جامعة الانبار

كلية : الصيدلة

قسم : فرع الادوية والسموم

اسم المادة باللغة العربية: السموم العامة

اسم المادة باللغة الإنكليزية: **General toxicology**

المرحلة: الرابعة

التدريسي: م.د. محمد مالك عبدالرحمن

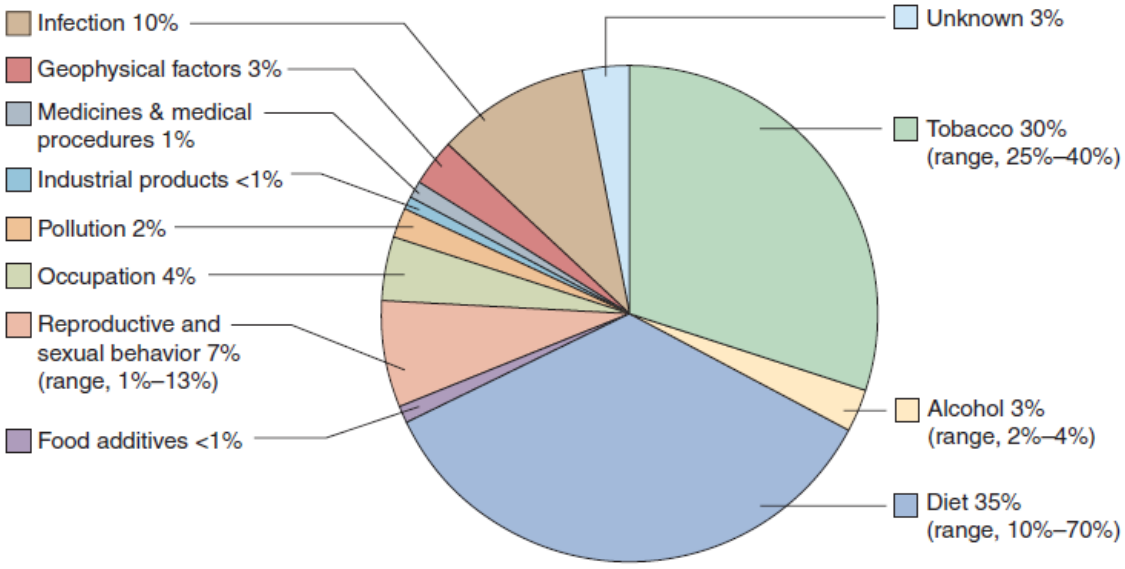
عنوان المحاضرة باللغة العربية: المسرطنات الكيميائية

عنوان المحاضرة باللغة الإنكليزية: **Chemical Carcinogenesis**

Chemical Carcinogenesis

Cancer is a disease characterized by mutation, modified gene expression, cell proliferation, and aberrant cell growth. Multiple causes of cancer have been established including infectious agents, radiation, and chemicals. Estimates suggest that 70% to 90% of all human cancers have a linkage to environmental, dietary, and behavioral factors (figure 1).

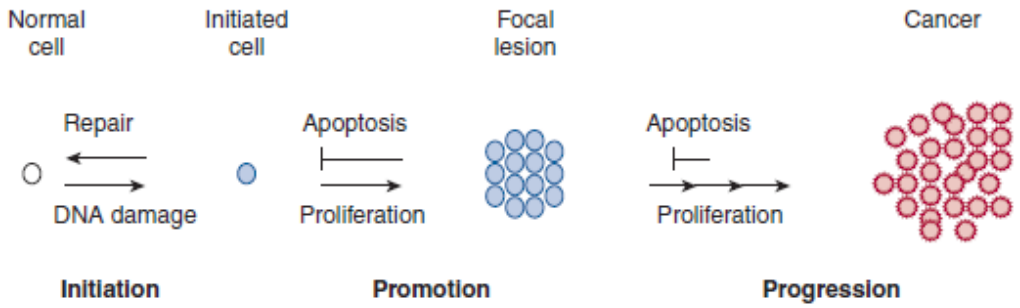
An understanding of the cellular and molecular aspects of the cancer process requires an understanding of the scientific terms involved in defining and describing the pathology of neoplasia
(Table1). Neoplasia is defined as new growth or autonomous growth of tissue. A neoplastic lesion is referred to as a neoplasm. A neoplasm can be either benign or malignant. Both types of lesions are induced by chemical carcinogens. Benign neoplasms (eg, adenomas) are lesions characterized by expansive growth, frequently exhibiting slow rates of proliferation that do not invade surrounding tissue or other organs. Benign neoplasms can impair and damage the normal function of an organ through its growth impeding of blood flow. A malignant neoplasm (eg, a carcinoma) demonstrates invasive growth characteristics, capable of spreading not only through the organ of origin but via metastasis to other organs. Metastases are secondary growths derived from the cells of the primary malignant neoplasm.



