41) Ho, T.-L. *Chem. Rev.* **1975**, *75*, 1.
(42) Carlson, R. M. *Environ. Health Perspect.* **1990**, *87*, 227.
(43) Beyerbach, A.; Farmer, P. B.; Sabbioni, G. *Chem. Res. Toxicol.* **2006**, *19*, 1611.
(44) Segal, A.; Solomon, J. J.; Maté, U.; Van Duuren, B. L. *Chem. Biol. Interact.* **1982**, *40*, 209.
(45) Geacintov, N. E.; Cosman, M.; Hingerty, B. E.; Shantu, A.; Brodye, S.; Patel, D. J. *Chem. Res. Toxicol.* **1997**, *10*, 111.
(46) Snyder, R. D.; McNulty, J.; Zairov, G.; Ewing, D. E.; Hendry, L. B. *Mutat. Res.* **2005**, *578*, 88.
(47) Snyder, R. D.; Arnone, M. R. *Mutat. Res.* **2002**, *503*, 21.
(48) Ferguson, L. R.; Denny, W. A. *Mutat. Res.* **1991**, *258*, 123.
(49) Snyder, R. D.; Ewing, D.; Hendry, L. B. *Mutat. Res.* **2006**, *609*, 47.
(50) Snyder, R. D. *Environ. Mol. Mutagen.* **2000**, *35*, 13.
(51) Geacintov, N. E. *Carcinogenesis* **1986**, *7*, 759.
(52) Xue, W.; Warshawsky, D. *Toxicol. Appl. Pharmacol.* **2005**, *206*, 73.
(53) Boysen, G.; Pachkowski, B. F.; Nakamura, J.; Swenberg, J. A. *Mutat. Res.* **2009**, *678*, 76.
(54) Bailey, E. A.; Iyer, R. S.; Stone, M. P.; Harris, T. M. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 1535.
(55) Lukin, M.; los Santos, C. *Chem. Rev.* **2006**, *106*, 607.
(56) Reddy, J. K.; Rao, M. S.; Azarnoff, D. L.; Sell, S. *Cancer Res.* **1979**, *39*, 152.
(57) Gonzalez, F. J.; Peters, J. M.; Cattley, R. C. *J. Natl. Cancer Inst.* **1998**, *90*, 1702.
(58) Reddy, J. K.; Rao, M. S. *Mutat. Res.* **1989**, *214*, 63.
(59) Corton, J. C.; Lapinskas, P.; Gonzalez, F. J. *Mutat. Res.* **2000**, *448*, 139.
(60) Upham, B. L.; Deocampo, N. D.; Wurl, B.; Trosko, J. E. *Int. J. Cancer* **1998**, *78*, 491.
(61) Trosko, J. E.; Ruch, R. J. *Front. Biosci.* **1998**, *3*, 208.
(62) Hernández, L. G.; van Steeg, H.; Luijten, M.; van Benthem, J. *Mutat. Res.* **2009**, *682*, 94.
(63) Butterworth, B. E.; Conolly, R. B.; Morgan, K. T. *Cancer Lett.* **1995**, *93*, 129.

(64) Larson, J. L.; Wolf, D. C.; Butterworth, B. E. *Fundam. Appl. Toxicol.* **1994**, *22*, 90.
(65) Cohen, S. M.; Ellwein, L. B. *Chem. Res. Toricol.* **1992**, *5*, 742.
(66) Fukushima, S.; Murai, T. *IARC Sci. Publ.* **1999**, *147*, 159.
(67) Heck, H. D.; Tyl, R. W. *Regul. Toxicol. Pharmacol.* **1985**, *5*, 294.
(68) Melnick, R. L.; Kohn, M. C.; Portier, C. J. *Environ. Health Perspect.* **1996**, *104*, 123.
(69) Buell, J. F.; Gross, T. G.; Woodle, E. S. *Transplantation* **2005**, *80*, 254.
(70) Botti, C.; Seregni, E.; Martinetti, A.; Bombardieri, E. *Int. J. Biol. Markers* **1998**, *13*, 51.
(71) Hojo, M.; Morimoto, T.; Maluccio, M.; Asano, T.; Morimoto, K.; Lagman, M.; Shimbo, T.; Suthanthiran, M. *Nature* **1999**, *397*, 530.
(72) Yager, J. D. *J. Natl. Cancer Inst. Monogr.* **2000**, *2000*, 67.
(73) Feigelson, H. S.; Henderson, B. E. *Carcinogenesis* **1996**, *17*, 2279.
(74) Bolton, J. L.; Thatcher, G. R. J. *Chem. Res. Toxicol.* **2008**, *21*, 93.
(75) Capen, C. C. *Prog. Clin. Biol. Res.* **1994**, *387*, 173.
(76) Capen, C. C.; Martin, S. L. *Toxicol. Pathol.* **1989**, *17*, 266.
(77) Valko, M.; Rhodes, C. J.; Moncol, J.; Izakovic, M.; Mazur, M. *Chem. Biol. Interact.* **2006**, *160*, 1.
(78) Upham, B. L.; Wagner, J. G. *Toxicol. Sci.* **2001**, *64*, 1.
(79) Klaunig, J. E.; Xu, Y.; Isenberg, J. S.; Bachowski, S.; Kolaja, K. L.; Jiang, J.; Stevenson, D. E.; Walborg, E. F., Jr. *Environ. Health Perspect.* **1998**, *106 S1*, 289.
(80) Jones, P. A. *Cancer Res.* **1996**, *56*, 2463.
(81) Jones, P. A. *Cancer Res.* **1986**, *46*, 461.
(82) Watson, R. E.; Goodman, J. I. *Toxicol. Sci.* **2002**, *67*, 11.
(83) Counts, J. L.; Goodman, J. I. *Cell* **1995**, *83*, 13.
(84) Woo, Y. T.; Lai, D. Y.; Argus, M. F.; Arcos, J. C. *Toxicol. Lett.* **1995**, *79*, 219.
