Mechanism-based Molecular Design of Chemicals with Low Carcinogenicity Potential

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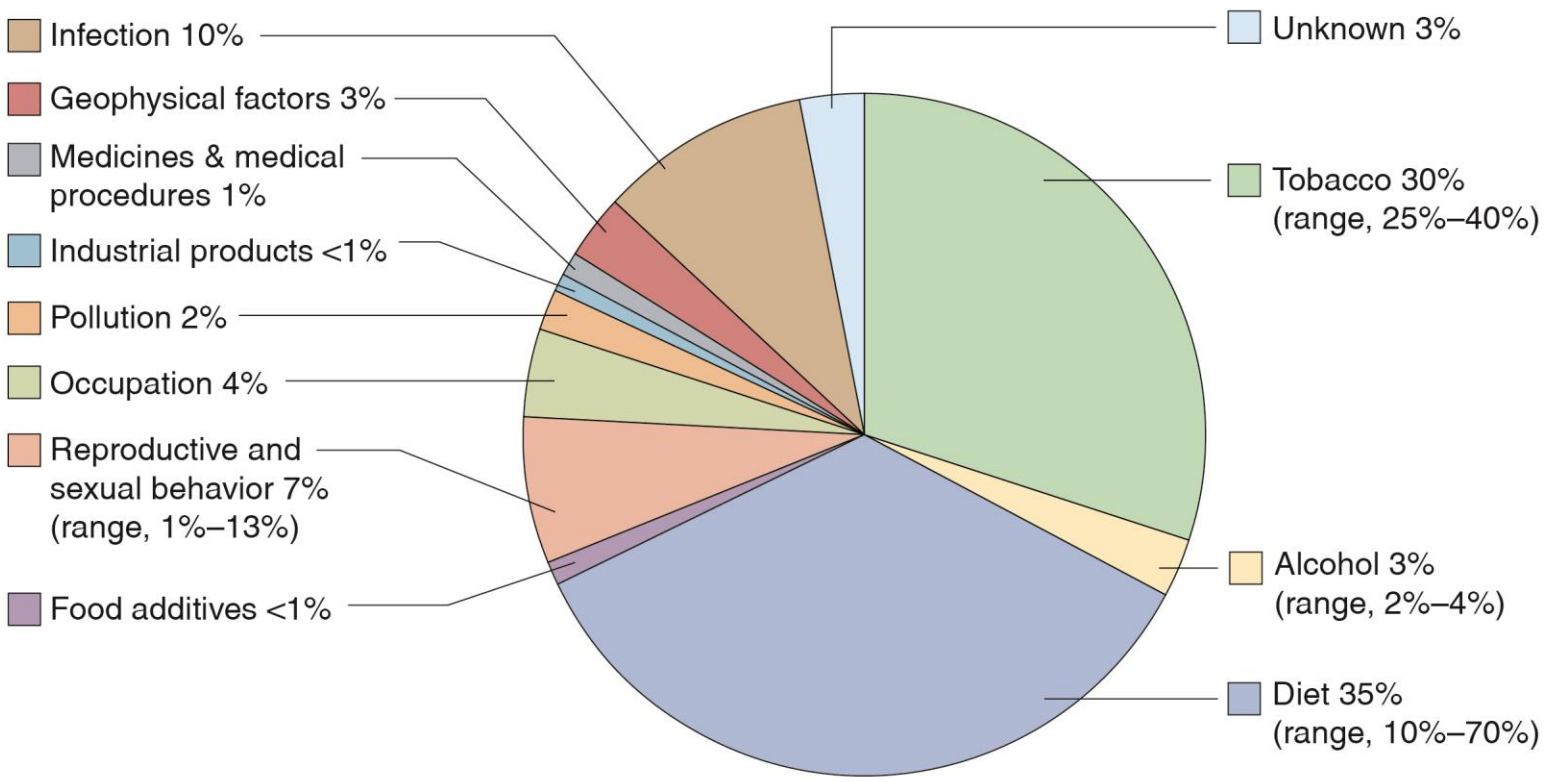


Figure 8-1. *Proportions of human cancer deaths attributed to various factors.* (Reproduced with permission from [no authors listed] Harvard reports on cancer prevention: causes of human cancer. Center for Cancer Prevention Harvard School of Public Health. *Cancer Causes and Control.* 1996;7 (Suppl 1):S3–S4, 1996.)

Table 8-32

Known Human Carcinogens: International Agency for Research on Cancer

Acetaldehyde
Acid mists, strong inorganic
Aflatoxins
Alcoholic beverages
Aluminum production
4-Aminobiphenyl
Areca nut
Aristolochic acid
Arsenic and inorganic arsenic compounds
Asbestos (all forms)
Auramine production
Azathioprine
Benzene
Benzidine and dyes metabolized to benzidine
Benzo[*a*]pyrene
Beryllium and beryllium compounds
Betel quid, with or without tobacco
Bis(chloromethyl)ether and chloromethyl methyl ether
Busulfan
1,3-Butadiene
Cadmium and cadmium compounds
Chlorambucil
Chlornaphazine
Chromium (VI) compounds
Clonorchis sinensis (infection with)
Coal, household combustion
Coal gasification
Coal tar distillation
Coal tar pitch
Coke production
Cyclophosphamide
Cyclosporine
Diethylstilbestrol
Epstein–Barr virus (infection with)
Erionite
Estrogen postmenopausal therapy
Estrogen–progestogen therapy
Estrogen–progestogen oral contraceptives (combined)
Ethanol in alcoholic beverages
Ethylene oxide
Etoposide
Etoposide in combination with cisplatin and bleomycin Fission products, including strontium-90
Formaldehyde
Haematite mining
Helicobacter pylori
Hepatitis B virus
Hepatitis C virus
Human immunodeficiency virus type 1
Human papilloma virus (HPV)
Human T-cell lymphotropic virus type I
Ionizing radiation
Iron and steel founding
Isopropyl alcohol
Kaposi sarcoma herpesvirus
Leather dust
Magenta production
Melphalan
Methoxsalen (8-methoxypsoralen) plus ultraviolet A radiation
4,4′-Methylenebis(chloroaniline) (MOCA)
Mineral oils, untreated or mildly treated
MOPP
2-Naphthylamine
Neutron radiation
Nickel compounds
N′-Nitrosonornicotine (NNN) and 4-(*N*-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) Opisthorchis viverrini (liver fluke)
Painter (workplace exposure)
3,4,5,3′,4′-Pentachlorobiphenyl (PCB-126)
2,3,4,7,8-Pentachlorodibenzofuran
Phenacetin (and mixtures containing it)
Phosphorus-32, as phosphate
Plutonium
Radioiodines, including iodine-131
Radionuclides, α-particle-emitting
Radionuclides, β-particle-emitting,
Radium-224 and its decay products
Radium-226 and its decay products
Radium-228 and its decay products
Radon-222 and its decay products
Rubber manufacturing industry
Salted fish (Chinese-style)
Schistosoma haematobium (flatworm)
Semustine (methyl-CCNU)
Shale oils
Silica dust, crystalline (cristobalite)
Solar radiation
Soot (exposure of chimney sweeps)
Sulfur mustard
Tamoxifen
2,3,7,8-Tetrachlorodibenzo-para-dioxin
Thiotepa
Thorium-232 and its decay products
Tobacco, smokeless
Tobacco smoke, secondhand
Tobacco smoking
ortho-Toluidine
Treosulfan
Ultraviolet (UV) including UVA, UVB, and UVC
Ultraviolet-emitting tanning devices
Vinyl chloride
Wood dust
X- and γ-radiation

EPA - New Chemicals Program

- Toxic Substances Control Act (TSCA) requires a manufacturer (including importer) of a new chemical substance to submit a "premanufacture notice" (PMN) to EPA 90 days before the date of intended start of production or import of the subject substance.

- During that 90-day review period, EPA assesses whether the manufacture, processing, distribution in commerce, use or disposal of the substance presents or may present an unreasonable risk to human health or the environment.

- Information required as part of a PMN: chemical identity, use, anticipated production volume, byproducts, exposure & release information, disposal practices, existing available health & environmental effects test data which are very limited.