Terminology	
Neoplasia	New growth or autonomous growth of tissue
Neoplasm	The lesion resulting from the neoplasia
Benign	Lesions characterized by expansive growth, frequently exhibiting slow rates of proliferation that do not invade surrounding tissues
Malignant	Lesions demonstrating invasive growth, capable of metastases to other tissues and organs
Metastases	Secondary growths derived from a primary malignant neoplasm
Tumor	Lesion characterized by swelling or increase in size, may or may not be neoplastic
Cancer	Malignant neoplasm
Carcinogen	A physical or chemical agent that causes or induces neoplasia
Genotoxic	Carcinogens that interact with DNA resulting in mutation
Nongenotoxic	Carcinogens that modify gene expression but do not damage DNA

MULTISTAGE CARCINOGENESIS

Extensive experimental studies with animal models and pathological evaluation of human cancers, have demonstrated that the carcinogenesis process involves a series of definable stages and steps Operationally, three defined stages, initiation, promotion, and progression have been identified in rodent studies (initially in skin and liver tumors models) (Fig. 2).



Initiation

The first stage of the cancer process involves initiation, a process that is defined as a stable, heritable change. This stage is a rapid, irreversible process that results in a carcinogen-induced mutational event. Chemical and physical agents that function at this stage are referred to as initiators or initiating agents.

Initiating agents lead to genetic changes including mutations and deletions. Chemical carcinogens that covalently bind to DNA and form adducts that result in mutations are initiating agents.
Included among chemicals classified as initiating carcinogens are compounds such as polycyclic hydrocarbons and nitrosamines, biological agents, certain viruses, and physical agents such as X-rays and ultraviolet (UV) light. Most chemical carcinogens that function at the initiation stage of the cancer process are indirect-acting genotoxic compounds that require metabolic activation in the target cell to produce the DNA- damaging event.

Initiation by itself does not appear to be sufficient for neoplastic formation. Once initiated cells are formed, their fate has multiple potential outcomes: (1) the initiated cell can remain in a static non dividing state through influences by growth control either via normal surrounding cells or through endocrine influence; (2) the initiated cell may possess mutations incompatible with viability or normal function and be deleted through apoptotic mechanisms; or (3) the cell, through stimuli such as intrinsic factors or from chemical exposure, may undergo cell division resulting in the growth in the proliferation of the initiated cell.

Promotion

The second stage of the carcinogenesis process, derived from either endogenous or exogenous stimuli of cell growth, involves the selective clonal expansion of initiated cells to produce a preneoplastic lesion. This is referred to as the promotion stage of the carcinogenesis process. Both exogenous and endogenous agents that function at this stage are referred to as tumor promoters.

Tumor promoters are not mutagenic and generally are not able to induce tumors by themselves; rather they act though several mechanisms involving gene expression changes

that result in sustained cell proliferation either through increases in cell proliferation and/ or the inhibition of apoptosis.

The growth of preneoplastic lesions requires repeated applications or continuous exposure to tumor promoting compounds. Although initial exposure to tumor promoters may result in an increase in cell proliferation and/or DNA synthesis in all tissues of the organ, this is usually a transient effect on the normal cells and with repeated applications of the tumor promoter only the initiated cells continue to clonally expand and divide. In addition, these agents demonstrate a well-documented threshold for their effects—below a certain dose or frequency of application; tumor promoters are unable to induce cell proliferation. Multiple chemical compounds as well as physical agents have been linked to the tumor promotion stage of the cancer process. Tumor promoters in general show organ-specific effects, for example, a tumor promoter of the liver, such as phenobarbital, will not function as a tumor promoter in the skin or other tissues.

Progression

The final stage of the carcinogenesis process, progression, involves the conversion of the benign preneoplastic lesions into a neoplastic cancer. In this stage, due to increase in DNA synthesis in cell proliferation in the preneoplastic lesions, additional genotoxic events may occur resulting in additional DNA damage including chromosomal damage such as aberrations and translocations.

These events result in the transfer from preneoplastic, clonally derived cell populations into neoplastic cell populations. Cells accumulate a variety of mutations and epigenetic changes that cause them to outgrow the surrounding cells and to attract all of the necessary nutrients via angiogenesis.