(85) Benigni, R., Bossa, C., Jeliazkova, N. G., Netzeva, T. I., Worth, A. P. EUR - Scientific and Technical Report Series; EUR 23241 EN; Office for the Official Publications of the European Communities: Luxembourg, 2008. http://ecb.jrc.it/documents/QSAR/EUR_23241_EN.pdf .
(86) Van Duuren, B. L. *Ann. N.Y. Acad. Sci.* **1988**, *534*, 620.
(87) IARC. IARC monographs on the evaluation of carcinogenic risks to humans. Hydrazine and Hydrogen Peroxide (Part one, Part two, Part three). [71]. 1999. Lyon, IARC.
(88) Brochhagen, F. *The Handbook of Environmental Chemistry*; Springer-Verlag: Heidelberg, Germany, 1991; Vol. 3.
(89) Vock, E. H.; Cantoreggi, S.; Gupta, R. C.; Lutz, W. K. *Toxicol. Lett.* **1995**, *76*, 17.
(90) Segal, A.; Solomon, J. J.; Li, F. J. *Chem. Biol. Interact.* **1998**, *69*, 359.
(91) Tamura, N.; Aoki, K.; Lee, M. S. *Mutat. Res.* **1992**, *283*, 97.
(92) Jones, A. S.; Warren, J. H. *Tetrahedron* **1970**, 791.
(93) Tamura, N.; Aoki, K.; Lee, M. S. *Carcinogenesis* **1990**, *11*, 2009.
(94) Zhang, Y. *Mutat. Res.* **2004**, *555*, 173.
(95) Kassie, F.; Knasmüller, S. *Chem. Biol. Interact.* **2000**, *127*, 163.
(96) Kassie, F.; Pool-Zobel, B.; Parzefall, W.; Knasmüller, S. *Mutagenesis* **1999**, *14*, 595.
(97) Musk, S. R.; Johnson, I. T. *Mutat. Res.* **1993**, *300*, 111.
(98) Musk, S. R.; Smith, T. K.; Johnson, I. T. *Mutat. Res.* **1995**, *348*, 19.
(99) NTP NTP Technical Report; TR 234; U.S. Department of Health and Human Services: Washington, DC, 1982.
(100) Murata, M.; Yamashita, N.; Inoue, S.; Kawanishi, S. *Free Radical Biol. Med.* **2000**, *28*, 797.
(101) Paolini, M.; Perocco, P.; Canistro, D.; Valgimigli, L.; Pedulli, G. F.; Iori, R.; Della Croce, C.; Cantelli-Forti, G.; Legator, M. S.; Abdel-Rahman, S. Z. *Carcinogenesis* **2004**, *25*, 61.
(102) Fishbein, L. *J. Toxicol. Environ. Health* **1980**, *6*, 1133.
(103) Woo, Y. T.; Arcos, J. C. *Carcinogenicity and Pesticides: Principles, Issues, And Relationships*; American Chemical Society: Washington DC, 1989; Chapter 11, p 175.
(104) Salazar-Arredondo, E.; de Jesús Solis-Heredia, M.; Rojas-García, E.; Hernández-Ochoa, I.; Quintanilla-Vega, B. *Reprod. Toxicol.* **2008**, *25*, 455.
(105) Vijayaraghavan, M.; Nagarajan, B. *Mutat. Res.* **1994**, *321*, 103.
(106) Dedek, W.; Grahl, R.; Schmidt, R. *Acta Pharmacol. Toxicol.* **1984**, *55*, 104.
(107) Mehl, A.; Rolseth, V.; Gordon, S.; Bjoraas, M.; Seeberg, E.; Fonnum, F. *Neurotoxicology* **2000**, *21*, 173.
(108) Degraeve, N.; Chollet, M. C.; Moutschen, J. *Environ. Health Perspect.* **1985**, *60*, 395.
(109) Çakir, S.; Sarikaya, R. *Food Chem. Toxicol.* **2005**, *43*, 443.
(110) Piña-Guzmán, B.; Solís-Heredia, M. J.; Rojas-García, A. E.; Urióstegui-Acosta, M.; Quintanilla-Vega, B. *Toxicol. Appl. Pharmacol.* **2006**, *216*, 216.
(111) Eder, E.; Kütt, W.; Deininger, C. *Chem. Biol. Interact.* **2001**, *137*, 89.
(112) Pacheco, D. Y.; Cook, C.; Hincks, J. R.; Gibson, N. W. *Cancer Res.* **1990**, *50*, 7555.
(113) Wyatt, M. D.; Pittman, D. L. *Chem. Res. Toxicol.* **2006**, *19*, 1580.
(114) Tong, W. P.; Ludlum, D. B. *Biochim. Biophys. Acta* **1980**, *608*, 174.
(115) Iwamoto, T.; Hiraku, Y.; Oikawa, S.; Mizutani, H.; Kojima, M.; Kawanishi, S. *Cancer Sci.* **2004**, *95*, 454.
(116) Ashby, J.; Richardson, C. R.; Lefevre, P. A.; Callander, R. D.; Styles, J. A. *Mutat. Res.* **1985**, *156*, 19.
(117) Coley, H. M.; Brooks, N.; Phillips, D. H.; Hewer, A.; Jenkins, T. C.; Jarman, M.; Judson, I. R. *Biochem. Pharmacol.* **1995**, *49*, 1203.
(118) Noll, D. M.; McGregor Mason, T.; Miller, P. S. *Chem. Rev.* **2006**, *106*, 277.
(119) IARC. IARC monographs on the evaluation of carcinogenic risks to humans. Some Aziridines, *N*-, *S*- & *O*-Mustards and Selenium. [9]. IARC, Lyon, 1975.
(120) Chabner, B. A.; Thomas, T. G., Jr. *Nat. Rev. Cancer* **2005**, *5*, 65.