Mechanism-based SAR Analysis

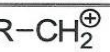
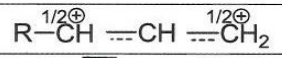
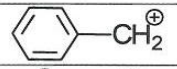
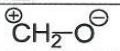
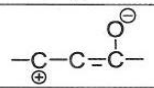
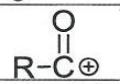
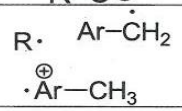
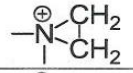
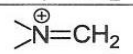
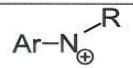
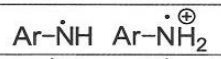
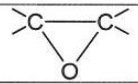
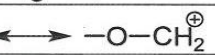
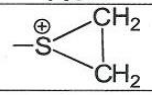
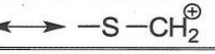
- Physical and chemical Properties
- Metabolism and Mechanisms of Action
- Carcinogenicity and SAR

Mechanisms of Chemical Carcinogenesis

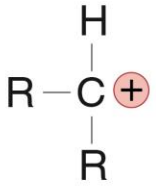
- Genotoxic (direct-acting/active metabolites)
  Interaction with DNA to cause mutation(s) in genes

- Non-genotoxic
  Receptor-mediated, oxidative stress, etc.

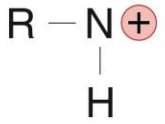
Table 1. Electrophilic species of some chemical carcinogens.

Electrophilic species	Structures	Examples of carcinogens
Alkylcarbonium ions		Diethylnitrosamine: dimethylsufate; 1,2-dimethylhydrazine
Arylcarbonium ions		Benzidine;1-(4-methoxyphenyl)-1,3-dimethyltriazene
Allylic carbonium ions		Allyl methanesulfonate: lasiocarpine; safrole
Benzylic carbonium ions		Benzyl chloride; 7,12-dimethylbenz(a)anthracene
Formaldehyde		Formaldehyde; hexamethylphosphoramide
A,β-Unsaturated carbonyls or carboxylates		Acrylates; arecoline;cyclophosphamide; diallate; ptaquiloside
Acylating moieties		Benzoyl chloride; dimethylcarbamyl chloride
Carbon-centered free radicals or radical cations		Carbon tetrachloride; 7.12-dimethylbenz(a)anthracene
Carbene		Carbon tetrachloride; safrole
Aziridinium ions		Aziridine; cyclophosphanude; nitrogen mustard
Iminium ions		Hexamethylmelamine; bis-(morpholino)-methane; hycanthone
Arylnitrenium ions		2-Acetylaminofluuorene; benzidine; N,N-dimethyl-4-aminoazobenzene
Nitrogen-centered free radicals or radicals cations		Benzidine; 2-naphthylamine
Epoxides		Afatoxic B_1;capsaicin. Ethylene oxide; vinyl chloride
Oxonium ions (α-haloethers)		Bis(chloromethyl)ether
Peroxy free radicals		Di-t-butyl peroxide
Episulfonium ions		1,2-dichloroethane; sulfur mustard
Sulfonium ions (α halothioesters)		Dichloromethane

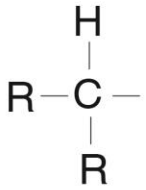
1. Carbonium ions



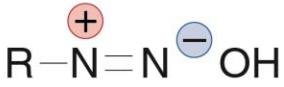
2. Nitrenium ions



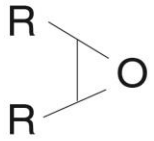
3. Free radicals



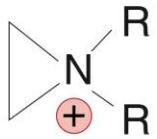
4. Diazonium ions



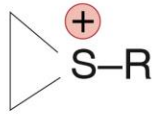
5. Epoxides



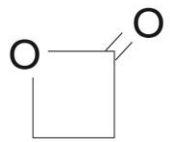
6. Aziridinium ions



7. Episultonium ions



8. Strained lactones



9. Sulfonates

RSO_2OCH_3

10. Halo ethers

$ClCH_2OCH_2Cl$

11. Enals

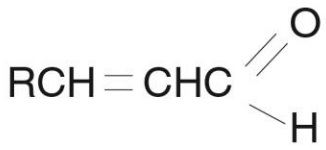


Figure 8-3. *Structures of reactive electrophiles.*

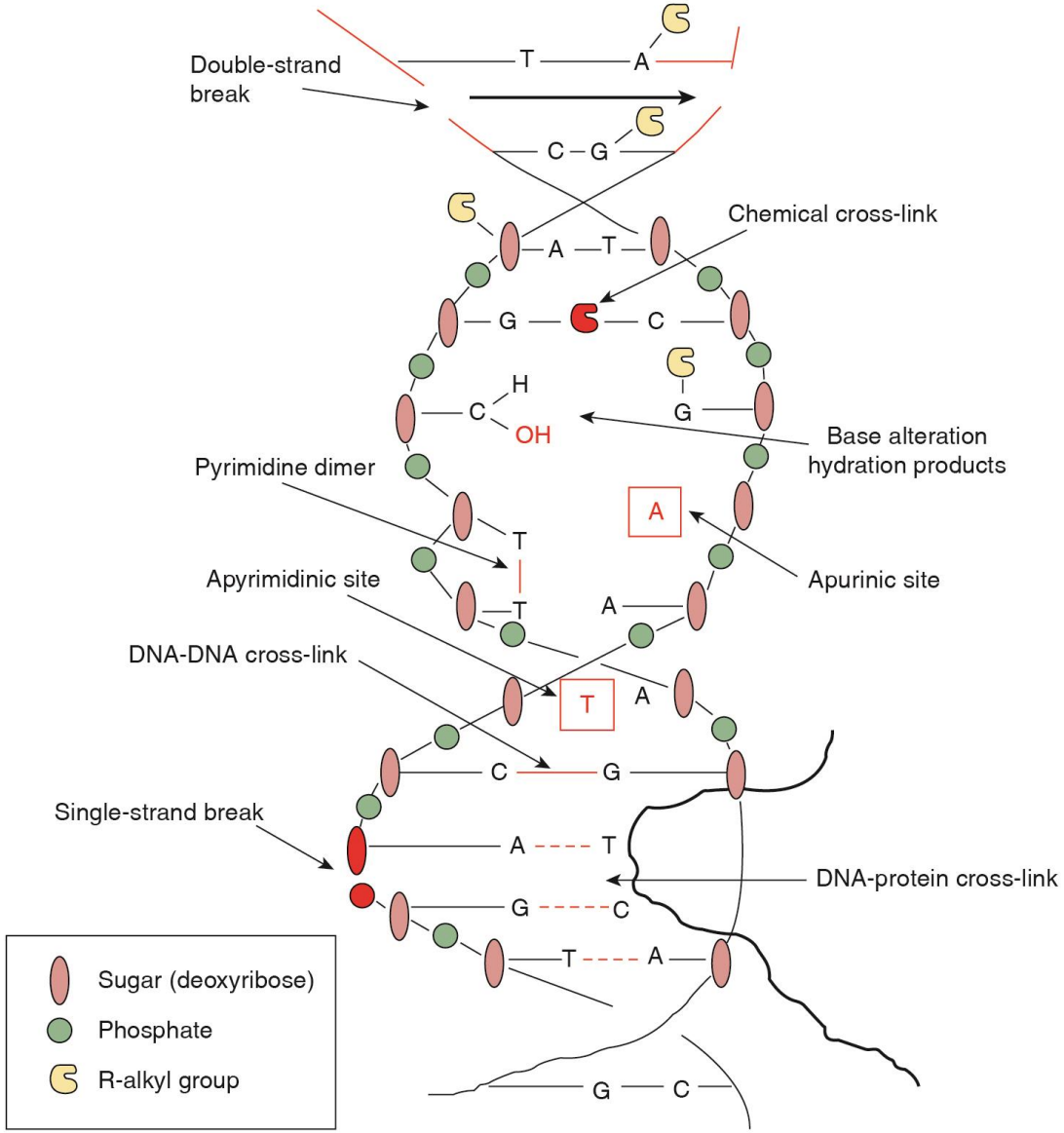


Figure 8-8. *Schematic representation of chemical and radiation-induced lesions in DNA.*

Critical Factors for Consideration

I. Nature of electrophiles:

- Reactivity
- Resonance stabilization of electrophilic metabolites
- Molecular flexibility
- Polyfunctionality and spacing/distance between reactive groups

Critical Factors for Consideration (cont.)