By definition, the progression stage is an irreversible stage in that neoplasm formation, whether benign or malignant, occurs. Complete carcinogens have the ability to produce initiation, promotion and progression and hence by definition have genotoxic properties.

MECHANISMS OF ACTION OF CHEMICAL CARCINOGENS

Chemicals that induce cancer have been classified into one of two broad categories as genotoxic or DNA-reactive agents, and nongenotoxic or epigenetic agents.

Genotoxic/DNA-Reactive Compounds

Genotoxic compounds interact with nuclear DNA of a target cell producing unrepaired DNA damage that is inherited in subsequent daughter cells. DNA-reactive carcinogens can be further subdivided according to whether they are active in their parent form (ie, direct-acting: agents that can directly bind to DNA without being metabolized) and those that require metabolic activation (ie, indirect-acting carcinogens: compounds that require metabolism in order to react with DNA.

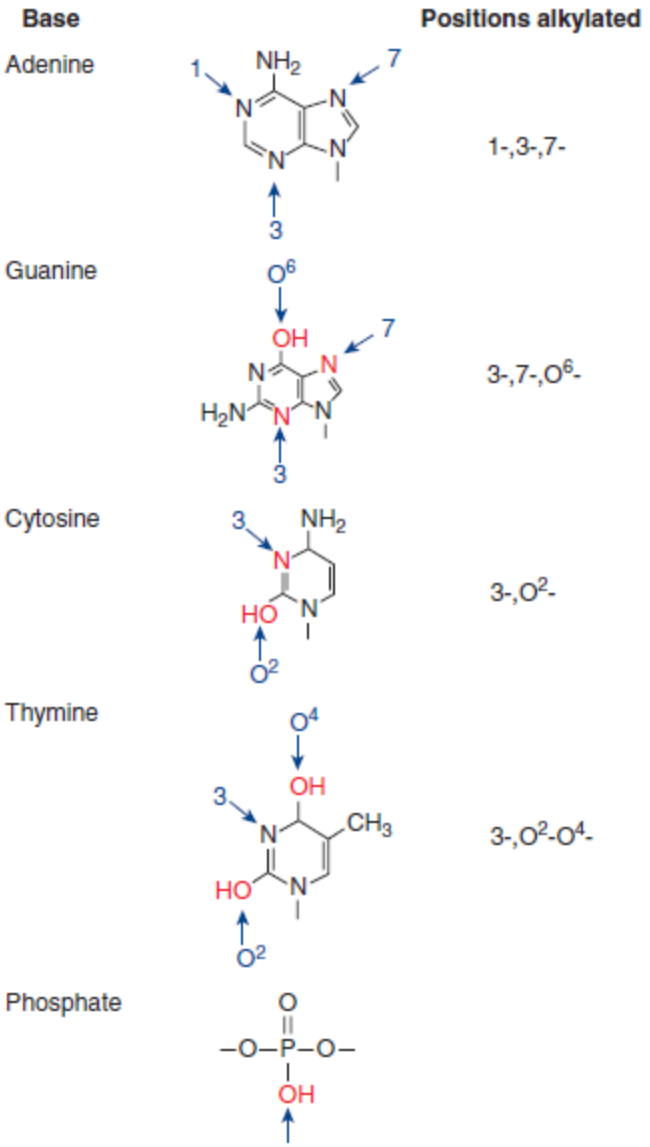
Damage by Alkylating Electrophiles

Most chemical carcinogens require metabolic activation to exert a carcinogenic effect. The ultimate carcinogenic forms of these chemicals are frequently strong electrophiles (Fig. 3 that can readily form covalent adducts with nucleophilic targets. Because of their unpaired electrons, S:, O:, and N: atoms are nucleophilic targets of many electrophiles.

In general, the stronger electrophiles display a greater range of nucleophilic targets (ie, they can attack weak and strong nucleophiles), whereas weak electrophiles are only capable of Alkylating strong nucleophiles (eg , S: atoms in amino acids).

An important and abundant source of nucleophiles is contained not only in the DNA bases, but also in the phosphodiester backbone. Although carcinogen–DNA adducts may be formed at all sites in DNA, the most common sites of alkylation include the N7 of guanine, the N3 of adenine, the N1 of adenine, the N3 of guanine, and the O6 of guanine. Alkylations of phosphate may also occur at a high frequency.