(121) Rajski, S. R.; Williams, R. M. *Chem. Rev.* **1998**, *98*, 2723.
(122) Olson, A. R.; Miller, R. J. *J. Am. Chem. Soc.* **1938**, *60*, 2687.
(123) Hemminki, K. *Chem. Biol. Interact.* **1981**, *34*, 323.
(124) Segal, A.; Solomon, J. J.; Mignano, J.; Dino, J. *Chem. Biol. Interact.* **1981**, *35*, 349.
(125) Segal, A.; Solomon, J. J.; Maté, U. *Chem. Biol. Interact.* **1980**, *29*, 335.
(126) Van Duuren, B. L. *Ann. N.Y. Acad. Sci.* **1969**, *163*, 633.
(127) Walles, S. A. *Toxicol. Lett.* **1980**, *5*, 161.
(128) Hemminki, K. *Carcinogenesis* **1983**, *4*, 901.
(129) Koskinen, M.; Plná, K. *Chem. Biol. Interact.* **2000**, *129*, 209.
(130) Barlow, T.; Dipple, A. *Chem. Res. Toxicol.* **1998**, *11*, 45.
(131) Kolman, A.; Chovanec, M.; Osterman-Golkar, S. *Mutat. Res.* **2002**, *512*, 173.
(132) Albertini, R. J.; Sweeney, L. M. *Crit. Rev. Toxicol.* **2007**, *37*, 489.
(133) Agurell, E.; Cederberg, H.; Ehrenberg, L.; Lindahl-Kliessing, K.; Rannug, U.; Törnqvist, M. *Mutat. Res.* **1991**, *250*, 229.
(134) Hata, T.; Hoshi, T.; Kanamori, K.; Matsumae, A.; Sano, Y.; Shima, T.; Sugawara, R. *J. Antibiot. (Tokyo)* **1956**, *9*, 141.
(135) Bizanek, R.; Chowdary, D.; Arai, H.; Kasai, M.; Hughes, C. S.; Sartorelli, A. C.; Rockwell, S.; Tomasz, M. *Cancer Res.* **1993**, *53*, 5127.
(136) Zang, H.; Gates, K. S. *Biochemistry* **2000**, *39*, 14975.
(137) Schultz, W. G.; Nieman, R. A.; Skibo, E. B. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 11854.
(138) Woo, Y. T.; Lai, D. Y.; McLain, J. L.; Ko Manibusan, M.; Dellarco, V. *Environ. Health Perspect.* **2002**, *110*, 75.
(139) Bolt, H. M.; Gansewendt, B. *Crit. Rev. Toxicol.* **1993**, *23*, 237.
(140) Chamberlain, M. P.; Lock, E. A.; Reed, C. J. *Toxicology* **1998**, *129*, 169.
(141) Chamberlain, M. P.; Sturgess, N. C.; Lock, E. A.; Reed, C. J. *Toxicology* **1999**, *139*, 27.
(142) NTP NTP Technical Report; TR 346; U.S. Department of Health and Human Services: Washington, DC, 1989.

(143) NTP NTP Technical Report; TR 363; U.S. Department of Health and Human Services: Washington, DC, 1989.
(144) Fedtke, N.; Certa, H.; Ebert, R.; Wiegand, H. J. *Arch. Toxicol.* **1994**, *68*, 217.
(145) Fedtke, N.; Certa, H.; Ebert, R.; Wiegand, H. J. *Arch. Toxicol.* **1994**, *68*, 158.
(146) Guengerich, F. P. *J. Biochem. Mol. Biol.* **2003**, *36*, 20.
(147) Ozawa, N.; Guengerich, F. P. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 5266.
(148) Guengerich, F. P. *Chem. Res. Toxicol.* **1991**, *4*, 391.
(149) Törnqvist, M.; Rannug, U.; Jonsson, A.; Ehrenberg, L. *Mutat. Res.* **1983**, *117*, 47.
(150) Dunkel, V. C.; Rogers-Back, A. M.; Lawlor, T. E.; Harbell, J. W.; Cameron, T. P. *Environ. Mol. Mutagen.* **1989**, *14*, 115.
(151) Kazius, J.; McGuire, R.; Bursi, R. *J. Med. Chem.* **2005**, *48*, 312.
(152) Koleva, Y. K.; Madden, J. C.; Cronin, M. T. D. *Chem. Res. Toxicol.* **2008**, *21*, 2300.
(153) Eder, E.; Hoffman, C.; Bastian, H.; Deininger, C.; Scheckenbach, S. *Environ. Health Perspect.* **1990**, *88*, 99.
(154) Kozekov, I. D.; Nechev, L. V.; Moseley, M. S.; Harris, C. M.; Rizzo, C. J.; Stone, M. P.; Harris, T. M. *J. Am. Chem. Soc.* **2003**, *125*, 50.
(155) Benigni, R.; Conti, L.; Crebelli, R.; Rodomonte, A.; Vari', M. R. *Environ. Mol. Mutagen.* **2005**, *46*, 268.
(156) IARC. IARC monographs on the evaluation of carcinogenic risks to humans. Formaldehyde, 2-Butoxyethanol and 1-tert-Butoxypropan-2-ol. [88], IARC, Lyon, France, 2006.
(157) Casanova, M.; Deyo, D. F.; Heck, H. D. *Fundam. Appl. Toxicol.* **1989**, *12*, 397.
(158) Speit, G.; Schütz, P.; Högel, J.; Schmid, O. *Mutagenesis* **2007**, *22*, 387.
(159) Stein, S.; Lao, Y.; Yang, I.-Y.; Hecht, S. S.; Moriya, M. *Mutat. Res.* **2006**, *608*, 1.
(160) Wang, M.; McIntee, E. J.; Cheng, G.; Shi, Y.; Villalta, P. W.; Hecht, S. S. *Chem. Res. Toxicol.* **2000**, *13*, 1149.