II. Environment Surrounding Electrophiles:

- Physicochemical Factors
  - Molecular weight
  - Physical state, size and shape
  - Solubility

- Nature and position of substituents
  - Steric hindrance
  - Enhancement of activation
  - Blocking of detoxification

Resonance stabilization of Reactive Intermediates

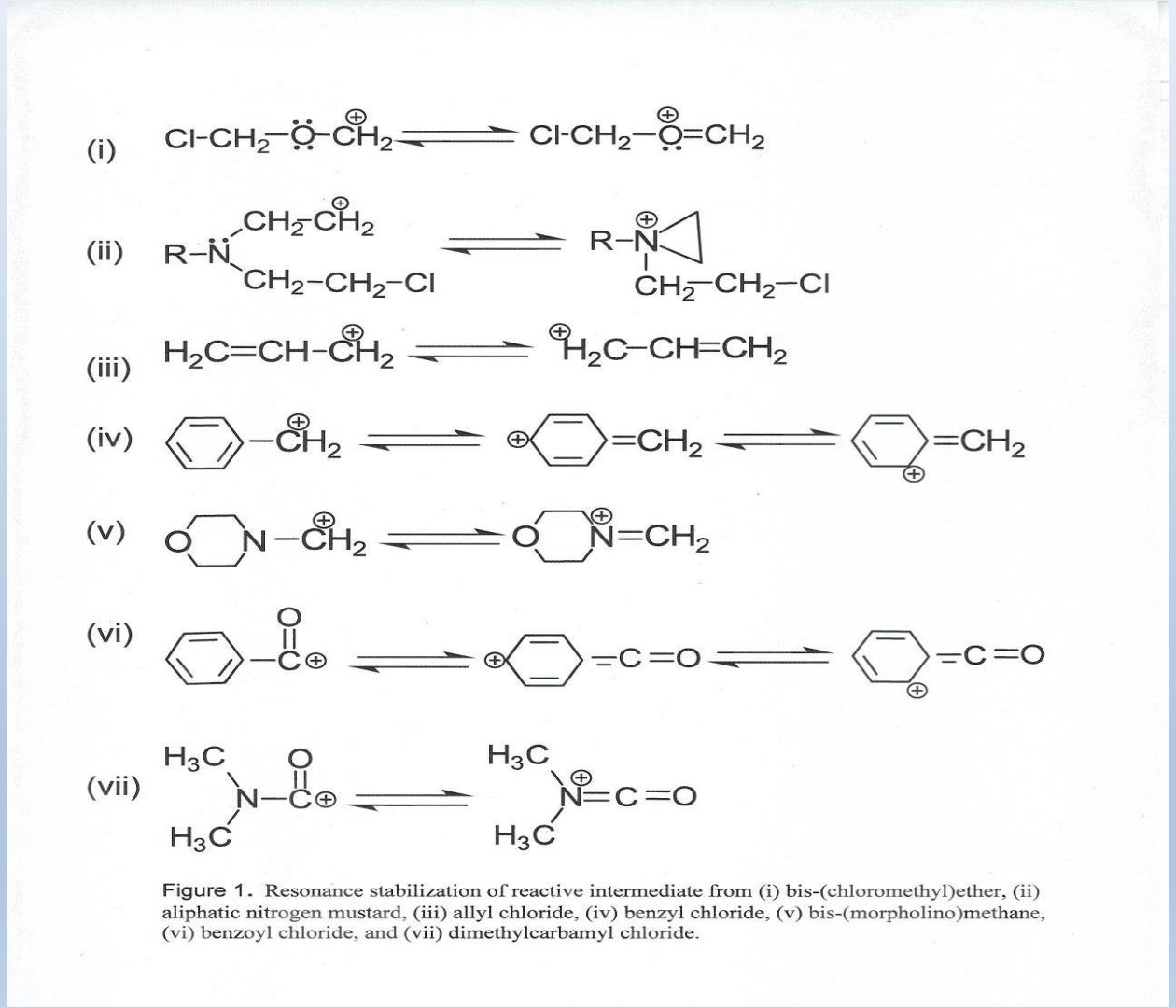
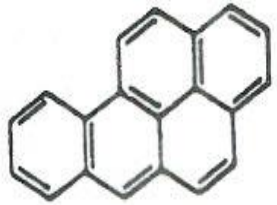
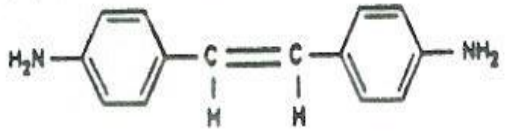
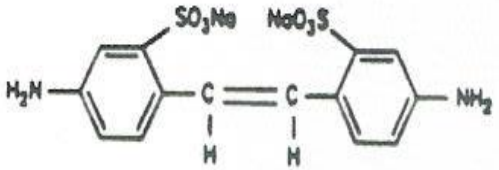
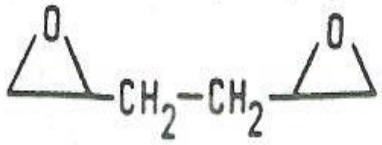
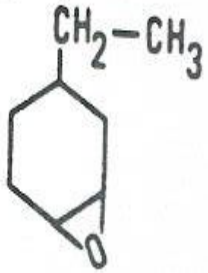


Figure 1. Resonance stabilization of reactive intermediate from (i) bis-(chloromethyl)ether, (ii) aliphatic nitrogen mustard, (iii) allyl chloride, (iv) benzyl chloride, (v) bis-(morpholino)methane, (vi) benzoyl chloride, and (vii) dimethylcarbamyl chloride.

TABLE 2. Molecular Parameters That Can Affect the Carcinogenicity of Chemicals

Carcinogenic	Noncarcinogenic
(a) Molecular size and shape	
 Benzo[a]pyrene	 Pentacene
(b) Substituent effect	
 4,4′-Diaminostilbene	 4,4′-Diamino-2,2′-stilbene-disulfonic acid, disodium salt
(c) Molecular flexibility and polyfunctionality of reactive groups	
 Diepoxyhexane	 3,4-Epoxyethylcyclohexane

Molecular Design of Chemicals of Low Carcinogenic Potential – General Approaches

Approaches	**Rationale**
1. Introduce bulky groups to increase the molecular size (*e.g.*, m.w. >1,000) or hydrophilic groups (*e.g.*, sulfonyl, carboxyl)	To reduce "bioavailability", *i.e*, its abililty to be absorbed, bioactivated and to reach target tissues and cells, and render it more polar so it cannot easily penetrate the lipid membrane of cells
2. Introduce substituent(s) adjacent to the electrophilic group	To exert electronic or steric effects on the bioactivation and reactivity/stability of ultimate electrophilic metabolites
3. For alkylating agents, substituting the alkyl group with group (s) higher than butyl	To decrease the alkylating potential
4. Positioning of all electrophilic functional groups in the middle of the molecule with none at any terminal position	To reduce molecular flexibility and accessibility of the electrophilic functional group(s) to interact with cellular nucleophiles such as DNA
5. For compounds which require metabolic activation, avoid structural features (*e.g.*, conjugated double bonds, conjugated system/aryl moiety)	Not to provide resonance stabilization of electrophilic metabolites to give the reactive intermediates a longer life time to remain reactive during transport from the site of activation to target macromolecules