Dimethylnitrosamine and diethylnitrosamine, for example, are metabolized by P450 oxidation to yield a methyl carbonium ion (CH 3 +) and an ethyl carbonium ion (CH 3 CH 3 +), respectively.
Another common modifi cation to DNA is the hydroxylation of DNA bases. Oxidative DNA adducts have been identified in all four DNA bases ; however, 8-hydroxyguanine is among the most prevalent oxidative DNA adduct. The source of oxidative DNA damage is typically formed from free radical reactions that occur endogenously in the cell or from exogenous sources.



Classes of Genotoxic Carcinogens

Polyaromatic Hydrocarbons PAHs are found at high levels in charcoal-broiled foods, cigarette smoke, and in diesel exhaust. Benzo(a)pyrene is a representative polycyclic hydrocarbon that has been extensively studied. The ultimate carcinogen is a diol epoxide of benzo(a)pyrene, formed following three separate enzymatic reactions. Benzo [a] pyrene is fi rst oxidized by cytochrome P4501A1 to form benzo [a] pyrene 7,8-oxide, further metabolized by epoxide hydrolase, yielding the 7,8-dihydrodiol. And then further metabolism by cytochrome P4501A1 to yield the ultimate carcinogen, the 7,8-dihydrodiol-9,10-epoxide.

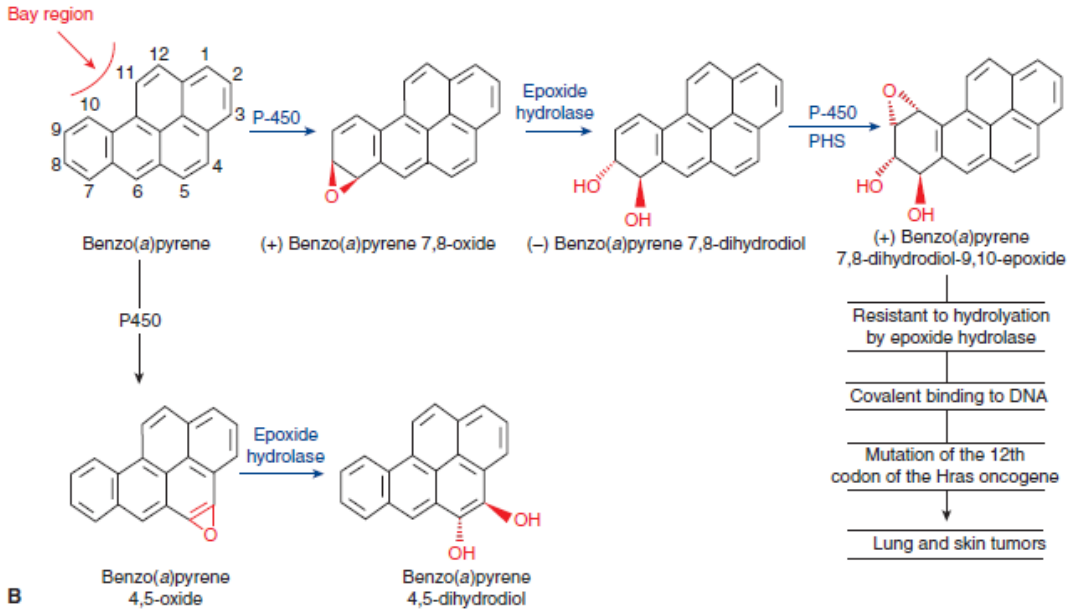
Alkylating Agents

Alkylating agents can be classified into several groups including the direct-acting alkylalkanesulfonates (methyl- and ethyl methanesulfonate) and nitrosamides (N - methyl- N -nitrosourea, N - ethyl-N -nitrosourea, N -methyl- N -nitro- N - nitrosoguanidine, and the indirect-acting nitrosamides (dimethyl- and diethylnitrosamines). The alkylating compounds (or in the case of diethylnitrosamine and dimethylnitrosamine and their metabolites) readily react with DNA at more than 12 sites. The N7 position of guanine and the N3 position of adenine are the most reactive sites for Alkylating chemicals to find to DNA.

Other alkylating chemicals including the nitrogen mustards (eg, chlorambucil, cyclophosphamide) have been used in cancer chemotherapy. They produce DNA adducts as well as induce the formation of DNA strand breaks. The alkylation of DNA by nitrogen mustards requires the formation of highly reactive metabolite.

Aromatic Amines and Amides

Aromatic amines and amides encompass a class of chemicals with varied structures (aromatic amines, eg, aniline dyes, 2- natphylamine, benzidine, 2-acetylaminofluofene).