(161) Kurtz, A. J.; Lloyd, R. S. *J. Biol. Chem.* **2003**, *278*, 5970.
(162) Siraki, A. G.; Chan, T. S.; O'Brien, P. J. *Toxicol. Sci.* **2004**, *81*, 148.
(163) Gaskell, M.; McLuckie, K. I. E.; Farmer, P. B. *Mutat. Res.* **2004**, *554*, 387.
(164) Gaskell, M.; McLuckie, K. I. E.; Farmer, P. B. *Chem. Biol. Interact.* **2005**, *153*−*154*, 267.
(165) Monks, T. J.; Hanzlick, R. P.; Cohen, G. M.; Ross, D.; Graham, D. G. *Toxicol. Appl. Pharmacol.* **1992**, *112*, 2.
(166) Shigenaga, M. K.; Ames, B. N. *Free Radical Biol. Med.* **1991**, *10*, 216.
(167) Guengerich, F. P. *Arch. Biochem. Biophys.* **2003**, *409*, 59.
(168) Albertini, R.; Clewell, H.; Himmelstein, M. W.; Morinello, E.; Olin, S.; Preston, J.; Scarano, L.; Smith, M. T.; Swenberg, J.; Tice, R.; Travis, C. *Regul. Toxicol. Pharmacol.* **2003**, *37*, 105.
(169) Elfarra, A. A.; Krause, R. J.; Last, A. R.; Lash, L. H.; Parker, J. C. *Drug Metab. Dispos.* **1998**, *26*, 779.
(170) Anders, M. W. *Chem. Res. Toxicol.* **2008**, *21*, 145.
(171) Dreessen, B.; Westphal, G.; Bünger, J.; Hallier, E.; Müller, M. *Mutat. Res.* **2003**, *539*, 157.
(172) Gamberini, M.; Cidade, M. R.; Valotta, L. A.; Armelin, M. C. S.; Leite, L. C. C. *Carcinogenesis* **1998**, *19*, 147.
(173) Kovacic, P.; Jason, D. J. *Curr. Med. Chem.* **2001**, *8*, 773.
(174) Choudhary, G.; Hansen, H. *Chemosphere* **1998**, *37*, 801.
(175) Gannet, P. M.; Shi, X.; Lawson, T.; Kolar, C.; Toth, B. *Chem. Res. Toxicol.* **1997**, *10*, 1372.
(176) Ito, K.; Yamamoo, K.; Kawanishi, S. *Biochemistry* **1992**, *31*, 11606.
(177) IARC. IARC monographs on the evaluation of carcinogenic risks to humans. Some Antithyroid and Related Substances, Nitrofurans and Industrial Chemicals. [7], 111−140, IARC, Lyon, 1974.
(178) Beland, F. A.; Benson, R. W.; Mellick, P. W.; Kovatch, R. M.; Roberts, D. W.; Fang, J.-L.; Doerge, D. R. *Food Chem. Toxicol.* **2005**, *43*, 1.
(179) Ghanayem, B. I. *Toxicol. Sci.* **2007**, *95*, 331.
(180) Hoffler, U.; El-Masri, H. A.; Ghanayem, B. I. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 557.
(181) Wang, T. C.; Chiou, C. M.; Chang, Y. L. *Mutagenesis* **1998**, *13*, 405.
(182) Lijinsky, W. *Chemistry and Biology of N-Nitroso Compounds*; Cambridge Monographs on Cancer Research; Cambridge University Press: Cambridge, U.K., 1992; pp 14−53.
(183) Kaya, B.; Marcos, R.; Yanikoglu, A.; Creus, A. *Mutat. Res.* **2004**, *557*, 53.
(184) Zimmerman, L. J.; Valentine, H. L.; Valentine, W. M. *Chem. Res. Toxicol.* **2004**, *17*, 258.
(185) Calderon-Segura, M. E.; Gomez-Arroyo, S.; Molina-Alvarez, B.; Villalobos-Pietrini, R.; Calderon-Ezquerro, C.; Cortes-Eslava, J.; Valencia-Quintana, P. R.; Lopez-Gonzalez, L.; Zuniga-Reyes, R.; Sanchez-Rincon, J. *Toxicol. in Vitro* **2007**, *21*, 1143.
(186) van Bemmel, D. M.; Visvanathan, K.; Beane Freeman, L. E.; Coble, J.; Hoppin, J. A.; Alavanja, M. C. R. *Environ. Health Perspect.* **2008**, *116*, 1541.
(187) Tinkler, J.; Gott, D.; Bootman, J. *Food Chem. Toxicol.* **1998**, *36*, 849.
(188) Franekic, J.; Bratulic, N.; Pavlica, M.; Papes, D. *Mutat. Res. Lett.* **1994**, *325*, 65.
(189) Shanthi, R.; Krishnamoorthy, M. *Teratog., Carcinog., Mutagen.* **2002**, *22*, 451.
(190) Osaba, L.; Rey, M. J.; Aguirre, A.; Alonso, A.; Graff, U. *Mutat. Res.* **2002**, *518*, 95.
(191) Calviello, G.; Piccioni, E.; Boninsegna, A.; Tedesco, B.; Maggiano, N.; Serini, S.; Wolf, F. I.; Palozza, P. *Toxicol. Appl. Pharmacol.* **2006**, *211*, 87.
(192) Gonzalez, M.; Soloneski, S.; Reigosa, M. A.; Larramendy, M. L. *Mutat. Res.* **2003**, *534*, 145.
(193) IARC. IARC monographs on the evaluation of carcinogenic risks to humans. Some Thyrotropic Agents. [79], IARC, Lyon, 2001.
(194) Woo, Y. T.; Lai, D. Y. *Predictive Toxicology*; Taylor and Francis: Boca Raton, FL, 2005; Chapter 10, p 385.
(195) Woo, Y. T.; Lai, D. Y.; Argus, M. F.; Arcos, J. C. *Toxicol. Lett.* **1995**, *79*, 219.