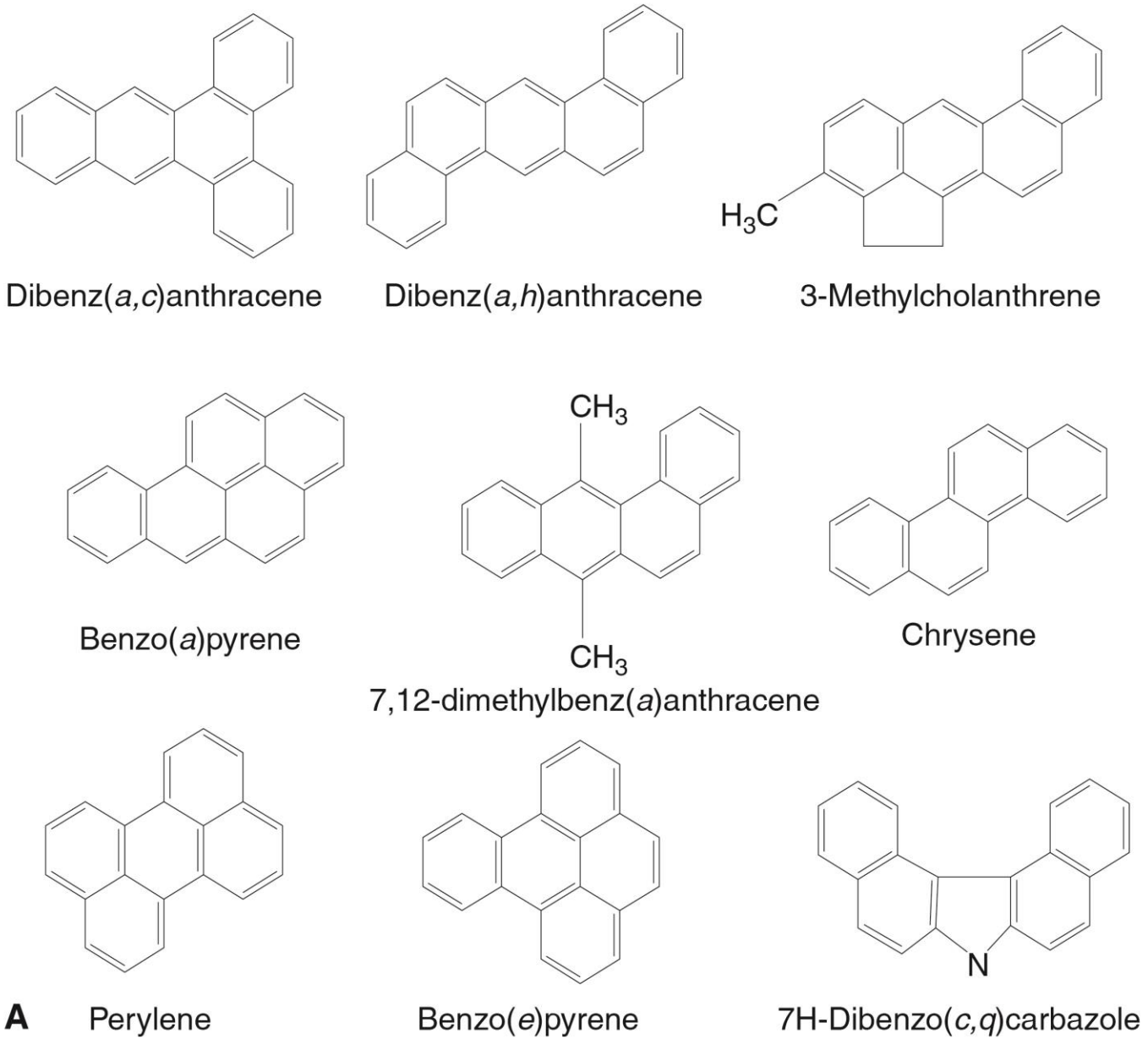


Figure 8-10. *(**A**), Chemical structures of selected carcinogenic polycyclic hydrocarbons. (**B**), Role of epoxide hydrolase in the inactivation of benzo[a]pyrene 4,5-oxide and in the conversion of benzo[a]pyrene to its tumorigenic Bay region diolepoxide.*

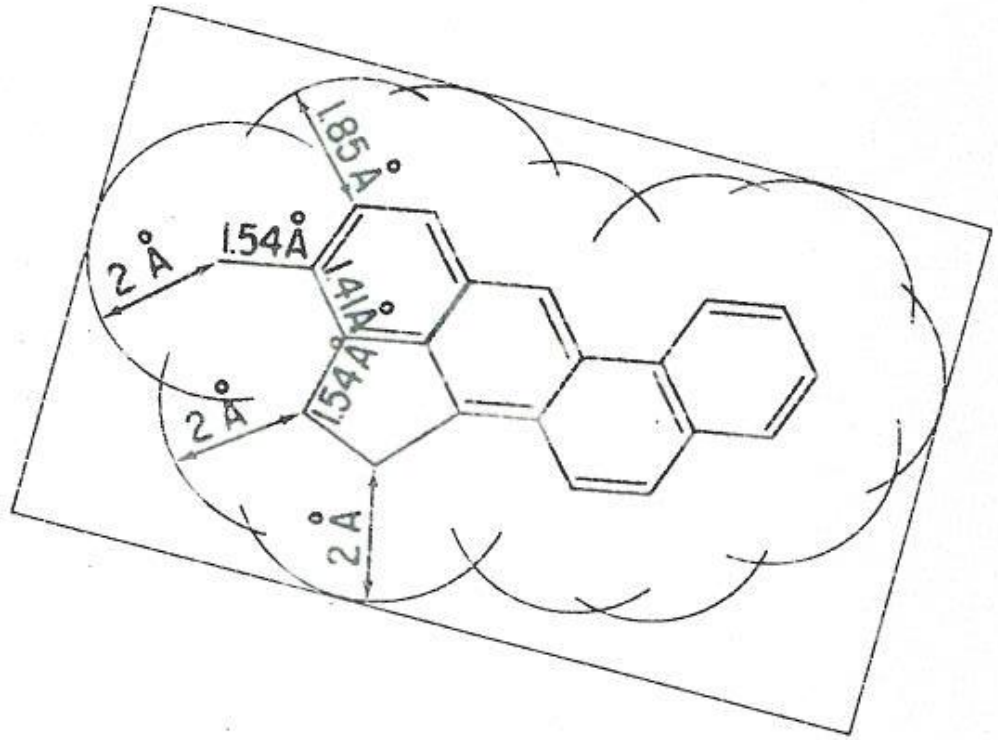


Fig. 34. Incumbrance area parallelogram of 20-methylcholanthrene. The values used for drawing the structure proportionally to molecular dimensions are shown.

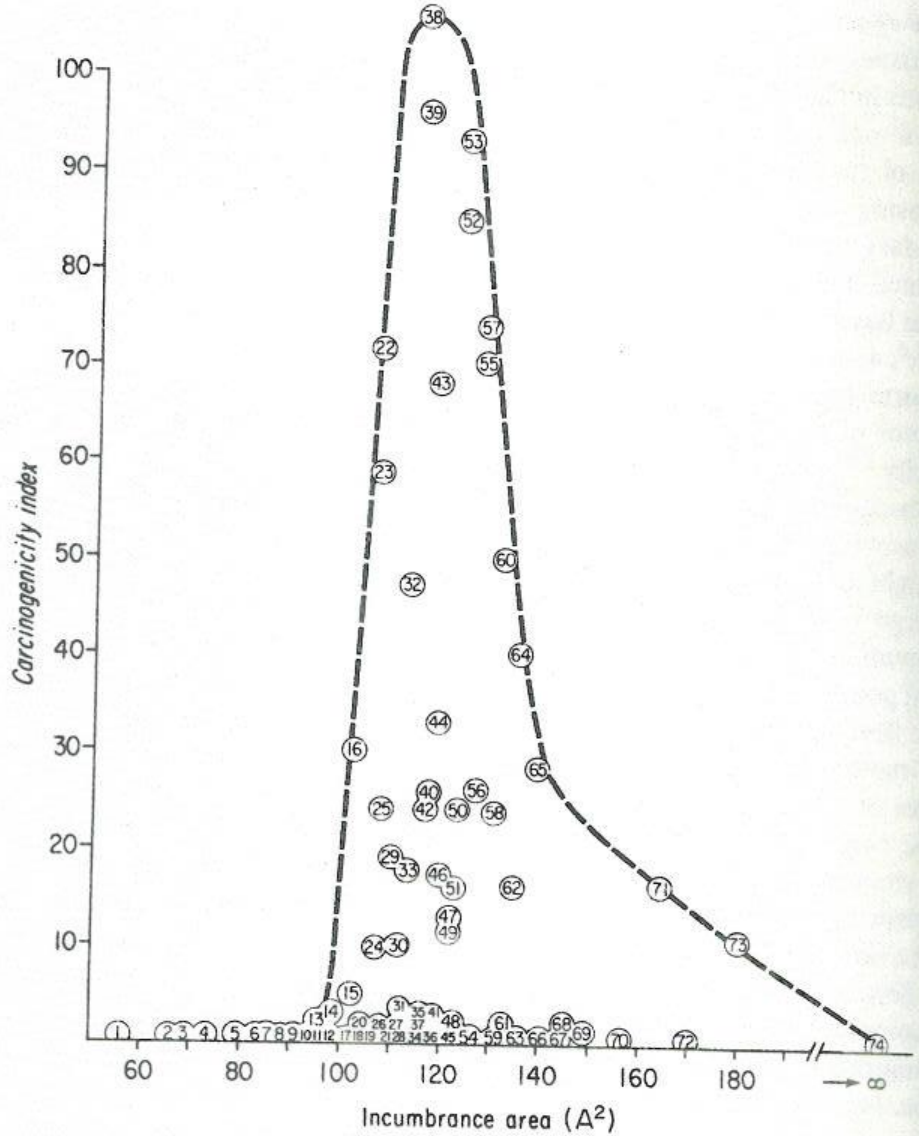


Fig. 39. The critical molecular size-range of condensed polycyclic aromatic hydrocarbons for carcinogenic activity. When available, the average of the sarcoma and epithelioma (+papil-