Because of their use in the dye industry and other industrial processes their carcinogen risk to humans was realized in the late 19th century.
The aromatic amines undergo both phase I and phase II metabolism. Phase I reactions occur mainly by cytochrome P450-mediated reactions, yielding hydroxylated metabolites that

are often associated with adduct formation in proteins and DNA, and produce liver and bladder carcinogenicity.

Classes and Mode of Action of Nongenotoxic (Epigenetic) Carcinogens

A number of chemicals that produce tumors in experimental animals following chronic treatment appear to act via mechanisms not involving direct binding, damage, or interaction of the chemical or its metabolites with DNA. Examples include chemicals that function via sustained cytotoxicity, receptor-mediated (eg, CAR, peroxisome proliferator–activated receptor alpha [PPARα], aryl hydrocarbon receptor [AhR]) effects, hormonal perturbation, as well as the induction of oxidative stress and modulation of methylation status.

Cytotoxicity

Chloroform-induced liver and kidney tumors and melamine-induced bladder tumors are classic examples of chemical carcinogens that are classified as functioning via a cytolethal mode of action. Chemicals that function through this mechanism produce sustained cell death, often related to metabolism of the chemical, that is accompanied by persistent regenerative growth, resulting in the potential for the acquisition of "spontaneous" DNA mutations and allowing mutated cells to accumulate and proliferate.

α2u-Globulin-Binding Drugs The carcinogens D -limonene, 1,4-dichlorobenzene, and trimethylpentane induce renal tumors selectively in the male rat, and provide excellent examples of the species, sex, and tissue specificity of non–DNA-reactive carcinogens. The mechanism for the species and sex specificity is related to the ability of these compounds to bind to α2u-globulin, a protein synthesized by the male rat liver at the onset of puberty, as the mechanism of tumorigenesis. α2u-Globulin is, filtered through the glomerulus, and only partially excreted (50%) in the urine.

The reabsorbed fraction accumulates in lysosomes of the P2 segment of the proximal tubules, where it is hydrolyzed to amino acids. Chemicals with the ability to bind to α2uglobulin decrease the rate of digestion of α2u-globulin and results in the accumulation in the lysosomes, dysfunction of this organelle and subsequent release of digestive enzymes and cell necrosis. The greater loss of tubule cells leads to increased cell proliferation in the P2 segment, which may be responsible for the tumor development and malignant transformation.

Receptor-Mediated

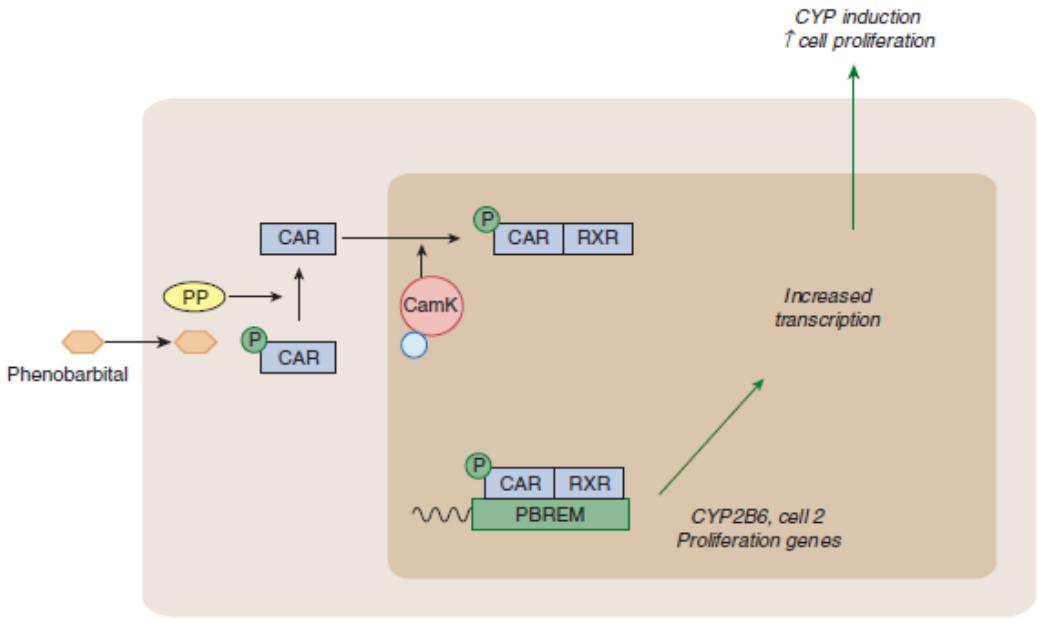
CAR Receptor-Mediated (Phenobarbital-Like Carcinogens)

Phenobarbital is a commonly studied non–DNA-reactive compound that is known to cause tumors by a nongenotoxic mechanism involving liver hyperplasia.
One feature seen following phenobarbital exposure is the induction of P450 enzymes, particularly Cyp2b. Recent evidence has shown that the induction of Cyp2b is mediated by

activation of the constitutive androstane receptor (CAR), a member of the nuclear receptor family.