(196) Hecht, S. S. *Proc. Soc. Exp. Biol. Med.* **1997**, *216*, 181.
(197) Cheng, G.; Wang, M.; Upadhyaya, P.; Villalta, P. W.; Hecht, S. S. *Chem. Res. Toxicol.* **2008**, *21*, 746.
(198) Carvalho, E.; Francisco, A. P.; Iley, J.; Rosa, E. *Bioorg. Med. Chem.* **2000**, *8*, 1725.
(199) Marchesi, F.; Turriziani, M.; Tortorelli, G.; Avvisati, G.; Torino, F.; De Vecchis, L. *Pharmacol. Res.* **2007**, *56*, 275.
(200) Malaveille, C.; Croisy, A.; Brun, G.; Bartsch, H. *Carcinogenesis* **1983**, *4*, 1477.
(201) Frei, E.; Pool, B. L.; Plesch, W. J.; Wiessler, M. *N-Nitroso Compounds: Occurence, Biological Effects and Relevance to Human Cancer*; IARC: Lyon, 1984; Chapter 57, p 491.
(202) Pool, B. L.; Frei, E.; Plesch, W. J.; Romruen, K.; Wiessler, M. *Carcinogenesis* **1984**, *5*, 814.
(203) Kazius, J.; McGuire, R.; Bursi, R. *J. Med. Chem.* **2005**, *48*, 312.
(204) Guengerich, F. P.; Johnson, W. W.; Shimada, T.; Ueng, Y.-F.; Yamazaki, Y.; Langouët, S. *Mutat. Res.* **1998**, *402*, 121.
(205) Conney, A. H. *Cancer Res.* **1982**, *42*, 4917.
(206) Wood, A. W.; Chang, R. L.; Levin, W.; Ryan, D. E.; Thomas, P. E.; Crolsy-Delcey, M.; Ittah, Y.; Yagi, H.; Jerina, D. M.; Conney, A. H. *Cancer Res.* **1980**, *40*, 2876.
(207) Cavalieri, E.; Rogan, E. *Environ. Health Perspect.* **1985**, *64*, 69.
(208) Cavalieri, E. L.; Rogan, E. G. *Xenobiotica* **1995**, *25*, 677.
(209) Leavitt, S. A.; George, M. H.; Moore, T.; Ross, J. A. *Mutagenesis* **2008**, *23*, 445.
(210) Penning, T. M.; Burczynski, M. E.; Hung, C.-F.; McCoull, K. D.; Palackal, N. T.; Tsuruda, L. S. *Chem. Res. Toxicol.* **1999**, *12*, 1.
(211) Bolton, J. L.; Trush, M. A.; Penning, T. M.; Dryhurst, G.; Monks, T. J. *Chem. Res. Toxicol.* **2000**, *13*, 135.
(212) Borosky, G. L. *J. Org. Chem.* **1999**, *64*, 7738.

(213) Lake, B. G. *Food Chem. Toxicol.* **1999**, *37*, 423.
(214) Born, S. L.; Caudill, D.; Smith, B. J.; Lehman-McKeema, L. *Toxicol. Sci.* **2000**, *58*, 23.
(215) Haack, T.; Erdinger, L.; Boche, G. *Mutat. Res.* **2001**, *491*, 183.
(216) Bickel, M. H. *Pharmacol. Rev.* **1969**, *21*, 325.
(217) Stöhrer, G.; Corbin, E.; Bosworth Brown, G. *Cancer Res.* **1972**, *32*, 637.
(218) Nelson, S. D. *J. Med. Chem.* **1982**, *25*, 753.
(219) Benigni, R.; Giuliani, A.; Franke, R.; Gruska, A. *Chem. Rev.* **2000**, *100*, 3697.
(220) Woo, Y. T.; Lai, D. Y. *Patty's Toxicology*, 5th ed.; John Wiley and Sons, Inc: New York, 2001; Chapter 58, p 969.
(221) Møller, P.; Wallin, H. *Mutat. Res.* **2000**, *462*, 13.
(222) Ziegler-Skylakakis, Z.; Nill, S.; Pan, J. F.; Andrae, U. *Environ. Mol. Mutagen.* **1998**, *31*, 362.
(223) Dearfield, K. L. *Mutat. Res.* **1994**, *317*, 111.
(224) Tithof, P. K.; Olivero, J.; Ruehle, K.; Ganey, P. E. *Toxicol. Sci.* **2000**, *53*, 40.
(225) Cicchetti, R.; Argentin, G. *Mutagenesis* **2003**, *18*, 127.
(226) EC - Scientific Committee on Food ; SCF/CS/CNTM/DIOXIN/8 Final; European Commission: Brussels, 2000. http://europa.eu.int/comm/food/fs/sc/scf/outcome_en.html.
(227) Poland, A.; Knutson, J. C. *Annu. Rev. Pharmacol. Toxicol.* **1982**, *22*, 517.
(228) Hansch, C.; Fujita, T. *J. Am. Chem. Soc.* **1964**, *86*, 1616.
(229) Hansch, C.; Hoekman, D.; Leo, A.; Weininger, D.; Selassie, C. D. *Chem. Rev.* **2002**, *102*, 783.
(230) Livingstone, D. J. *J. Chem. Inf. Comput. Sci.* **2000**, *40*, 195.
(231) Todeschini, R.; Lasagni, M.; Marengo, E. *J. Chemom.* **1994**, *8*, 263.
(232) Basak, S. C.; Mills, D. *SAR QSAR Environ. Res.* **2009**, *20*, 119.
(233) Estrada, E. *J. Phys. Chem. A* **2008**, *112*, 5208.
(234) Perez-Garrido, A.; Helguera, A. M.; Lopez, G. C.; Cordeiro, M. N.; Escudero, A. G. *Toxicology* **2010**, *268*, 64.