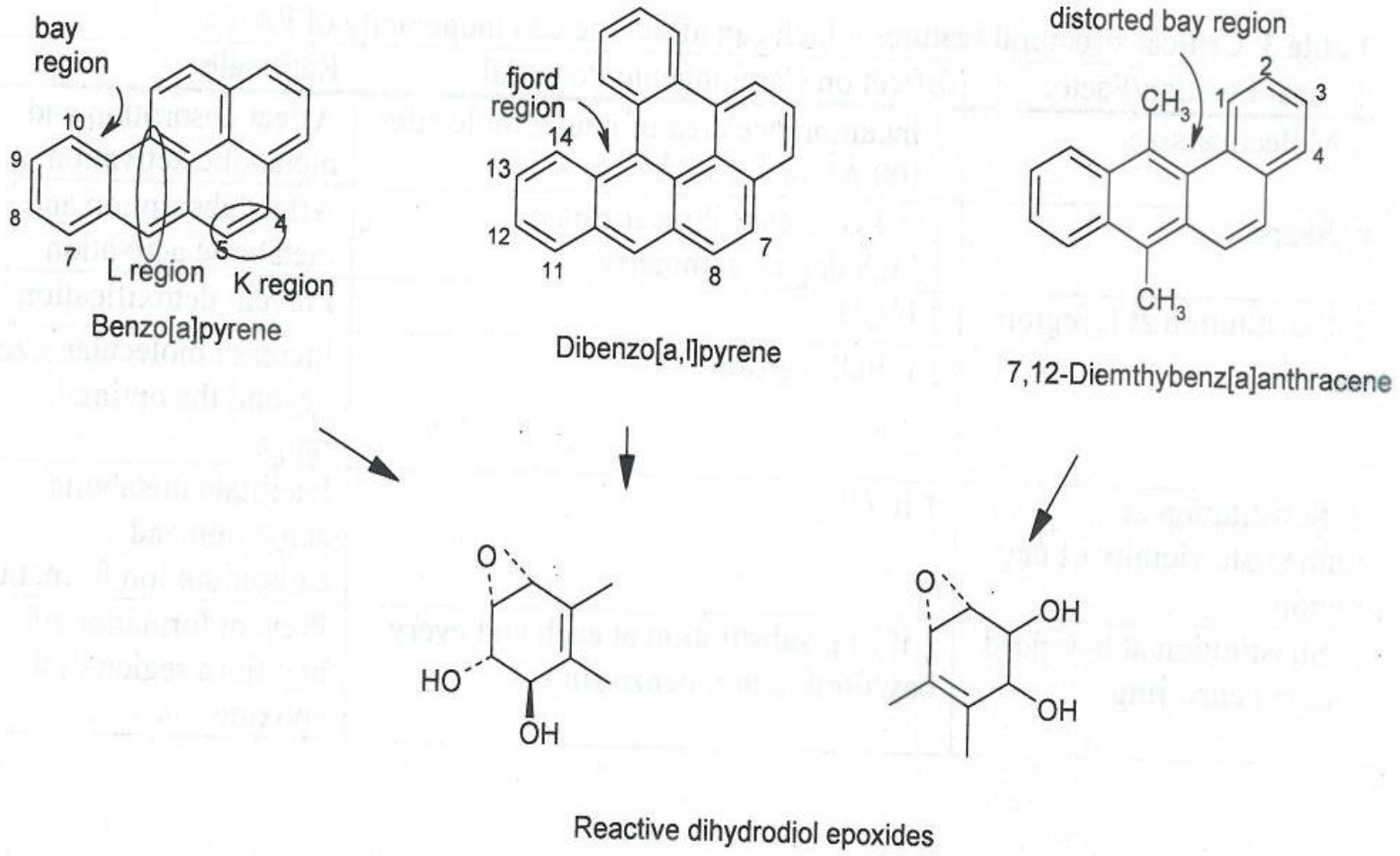


Figure 3. Potent PAH carcinogens with a bay/fjord region.

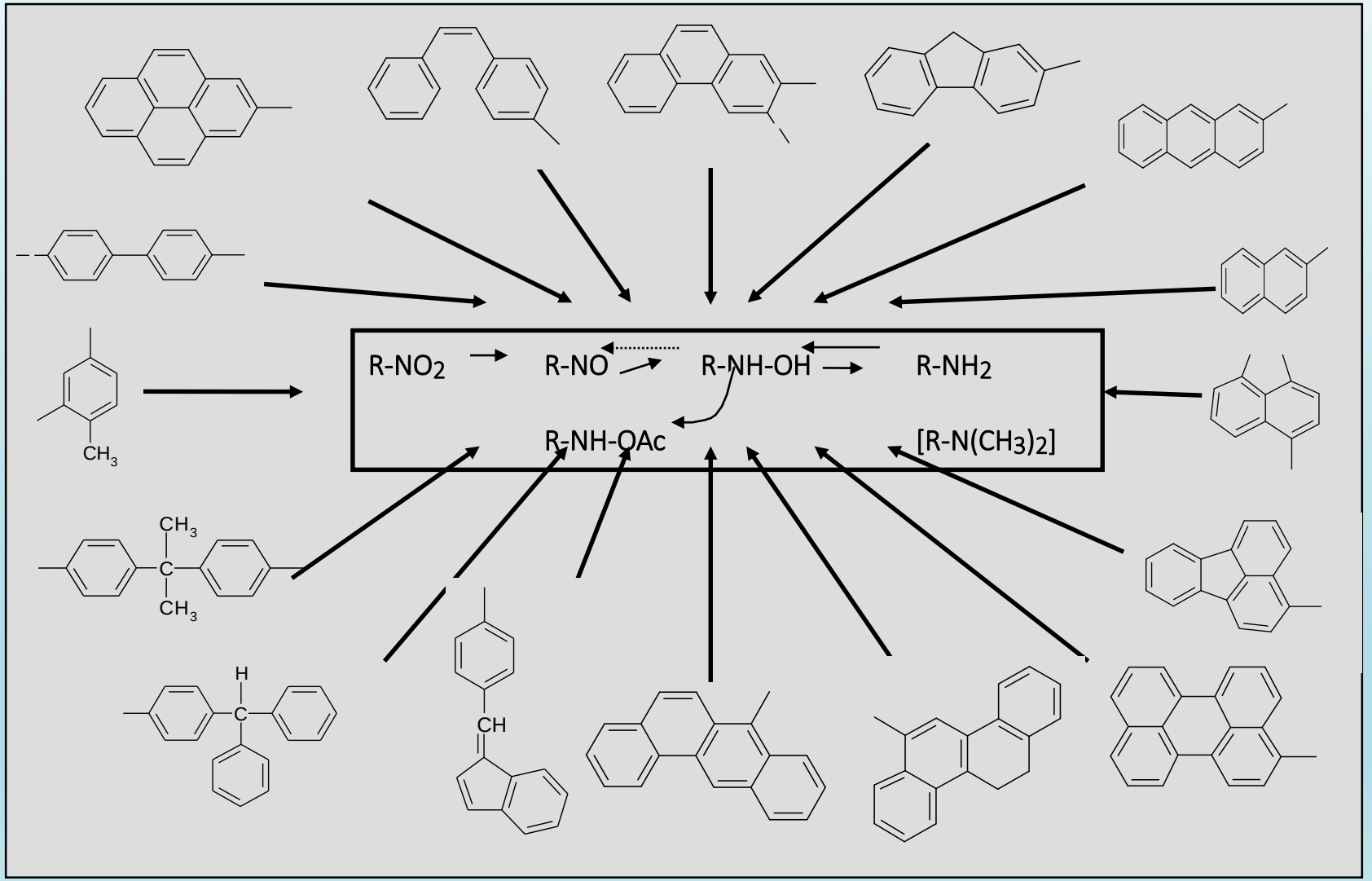
Critical Structural Features which can Affect the Carcinogenicity of PAHs