Peroxisome Proliferator–Activated Receptor α A wide array of chemicals are capable of increasing the number and volume of peroxisomes in the cytoplasm of cells. These agents, termed peroxisome proliferators, include chemicals such as herbicides, chlorinated solvents (eg, trichloroethylene and perchloroethylene) plasticizers (eg, diethylhexylphthalate and other phthalates), lipid-lowering fibrate drugs (eg, ciprofi brate, clofi brate), and natural products. The currently accepted mode of action for this class of chemicals involves agonist binding to a nuclear hormone receptor, the PPARα. Largely through the use of PPARα-knockout mice, it has been demonstrated that the activation of PPARα by agonists is needed for these chemicals to induce peroxisome proliferation and tumorigenesis in rodents. PPARα is highly expressed in cells that have active fatty acid oxidation capacity (eg, hepatocytes, cardiomyocytes, enterocytes). It is well documented that PPARα plays a central role in lipid metabolism and acts as a transcription factor to modulate gene expression following ligand activation. This latter effect arises through the heterodimerization of PPARα and RXRα, which results in binding to response elements (PPREs) and subsequent modulation of target gene transcription. Following this event is the induction of cell proliferation and suppression of apoptosis.

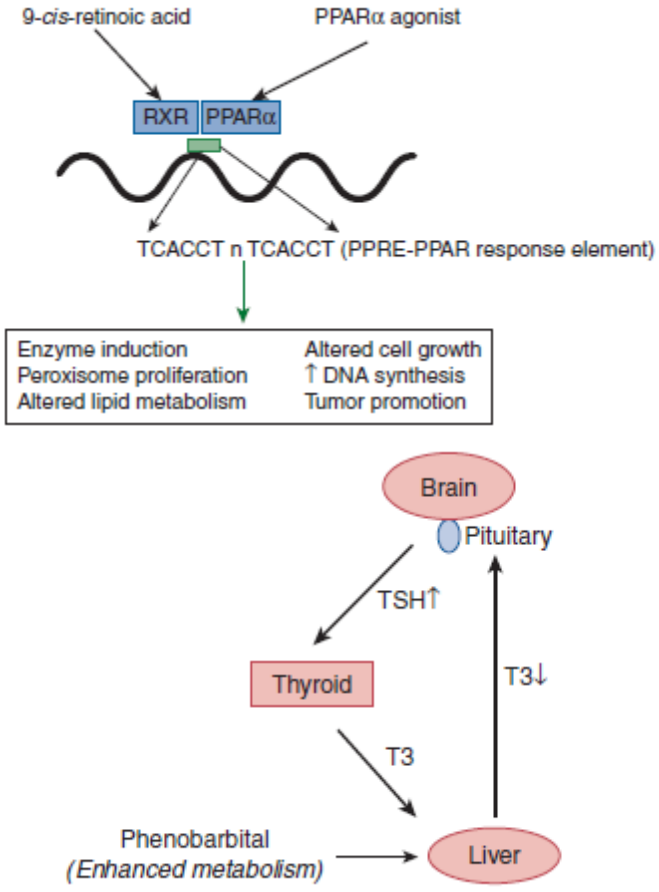
Although the same events would be expected to occur in exposed humans, several species differences have been noted including a lack of induction of cell proliferation in nonhuman primates, and the finding that PPARα in human liver is at least 10-fold lower compared with the rat or mouse. Based on these kinetic and dynamic differences between species, it has been concluded that tumors are not likely to occur in humans by PPARα agonists.