(235) Dunn, W. J.; Wold, S. *J. Med. Chem.* **1980**, *23*, 595.
(236) Wold, S. *Chemom. Intell. Lab. Syst.* **1995**, *30* (1), 109.
(237) Manallack, D. T.; Ellis, D. D.; Livingstone, D. J. *J. Med. Chem.* **1994**, *37*, 3758.
(238) Carlsson, L.; Helgee, E. A.; Boyer, S. *J. Chem. Inf. Model.* **2009**, *49*, 2551.
(239) Michielan, L.; Moro, S. *J. Chem. Inf. Model.* **2010**, *50*, 961.
(240) Hansch, C.; Leo, A. *Exploring QSAR. 1. Fundamentals and Applications in Chemistry and Biology*; American Chemical Society: Washington, DC, 1995.
(241) Benigni, R. *Quantitative Structure-Activity Relationship (QSAR) Models of Mutagens and Carcinogens*; CRC Press: Boca Raton, FL, 2003.
(242) Kubinyi, H. *3D QSAR in Drug Design: Theory, Methods and Applications*; ESCOM: Leiden, 1993.
(243) Tute, M. S. *Comprehensive Medicinal Chemistry*; Pergamon: Oxford, 1990; Chapter 17.1, p 1.
(244) Franke, R. *Theoretical Drug Design Methods*; Elsevier: Amsterdam, 1984.
(245) Franke, R.; Gruska, A. *Quantitative Structure-Activity Relationship (QSAR) Models of Mutagens and Carcinogens*; CRC Press: Boca Raton, FL, 2003; Chapter 1, p 1.
(246) Benigni, R.; Netzeva, T. I.; Benfenati, E.; Bossa, C.; Franke, R.; Helma, C.; Hulzebos, E.; Marchant, C. A.; Richard, A. M.; Woo, Y. T.; Yang, C. *J. Environ. Sci. Health, Part C: Environ. Carcinog. Ecotoxicol. Rev.* **2007**, *25*, 53.
(247) Hansch, C. *Sci. Tot. Environ.* **1991**, *109/110*, 17.
(248) Benigni, R. *Chem. Rev.* **2005**, *105*, 1767.
(249) LaLonde, R. T.; Leo, H.; Perakyla, H.; Dence, C. W.; Farrell, R. P. *Chem. Res. Toxicol.* **1992**, *5*, 392.
(250) Tuppurainen, K. *Chemosphere* **1999**, *38*, 3015.
(251) Hooberman, B. H.; Chakraborty, P. K.; Sinsheimer, J. E. *Mutat. Res.* **1993**, *299*, 85.
(252) Sugiura, K.; Goto, M. *Chem. Biol. Interact.* **1981**, *35*, 71.
(253) Tamura, N.; Takahashi, K.; Shirai, N.; Kawazoe, Y. *Chem. Pharm. Bull.* **1982**, *30*, 1393.
(254) Gonzalez, M. P.; Helguera, A. M.; Molina, R. R.; Garcia, J. F. *Polymer* **2004**, *45*, 2773.
(255) Gonzalez, M. P.; Dias, L. C.; Helguera, A. M. *Polymer* **2004**, *15*, 5353.
(256) Benigni, R.; Passerini, L.; Rodomonte, A. *Environ. Mol. Mutagen.* **2003**, *42*, 136.
(257) Perez-Garrido, A.; Helguera, A. M.; Lopez, G. C.; Cordeiro, M. N.; Escudero, A. G. *Toxicol.* **2010**, *268*, 64.
(258) Singer, G. M.; Andrews, A. W.; Guo, S. *J. Med. Chem.* **1986**, *29*, 40.
(259) Dunn, W. J., III; Wold, S. *J. Chem. Inf. Comput. Sci.* **1981**, *21*, 8.
(260) Frecer, V.; Miertus, S. *Neoplasma* **1988**, *35*, 525.
(261) Helguera, A. M.; Gonzalez, M. P.; Cordeiro, M. N.; Cabrera, M. A. *Toxicol. Appl. Pharmacol.* **2007**, *221*, 189.
(262) Woo, Y. T.; Lai, D. Y.; McLain, J. L.; Ko Manibusan, M.; Dellarco, V. *Environ. Health Perspect.* **2002**, *110*, 75.
(263) Crebelli, R.; Andreoli, C.; Carere, A.; Conti, L.; Crochi, B.; Cotta-Ramusino, M.; Benigni, R. *Chem. Biol. Interact.* **1995**, *98*, 113.
(264) He, L.; Jurs, P. C.; Custer, L. L.; Durham, S. K.; Pearl, G. M. *Chem. Res. Toxicol.* **2003**, *16*, 1567.
(265) Gramatica, P.; Pilutti, P.; Papa, E. *SAR QSAR Environ. Res.* **2007**, *18*, 169.
(266) Gramatica, P.; Papa, E.; Marrocchi, A.; Minuti, L.; Taticchi, A. *Ecotoxicol. Environ. Saf.* **2007**, *66*, 353.
(267) Zhang, L.; Sannes, K.; Shusterman, A. J.; Hansch, C. *Chem. Biol. Interact.* **1992**, *81*, 149.
(268) Norden, B.; Edlund, U.; Wold, S. *Acta Chem. Scand. Ser. B* **1978**, *32*, 602.
(269) Gallegos, A.; Robert, D.; Girones, X.; Carbo-Dorca, R. *J. Comput.-Aided Mol. Des.* **2001**, *15*, 67.
(270) Villemin, D.; Cherqaoui, D.; Mesbah, A. *J. Chem. Inf. Comput. Sci.* **1994**, *34*, 1288.
(271) Richard, A. M.; Woo, Y. T. *Mutat. Res.* **1990**, *242*, 285.
(272) Yuan, M.; Jurs, P. C. *Toxicol. Appl. Pharmacol.* **1980**, *52*, 294.