Critical Position/Factor	**Effect on Carcinogenic Potential**	**Rationale**
a. Molecular size	↓ incumbrance area of planar molecule <100 $Å^2$ or > 150 $Å^2$	Affect absorption and metabolic activation.
b. Shape	↓ > 4 condensed linear rings; ↓ high degree symmetry	Affect absorption and metabolic activation
c. Substitution at bay/fjord region benzo ring	↓ if ring substitution at each and every bay/fjord region benzo ring	Prevent formation of bay/fjord region diol epoxide
d. Substitution at L region	↑ if CH_3	Prevent detoxification
	↓ if bulky group	Increase molecular size beyond the optimal range

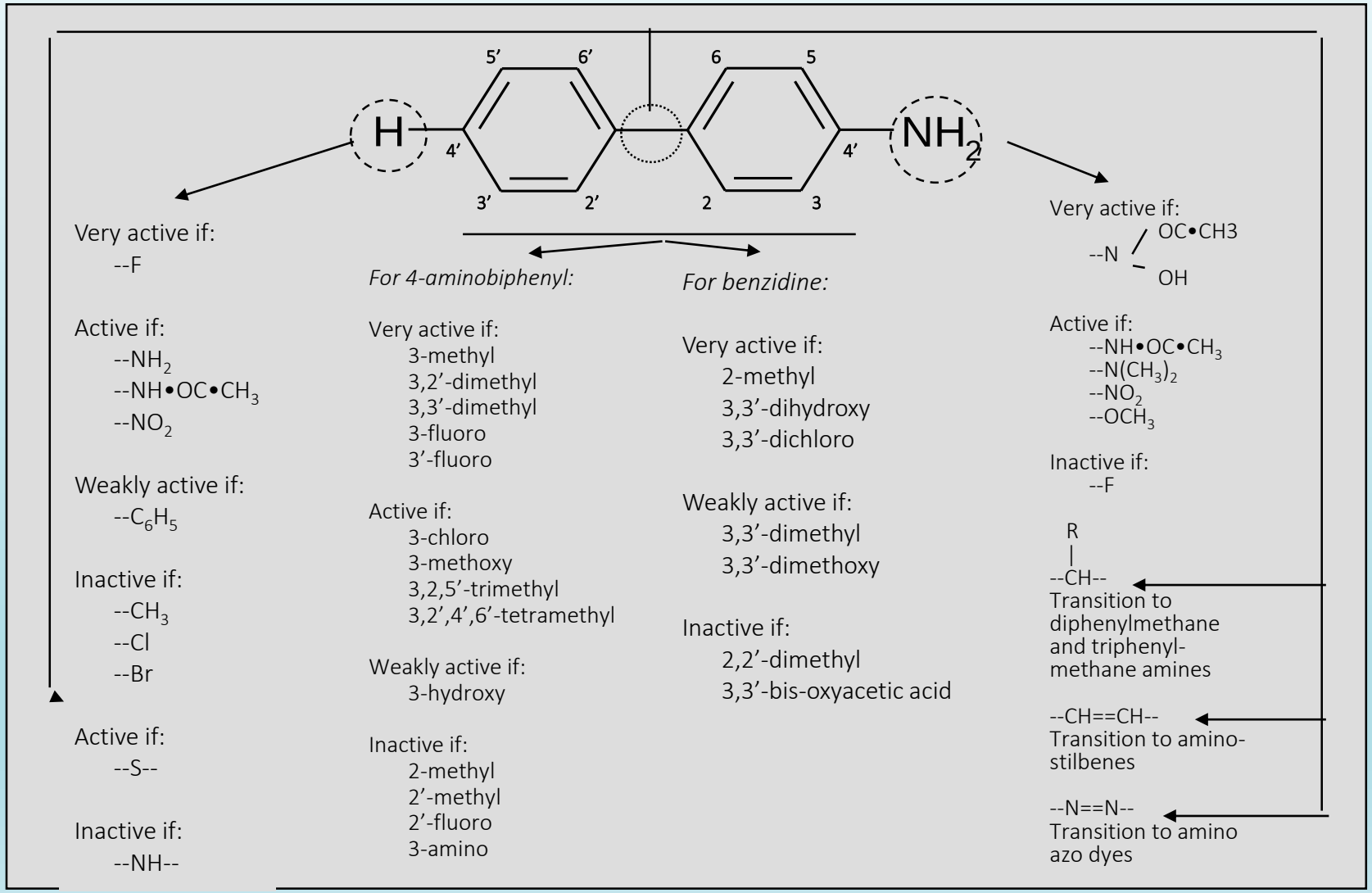
Molecular Design of Chemicals of Low Carcinogenic Potential - PAHs

Approaches	**Rationale**
1. Modify (a) the size of the incumbrance area of planar molecule to <100 $Å^2$ or > 150 $Å^2$, and (b) the shape to contain > 4 condensed linear rings.	Make the chemical less favorable for absorption, enzyme activation and DNA interaction.
2. Avoid compounds with a bay/fjord region. If a bay/fjord region is absolutely needed, add substituent(s) at each and every bay/fjord region benzo ring(s).	Since metabolic activation to bay/fjord ring diol epoxide is the key pathway for the carcinogenesis of PAHs.
3. Avoid methyl group or other small substituents at L-region	As substitutions at L-region will prevent detoxicification

Some Hydrocarbon Moieties Present in Carcinogenic Aromatic Amines



Synoptic Tabulation of Structural Requirements for Carcinogenic Activity of 4-Aminobiphenyl and Benzidine Derivatives



Very active if:
--F

Active if:
--NH_2
--NH•OC•CH_3
--NO_2

Weakly active if:
--C_6H_5

Inactive if:
--CH_3
--Cl
--Br

Active if:
--S--

Inactive if:
--NH--

For 4-aminobiphenyl:

Very active if:
3-methyl
3,2′-dimethyl
3,3′-dimethyl
3-fluoro
3′-fluoro

Active if:
3-chloro
3-methoxy
3,2,5′-trimethyl
3,2′,4′,6′-tetramethyl

Weakly active if:
3-hydroxy

Inactive if:
2-methyl
2′-methyl
2′-fluoro
3-amino

For benzidine:

Very active if:
2-methyl
3,3′-dihydroxy
3,3′-dichloro

Weakly active if:
3,3′-dimethyl
3,3′-dimethoxy

Inactive if:
2,2′-dimethyl
3,3′-bis-oxyacetic acid

Very active if:
--N(OC•CH3)(OH)

Active if:
--NH•OC•CH_3
--$N(CH_3)_2$
--NO_2
--OCH_3

Inactive if:
--F

R
|
--CH--
Transition to diphenylmethane and triphenyl-methane amines

--CH==CH--
Transition to amino-stilbenes

--N==N--
Transition to amino azo dyes

Factors Affecting Carcinogenicity of Aromatic Amines

- Number of aromatic ring(s)
- Molecular size, shape, planarity
- Number and position of amino or amine-generating groups(s) - position of amino group relative to longest resonance pathway - type of substituents on amino group
- Nature, number, position of other ring substituent(s) - steric hindrance - hydrophilicity
- Nature of aromatic ring(s) - homocyclic vs. heterocyclic - nature and position of heteroatoms