Hormonal Mode of Action Hormonally active chemicals include steroids and peptide hormones that produce tissue-specific changes through interaction with a receptor. A

number of non–DNA-reactive chemicals induce neoplasia through receptor mediated mechanisms, and/or perturbation of hormonal balance. Trophic hormones are known to induce cell proliferation at their target organs. Several well studied examples include the induction of ovarian neoplasms via decreased estradiol and increased luteinizing hormone (LH) levels, and the induction of thyroid tumors in rats by phenobarbital-type P450 inducers (which modulate T3 and T4). Estrogenic agents can induce tumors in estrogen-dependent tissue. Oral administration of 17α-estradiol to female mice increases the incidence of mammary tumors whereas subcutaneous administration of estradiol to young female mice produced tumors of the cervix and vagina. Species and tissue specificity in response to receptor- and hormone- mediated carcinogenesis is often observed. To further exemplify the complexity of the role of estrogen in cancer development, estrogens have also been shown to act as a protective agent in prostate cancer.

A reduction of thyroid hormone concentrations (T4 and/or T3) and increased thyroid-stimulating hormone (TSH) have been shown to induce neoplasia in the rodent thyroid. TSH increases proliferative activity in the thyroid. After chronic treatment chemical-induced TSH increases leading to follicular cell hypertrophy, hyperplasia, and eventually neoplasia. Inducers of metabolic enzymes in the liver, a classic and well-studied example being Phenobarbital, result in increased thyroid hormone metabolism and as such lead to increase in TSH levels.



DNA Methylation and Carcinogenesis Post-DNA synthetic methylation of the five position on cytosine (5-methylcytosine; 5mC) is a naturally occurring modification to DNA in higher eukaryotes that influences gene expression. Under normal conditions, DNA is methylated symmetrically on both strands. The degree of methylation within a gene inversely correlates with the expression of that gene; hypermethylation of genes is associated with gene silencing whereas hypomethylation results in an enhanced expression of genes. During carcinogenesis, both hypomethylation and hypermethylation of the genome have been observed. Choline and methionine, which can be derived from dietary sources, provide a source of methyl groups used in methylation reactions. Rats exposed to choline and/or methionine-deficient diets resulted in hepatocellular proliferation and neoplasia.

Oxidative Stress and Chemical Carcinogenesis Experimental evidence has shown that increases in reactive oxygen in the cell, through either physiological modification or chemical carcinogen exposure, contribute to the carcinogenesis processes. Reactive oxygen species encompass a series of reactive compounds including the superoxide anion

(O_2 -), hydroperoxyl radical (HO_2), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH), all derived through the reduction of molecular oxygen. Oxygen radicals can be produced by both endogenous and exogenous sources and are typically counterbalanced by antioxidants. Antioxidant defenses are both enzymatic (eg, superoxide dismutase, glutathione peroxidase, and catalase) and nonenzymatic (eg, vitamin E, vitamin C, β-carotene, and glutathione); importantly, many of these antioxidants are provided through dietary intake.

Endogenous sources of reactive oxygen species include oxidative phosphorylation, P450 metabolism, peroxisomes, and inflamatory cell activation. Within the mitochondria, a small percentage of oxygen is converted into the superoxide anion via 1-electron reduction of molecular oxygen. Superoxide can be dismutated by superoxide dismutase to yield hydrogen peroxide. In the presence of partially reduced metal ions hydrogen peroxide is converted into the highly reactive hydroxyl radical through Fenton and Haber–Weiss reactions. Neutrophils, eosinophils, and macrophages represent another intracellular source of reactive oxygen species. The release of cytokines and reactive oxygen intermediates from activated Kupffer cells (the resident macrophage of the liver) has been implicated in hepatotoxicological and hepatocarcinogenic events.

Pathways for Intercellular Oxidant Generation

Generation of Reactive Oxygen Species via Reduction of Molecular Oxygen

$O_2 + e^- \rightarrow O_2^{\bullet-}$ (superoxide anion)
$O_2^{\bullet-} + H_2O \rightarrow HO_2^{\bullet}$ (hydroperoxyl radical)
$HO_2^{\bullet} + e^- + H \rightarrow H_2O_2$ (hydrogen peroxide)
$H_2O_2 + e^- \rightarrow OH^- + {}^{\bullet}OH$ (hydroxyl radical)

A series of oxygen radicals are produced by the reduction of molecular oxygen. Of the radicals produced, the hydroxyl radical, hydroperoxyl radical, and the superoxide anion are sufficiently reactive and may interact with biomolecules.