(273) Lai, D. Y.; Woo, Y. T.; Argus, M. F.; Arcos, J. C. *Designing Safer Chemicals. Green Chemistry for Pollution Prevention*; American Chemical Society: Washington, DC, 1996; Chapter 3, p 1.
(274) Woo, Y. T.; Lai, D. Y. *Patty's Toxicology*, 5th ed.; John Wiley and Sons, Inc: New York, 2001; Chapter 58, p 969.
(275) Trieff, N. M.; Biagi, G. L.; Sadagopa Ramanujam, V. M.; Connor, T. H.; Cantelli-Forti, G.; Guerra, M. C.; Bunce, H., III; Legator, M. S. *Mol. Toxicol.* **1989**, *2*, 53.
(276) Ford, G. P.; Griffin, G. R. *Chem. Biol. Interact.* **1992**, *81*, 19.
(277) Debnath, A. K.; Debnath, G.; Shusterman, A. J.; Hansch, C. *Environ. Mol. Mutagen.* **1992**, *19*, 37.
(278) Klopman, G.; Frierson, M. R.; Rosenkranz, H. S. *Environ. Mutagen.* **1985**, *7*, 625.
(279) Basak, S. C.; Grunwald, G. D. *Chemosphere* **1995**, *31*, 2529.
(280) Hatch, F. T.; Knize, M. G.; Felton, J. S. *Environ. Mol. Mutagen.* **1991**, *17*, 4.
(281) Hatch, F. T.; Colvin, M. E.; Seidl, E. T. *Environ. Mol. Mutagen.* **1996**, *27*, 314.
(282) Felton, J. S.; Knize, M. G.; Hatch, F. T.; Tanga, M. J.; Colvin, M. E. *Cancer Lett.* **1999**, *143*, 127.
(283) Hatch, F. T.; Knize, M. G.; Colvin, M. E. *Environ. Mol. Mutagen.* **2001**, *38*, 268.
(284) Maran, U.; Karelson, M.; Katritzky, A. R. *Quant. Struct.-Act. Relat.* **1999**, *18*, 3.
(285) Bhat, K. L.; Hayik, S.; Sztandera, L.; Bock, C. W. *QSAR Comb. Sci.* **2005**, *24*, 831.
(286) Garg, A.; Bhat, K. L.; Bock, C. W. *Dyes Pigm.* **2002**, *55*, 35.
(287) Benigni, R.; Andreoli, C.; Giuliani, A. *Environ. Mol. Mutagen.* **1994**, *24*, 208.
(288) Benigni, R.; Bossa, C.; Netzeva, T. I.; Rodomonte, A.; Tsakovska, I. *Environ. Mol. Mutagen.* **2007**, *48*, 754.
(289) Yuta, K.; Jurs, P. C. *J. Med. Chem.* **1981**, *24*, 241.

(290) Loew, G. H.; Poulsen, M.; Kirkjian, E.; Ferrel, J.; Sudhindra, B. S.; Rebagliati, M. *Environ. Health Perspect.* **1985**, *61*, 69.
(291) Benigni, R.; Giuliani, A.; Franke, R.; Gruska, A. *Chem. Rev.* **2000**, *100*, 3697.
(292) Franke, R.; Gruska, A.; Giuliani, A.; Benigni, R. *Carcinogenesis* **2001**, *22*, 1561.
(293) Benigni, R.; Worth, A. P.; Netzeva, T. I.; Jeliazkova, N. G.; Bossa, C.; Gruska, A.; Franke, R. *Environ. Mol. Mutagen.* **2009**, *50*, 152.
(294) Benigni, R.; Passerini, L.; Gallo, G.; Giorgi, F.; Cotta-Ramusino, M. *Environ. Mol. Mutagen.* **1998**, *32*, 75.
(295) Zhang, Y. P.; Klopman, G.; Rosenkranz, H. S. *Environ. Mol. Mutagen.* **1993**, *21*, 100.
(296) Lewis, D. F. V.; Ioannides, C.; Walker, R.; Parke, D. V. *Food Addit. Contam.* **1995**, *12*, 715.
(297) Glende, C.; Schmitt, H.; Erdinger, L.; Engelhardt, G.; Boche, G. *Mutat. Res.* **2001**, *498*, 19.
(298) Biagi, L. G.; Hrelia, P.; Gerra, M. G.; Paolini, M.; Barbaro, A. M.; Cantelli-Forti, G. *Arch. Toxicol.* **1986**, No. Suppl. 9, 425.
(299) Walsh, D. B.; Claxton, L. D. *Mutat. Res.* **1987**, *182*, 55.
(300) Maynard, A. T.; Pedersen, L. G.; Posner, H. S.; Mckinney, J. D. *Mol. Pharmacol.* **1986**, *29*, 629.
(301) Compadre, R. L.; Shusterman, A. J.; Hansch, C. *Int. J. Quantum Chem.* **1988**, *34*, 91.
(302) Lopez de Compadre, R. L.; Debnath, A. K.; Shusterman, A. J.; Hansch, C. *Environ. Mol. Mutagen.* **1990**, *15*, 44.
(303) Debnath, A. K.; de Compadre, R. L. L.; Debnath, G.; Shusterman, A.; Hansch, C. *J. Med. Chem.* **1991**, *34*, 786.
(304) Debnath, A. K.; Lopez de Compadre, R. L.; Shusterman, A. J.; Hansch, C. *Environ. Mol. Mutagen.* **1992**, *19*, 53.
(305) Debnath, A. K.; Hansch, C. *Environ. Mol. Mutagen.* **1992**, *20*, 140.
(306) Debnath, A. K.; Hansch, C.; Kim, K. H.; Martin, Y. C. *J. Med. Chem.* **1993**, *36*, 1009.
(307) Caliendo, G.; Fattorusso, C.; Greco, G.; Novellino, E.; Perissutti, E.; Santagada, V. *SAR QSAR Environ. Res.* **1995**, *4*, 21.