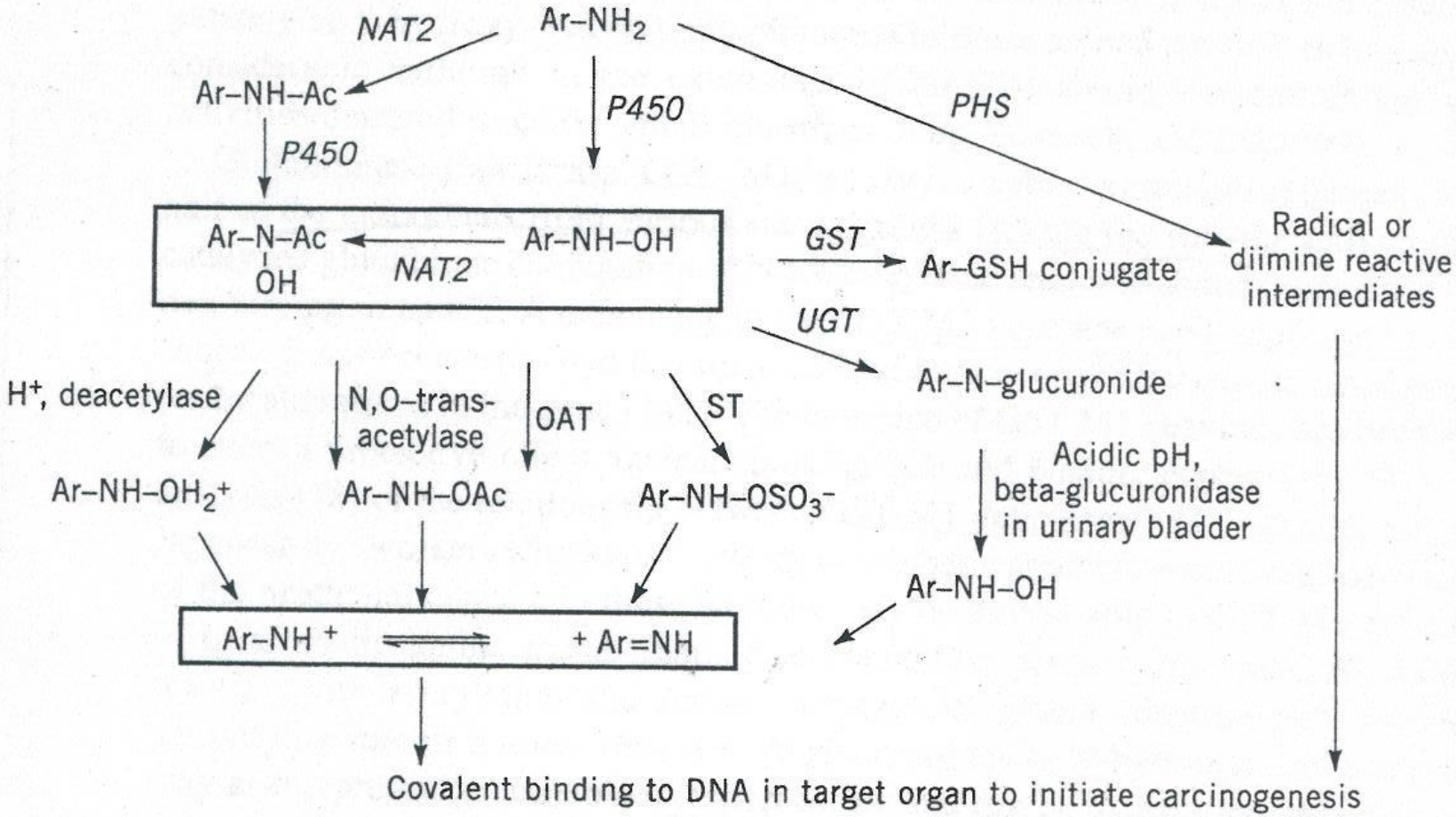
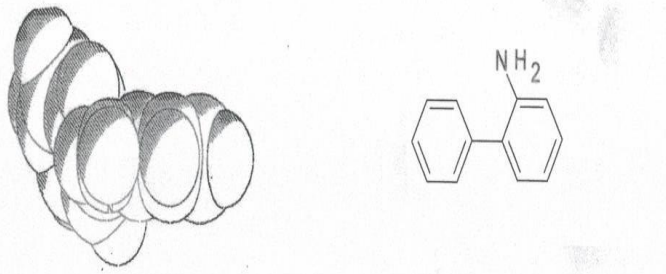
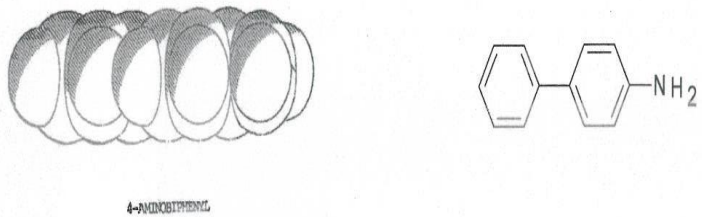


Figure 58.1. Metabolic pathways of typical aromatic amines. (abbreviations used: GST = glutathione *S*-transferase; Nat2 = *N*-acetyltransferase-2; OAT = *O*- Acetyltransferase; P450 = Cytochrome P450; PHS = prostaglandin H synthetase; ST = sulfotransferase; UGT = UDP glucuronyl transferase).



2-AMINOBIPHENYL

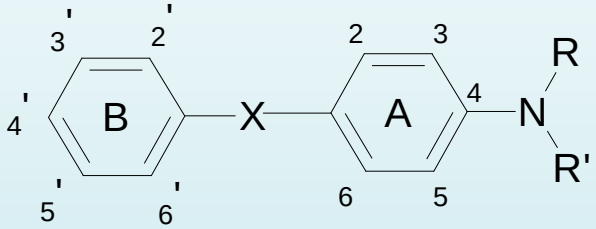
3-AMINOBIPHENYL



4-AMINOBIPHENYL

Fig. 4. Space-filled models of the isomeric forms of aminobiphenyl. Models were drawn using the PLUTO computer program and utilizing the following van der Waals radii to generate the computer graphical plots of the molecular geometry, carbon 1.6 Å, hydrogen 1.2 Å and nitrogen 1.5 Å.

Critical Structural Features which can Affect the Carcinogenicity of Aromatic Amines.



Critical Position/Factor	**Effect on Carcinogenic Potential**	**Rationale**
a. Substitution on the amino group	↑ if R/R'= H, CH_3	Enhance metabolic activation
	↓ if R/R' = *t*-alkyl or C>2	Steric/electronic hindrance of metabolic activation
b. Position of amino group	relative activity: 4- >> 3- ≥ 2-	Resonance stabilization and planarity
c. Substitution on 3- and 5- positions	↑ if one CH_3	Enhance metabolic activation.
	↓ if bulky group(s)	Hindrance or flanking effect
d. Intercyclic group (X)	↑ if —, —O—, —S—	Allows conjugation
	↓ if —(CH_2)n —, n>1	Electron insulating
e. Substitution on 2-, 2'-, and/or 6-, 6'-positions	↓ if bulky group	Distorts planarity
f. Substitution on 4'-position	↑ if -NH_2. ↑ if –F	Extended conjugation. blocks detoxification

Molecular Design of Chemicals of Low Carcinogenic Potential – Aromatic Amines

Approaches	**Rationale**
1. Introduce bulky N-substituent(s) to the amine/amine-generating group(s)	Make the dye a poor substrate for the bioactivation enzymes
2. Introduce bulky substituent(s) *ortho* to the amine/amine-generating group(s)	Provide steric hindrance to inhibit bioactivation
3. Introduce bulky groups *ortho* to the intercyclic linkages to distort the planarity of the molecule	Make it less accessible to the bioactivation enzymes
4. Alter the position of the amine/amine-generating group(s) in the aromatic ring(s)	To distort the planarity of the compound and reduce the force of conjugation and thus the resonance stability of the electrophilic nitrenium ion.
5. Replace electron-conducting intercyclic linkages by electron-insulating intercyclic linkages	To disrupt the conjugation path and thus reduce the force of conjugation which facilitate the departure of the electrophilic ion