Reactive Oxygen Species Generation and Removal in the Cell

Cellular oxidants

Endogenous	Exogenous
Mitochondria	Redox Cycling Compounds
$O_2^{\bullet-}$, H_2O_2, $^{\bullet}OH$	$O_2^{\bullet-}$
Cytochrome P450	Metals (Fenton Reaction)
$O_2^{\bullet-}$, H_2O_2	$H_2O_2 + Fe^{2+} \rightarrow OH^- + {}^{\bullet}OH + Fe^{3+}$
Macrophage/Inflammatory Cells	Radiation
$O_2^{\bullet-}$, $^{\bullet}NO$, H_2O_2, OCl^-	$^{\bullet}OH$
Peroxisomes	
H_2O_2	

Cellular antioxidants

Enzymatic	Non-Enzymatic
Superoxide Dismutase	Vitamin E
Catalase	Glutathione
Glutathione Peroxidase	Vitamin C
Glutaredoxin	Catechins
Thioredoxin	

Oxidants > Antioxidants → Oxidative Damage (DNA, RNA, Lipid, Protein)

Oxidants can be produced via both endogenous and exogenous sources. Antioxidants function to maintain the cellular redox balancing. However, excess production of oxidants and/or inadequate supplies of antioxidants result in damage to cellular biomolecules and may impact on neoplastic development.

CHEMICAL CARCINOGENESIS IN HUMANS

Recently, the International Agency for Research on Cancer (IARC) (Vol 100) has reported a review of chemically induced cancer in humans. This body has identified and classified

103 compounds as carcinogenic in humans.
Poor diets whether high-fat, low-protein, high-calories or diets lacking in needed antioxidants and minerals account for anywhere from 10% to 70% of human cancers. Diet contaminated by molds such as Aspergillus fl avis (which produces aflatoxin B1) have been linked epidemiologically to a higher incidence of liver cancer. It also appears that aflatoxin B1 exposure coupled with hepatitis B virus infection produces an increased incidence of liver cancer compared to afl atoxin B1 or hepatitis B exposure individually. In particular, high-fat and high-calorie diets have been linked to breast, colon, and gall bladder cancer in humans. Diets poor in antioxidants and/or vitamins such as vitamin A and vitamin E probably also contribute to the onset of cancer. The method of cooking may also influence the production of carcinogens produced in the cooking process. Carcinogenic HCAs and PAHs are formed during broiling and grilling of meat. Acrylamide, a suspected human carcinogen, has been found in fried foods at low concentrations. The linkage between occupational exposure to asbestos and the development of bronchiogenic carcinoma and as well as malignant mesothelioma has been clearlyestablished.
The appearance of bronchiogenic carcinoma was much higher in shipyard workers who were exposed to both asbestos and cigarette smoking. Prolonged high exposure to benzene in an occupational setting has been linked to the formation of acute myelogenous leukemia in humans.

A number of drugs and medical diagnostic tools have also been linked to the induction of human cancer. Anticancer drugs such as the alkylating agent cyclophosphamide have been associated with bladder tumors and leukemia in patients receiving these treatments. The use of oral contraceptives containing synthetic estrogens as their major or only component has been implicated in the induction of liver cell adenomas. Androgenic steroids and synthetic testosterone compounds have been implicated in hepatocellular carcinoma induction.

CLASSIFICATION EVALUATION OF CARCINOGENICITY IN HUMANS

IARC Classification of the Evaluation of Carcinogenicity for Human Beings

GROUP	EVIDENCE
1. Agent is carcinogenic to humans	Human data strong Animal data strong
2A. Agent is probably carcinogenic to humans	Human epidemiology data suggestive Animal data positive
2B. Agent is possibly carcinogenic to humans	Human epidemiology data weak Animal data positive
3. Agent is not classifiable as to carcinogenicity to humans	Human and animal data inadequate
4. Agent is probably not carcinogenic to humans	Human and animal data negative

USEPA Cancer Guidelines Descriptors

Carcinogenic to humans:
- strong evidence of human carcinogenicity, including convincing epidemiological evidence of a causal association between human exposure and cancer
- the mode(s) of carcinogenic action and associated key precursor events have been identified in animals, and there is strong evidence that the key precursor events in animals are anticipated to occur in humans

Likely to be carcinogenic to humans:
- weight of the evidence is adequate to demonstrate carcinogenic potential to an agent in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans

Suggestive evidence of carcinogenic potential:
- the weight of evidence is suggestive of carcinogenicity; a concern for potential carcinogenic effects in humans is raised, but the data are judged not sufficient for a stronger conclusion

Inadequate information to assess carcinogenic potential:
- available data are judged inadequate for applying one of the other descriptors

Not likely to be carcinogenic to humans:
- this descriptor is appropriate when the available data are considered robust, there is no basis for human hazard concern, evidence in both humans and animals that the agent is not carcinogenic