(308) Fan, M.; Byrd, C.; Compadre, C. M.; Compadre, R. L. *SAR QSAR Environ. Res.* **1998**, *9*, 187.
(309) Mersch-Sundermann, V.; Rosenkranz, H. S.; Klopman, G. *Mutat. Res.* **1994**, *304*, 271.
(310) Helguera, A. M.; Perez, M. A.; Combes, R. D.; Gonzalez, M. P. *Toxicology* **2006**, *220*, 51.
(311) Ariens, E. J. *Drug Metab. Rev.* **1984**, *15*, 425.
(312) Fung, V. A.; Huff, J.; Weisburger, E. K.; Hoel, D. G. *Fundam. Appl. Toxicol.* **1993**, *20*, 413.
(313) Greene, N.; Judson, P. N.; Langowski, J. J.; Marchant, C. A. *SAR QSAR Environ. Res.* **1999**, *10*, 299.
(314) Woo, Y. T.; Lai, D. Y. *Predictive Toxicology*; Taylor and Francis: Boca Raton, FL, 2005; Chapter 10, p 385.
(315) Woo, Y. T.; Lai, D. Y.; Argus, M. F.; Arcos, J. C. *J. Environ. Sci. Health, Part C: Environ. Carcinog. Ecotoxicol. Rev.* **1998**, *C16*, 101.
(316) Benigni, R.; Richard, A. M. *Methods* **1998**, *14*, 264.
(317) Cronin, M. T. D.; Dearden, J. C. *Quant. Struct.-Act. Relat.* **1995**, *14*, 518.
(318) Greene, N. *Adv. Drug Delivery Rev.* **2002**, *54*, 417.
(319) Hansch, C. *Biological Activity and Chemical Structure*; Elsevier: Amsterdam, 1977.
(320) Helma, C. *Mol. Diversity* **2006**, *10*, 147.
(321) Hulzebos, E. M.; Posthumus, R. *SAR QSAR Environ. Res.* **2003**, *14*, 285.
(322) Serafimova, R., Fuart Gatnik, M., Worth, A. JRC Technical Report EUR 24427 EN; Publications Office of the European Union: Luxembourg, 2010. http://ecb.jrc.ec.europa.eu/DOCUMENTS/QSAR/EUR_24427_EN.pdf.
(323) Pearl, G. M.; Livingstone-Carr, S.; Durham, S. K. *Curr. Top. Med. Chem.* **2001**, *1*, 247.
(324) Richard, A. M. *Toxicol. Lett.* **1998**, *102−103*, 611.
(325) Snyder, R. D.; Pearl, G. M.; Mandakas, G.; Choy, W. N.; Goodsaid, F.; Rosenblum, I. Y. *Environ. Mol. Mutagen.* **2004**, *43*, 143.
(326) Valerio, L. G.; Arvidson, K. B.; Chanderbhan, R. F.; Contrera, J. F. *Toxicol. Appl. Pharmacol.* **2007**, *222*, 1.
(327) Zeiger, E.; Ashby, J.; Bakale, G.; Enslein, K.; Klopman, G.; Rosenkranz, H. S. *Mutagenesis* **1996**, *11*, 474.
(328) Benigni, R., Bossa, C., Netzeva, T. I., Worth, A. P. EUR - Scientific and Technical Research Series; EUR 22772 EN; Office for the Official Publications of the European Communities: Luxembourg, 2007. http://ecb.jrc.it/documents/QSAR/EUR_22772_EN.pdf.
(329) Benigni, R.; Zito, R. *Curr. Top. Med. Chem.* **2003**, *3*, 1289.
(330) Piegorsch, W. W.; Zeiger, E. *Statistical Methods in Toxicology*; Springer-Verlag: Heidelberg, 1991; Chapter 43, p 35.
(331) Benigni, R.; Bossa, C.; Richard, A. M.; Yang, C. *Ann. Ist. Super. Sanita* **2008**, *44*, 48.
(332) Baan, R. A.; Grosse, Y.; Straif, K.; Secretan, B.; el Ghissassi, F.; Bouvard, V.; Benbrahim-Tallaa, L.; Guha, N.; Freeman, C.; Galichet, L.; Cogliano, V. J. *Lancet Oncol.* **2009**, *10*, 1143.
(333) Benigni, R.; Bossa, C. *J. Chem. Inf. Model.* **2008**, *48*, 971.
(334) Doll, R. *Rev. Epidemiol. Sante' Publique* **1976**, *24*, 107.
(335) Ashby, J.; Waters, M. D.; Preston, J.; Adler, I. D.; Douglas, G. R.; Fielder, R.; Shelby, M. D.; Anderson, D.; Sofuni, T.; Gopalan, H. N. B.; Becking, G.; Sonich-Mullin, C. *Mutat. Res.* **1996**, *352*, 153.
(336) Combes, R.; Grindon, C.; Cronin, M. T. D.; Roberts, D. W.; Garrod, J. *ATLA, Altern. Lab. Anim.* **2007**, *35*, 267.
(337) OECD. Detailed review paper on cell transformation assays for detection of chemical carcinogens. [31]; OECD Series on Testing and Assessment; OECD: Paris, 2007.
(338) Judson, R. S.; Houck, K. A.; Kavlock, R. J.; Knudsen, T. B.; Martin, M. T.; Mortensen, H. M.; Reif, D. M.; Rotroff, D. M.; Shah, I.; Richard, A. M.; Dix, D. J. *Environ. Health Perspect.* **2010**, *218*, 485.
(339) Davalos, V.; Esteller, M. *Curr. Opin. Oncol.* **2010**, *22*, 35.
(340) Benigni, R.; Bossa, C.; Giuliani, A.; Tcheremenskaia, O. *J. Environ. Sci. Health, Part C: Environ. Carcinog. Ecotoxicol. Rev.* **2010**, *28*, 272.