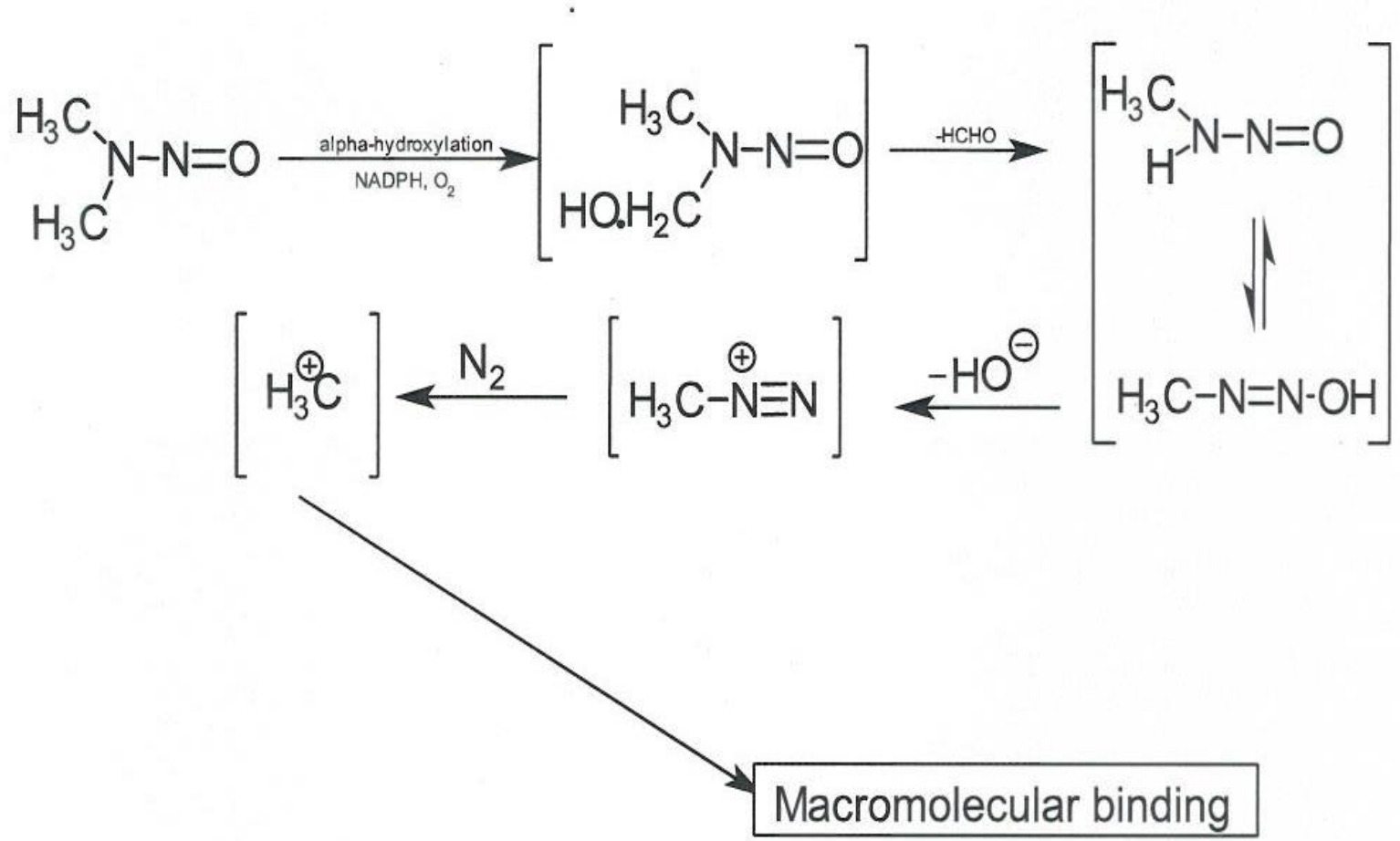
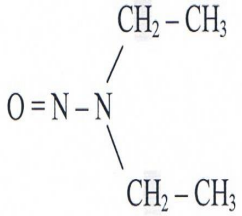


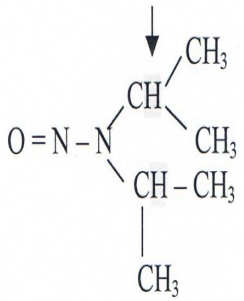
Figure 4. Bioactivation of N-Nitrosamines.

α-hydrogens

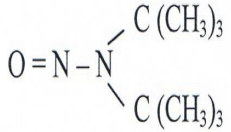


Diethylnitrosamine (DEN) : potent carcinogen

α-hydrogen



Di-*sec*-propylnitrosamine : weak carcinogen



Di-*tert*-butylnitrosamine : non-carcinogen.

Critical Structural Features which can Affect the Carcinogenicity of N-Nitrosamines

Critical Position/Factor	**Effect on Carcinogenic Potential**	**Rationale**
a. Substitution at the α-carbon	↓ if acidic group, fluoro group, bulky group(s)	Steric/electronic hindrance of metabolic activation
b. Substitution in the vicinity of the α-carbon	↓ if branching of alkyl groups/bulky substituents, or total number of C>15	Steric/electronic hindrance of metabolic activation

Molecular Design of Chemicals of Low Carcinogenic Potential – Nitrosamines

Approaches	**Rationale**
1. Not to include α-carbon in the molecule	No α-hydroxylation and bioactivation
2. Substitution in the vicinity of the α-carbon with branched alkyl group(s) or bulky substitutent(s)	Will hinder metabolic activation and reduce or abolish the carcinogenicity
3. Substitution at the α-carbon with acidic group(s), fluoro group(s), or bulky group(s)	Substitutents known to reduce/ eliminate carcinogenicity potential
4. Introduce large alkyl groups with a total of more than 15 carbons	Carcinogenicity decreases with the increase of carbon atoms

References

Lai, D. Y. and Woo, Y.-T (2012). Reducing Carcinogenicity and Mutagenicity Through Mechanism-based Molecular Design of Chemicals. In: "Handbook of Green Chemistry (P. T. Anastas, ed.), Volume 9: Designing Safer Chemicals." (R. Boethling & A. Voutchkova, eds.) , Chapter 14, Wiley-VCH. Pp.375-406.

Woo, Y.-T. and Lai, D. Y. (2012). Aromatic Amino and Nitro-amino Compounds and their Halogenated Derivatives. In: "*Patty's Industrial Hygiene and Toxicology*", Sixth edition, (E. Bingham, B. Cohrssen, C.H. Powell, eds.), Vol.2, Chapter 36, John Wiley & Sons, Inc. pp. 609-704.

Woo, Y.-T. and Lai, D.Y. (2010). QSAR Analysis of Genotoxic and Nongenotoxic Carcinogens: A State-of-the-Art Overview. In: "*Cancer Risk Assessment: Chemical Carcinogenesis: From Biology to Standards Quantification."* (G. Hsu and T. Stedeford, eds.), Chapter 20, Johns Wiley and Sons Ltd. pp.517-556.

Woo, Y.-T., and Lai, D.Y. (2003). Mechanism of Action of Chemical Carcinogens and their Role in Structure Activity Relationships (SAR) Analysis and Risk Assessment. In: *Quantitative Structure-Activity Relationship (QSAR) Models of Mutagens and Carcinogens.* R. Benigni, ed., CRC Press, Boca Raton, FL., pp. 41-80.

Lai, D. Y. and Woo, Y.-T. (2001). Structure-Activity Relationship (SAR) and Its Roles in Cancer Risk Assessment. *In*: "*Genetic Toxicology and Cancer Risk Assessment*" (W.N. Choy, ed.), Chapter 4, Marcel Dekker, Inc. Pp.73-91.

Lai, D.Y., Woo, Y,-T., Argus, M.F. and Arcos, J.C. (1996). Cancer Risk Reduction Through Mechanism-based Molecular Design of Chemicals. In:"*Designing Safer Chemicals*" (S. DeVito and R. Garrett, eds.), Chapter 3. American Chemical Society Symposium series No. 640, American Chemical Society Washington, DC.

Woo, Y.-T., Arcos, J.C. and Lai, D.Y. (1988). Metabolic and Chemical Activation of Carcinogens. An Overview. In:"*Chemical Carcinogens: Activation Mechanisms, Structural and Electronic Factors, and Reactivity*" (P. Politzer and L. Roberts, eds.),Elsevier, Amsterdam, Chapter 1, pp.1-31.

Lai, D.Y. and Arcos, J.C.(1980). Minireview: Dialkylnitrosamine Bioactivation and Carcinogenesis. *Life Sciences*, 27, 2149-2165.