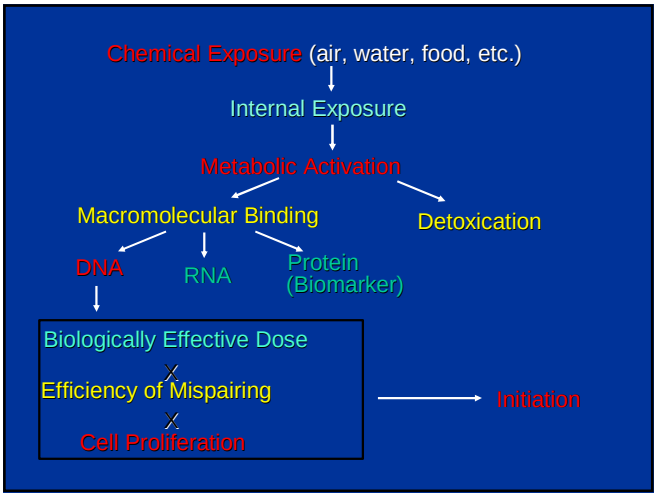
Environmental factors

- **CHEMICALS**
- **RADIATION**
- **NATURAL TOXINS**
- **VIRUSES**



What factors influence the development of cancer?

- Dose--amount and length of exposure. The lower the dose the least likely you are to develop cancer or related diseases.
- Environmental or "lifestyle" factors.
  - Cigarette smoking (co-carcinogen)
  - Alcohol consumption (co-carcinogen)
  - Diet--high fat consumption, natural antioxidants
  - Geographic location--industrial areas, UV light
  - Therapeutic drugs--some are known carcinogens
  - Inherited conditions

Human carcinogens - environmental

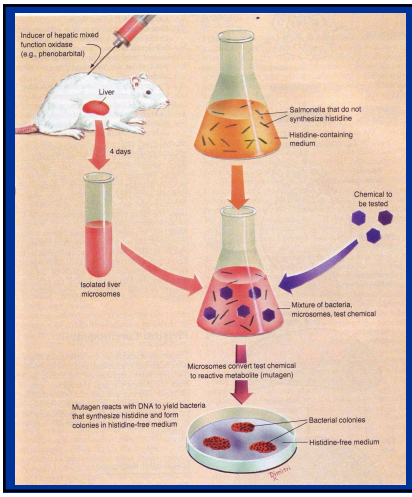
- Aflatoxins
- Asbestos
- Benzene
- Cadmium
- Coal tar
- Creosote
- DDT
- Polycyclic aromatic hydrocarbons
- Radon
- Solar radiation

Human carcinogens - drugs/therapeutic agents

- Adriamycin (doxorubicin)
- Androgenic steroids
- Chlorambucil
- Cisplatin
- Cyclophosphamide
- Cyclosporin A
- Diethylstilbestrol
- Ethylene oxide
- Melphalan
- Tamoxifen

How is chemical carcinogenicity determined?

- Epidemiological studies determine the relationship between a cancer suspect chemical and a human population over a long period of time.
- Animal studies directly induce cancer in test animals using a large sample of animals, usually of two or more species with varying dose and time parameters.
- Experiments with animals are based on the premise that chemicals that produce cancer in animals will have similar effects on human cells. Most known human carcinogens produce cancer in experimental animals.



Ames' test to detect the mutagenic property of a chemicals

CHEMICAL CARCINOGENESIS

Specific carcinogenic agents implicated in the causation of certain cancers[c]

Cancer	Exposure
Scrotal carcinomas	chimney smoke condensates
Liver angiosarcoma	vinyl chloride
Acute leukemias	benzene
Nasal adenocarcinoma	hardwood dust
Osteosarcoma	radium
Skin carcinoma	arsenic
Mesothelioma	asbestos
Vaginal carcinoma	diethylstilbestrol
Oral carcinoma	snuff

Which classes of chemicals tend to be carcinogens?

- Epoxides:
  - Ethylene oxide
  - Propylene oxide
- Organohalogen comp.:
  - Vinyl chloride
  - Carbon tetrachloride
  - Chloroform
  - Hexachlorobenzene
  - Trichloroethylene
- Hydrazines:
  - Hydrazine (and salts)
  - 1,2-Dimethylhydrazine
- N-Nitroso compounds:
  - N-Nitrosodimethylamine
- Aromatic Amines:
  - Benzidine
  - Aniline
  - o-Anisidine
  - o-Toluidine
- Aromatic hydrocarbons:
  - Benzene
  - Benz[a]anthracene
  - Benzo[a]pyrene

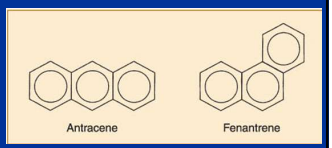
Classes of carcinogens (cont.)

- Misc. organic compounds:
  - Formaldehyde
  - Acetaldehyde
  - 1,4-Dioxane
  - Ethyl carbamate
  - 2-Nitropropane
  - Styrene
  - Thiourea
  - Thioacetamide
- Misc. inorganic comp.:
  - Arsenic and compounds
  - Chromium and comp.
  - Thorium dioxide
  - Beryllium and compounds
  - Cadmium and compounds
  - Lead and compounds
  - Nickel and compounds
  - Selenium sulfide

CHEMICAL CARCINOGENESIS

In 1930 Sir Ernest Kennaway and collaborators obtained 7 gr of powder by distilling 2 tons of tar: the powder was proven to contain Polycyclic Aromatic Hydrocarbons (PAH), of which 3,4-benzopirene was the most prominent.

In 1930, Saraki e Yoshida, showed that feeding rats with the dye o-amino-azotoluene could cause cancer in the LIVER, not in the intestine.



Thus, cancer developed in an organ different and distant from the organ of absorption ! This observation indicates that some (pro-)carcinogen need to be metaboliized in specific organs to become active.

In 1969 Elizabeth e James Miller suggested that in order to cause a mutation, the CHEMICAL CARCINOGEN must form an adduct with the Nitrous Base, and to this end it must possess electrophilic properties.

Electrophilic molecules can easily bind to electron-rich macromolecules (containing numerous N) such as Nucleic Acids and Proteins.

Some carcinogens intrinsically possess the electrophilic property, others need to be transformed (activated) by the DMS to acquire an electrophilic group (such as the expoxide).

Thus CHEMICAL CARCINOGENS are classified as DIRECT or INDIRECT (or PRO-Carcinogen).

An adduct to the Nucleic Base can also be formed through covalent binding of an 'alkyl group' = alkylation.

Table 7–8. MAJOR CHEMICAL CARCINOGENS

DIRECT-ACTING CARCINOGENS

Alkylating Agents
- Beta-propiolactone
- Dimethyl sulfate
- Diepoxybutane
- Anticancer drugs (cyclophosphamide, chlorambucil, nitrosoureas, and others)

Acylating Agents
- 1-Acetyl-imidazole
- Dimethylcarbamyl chloride

PROCARCINOGENS THAT REQUIRE METABOLIC ACTIVATION

Polycyclic and Heterocyclic Aromatic Hydrocarbons
- Benz(a)anthracene
- Benzo(a)pyrene
- Dibenz(a,h)anthracene
- 3-Methylcholanthrene
- 7,12-Dimethylbenz(a)anthracene

Aromatic Amines, Amides, Azo Dyes
- 2-Naphthylamine (beta-naphthylamine)
- Benzidine
- 2-Acetylaminofluorene
- Dimethylaminoazobenzene (butter yellow)

Natural Plant and Microbial Products
- Aflatoxin B_1
- Griseofulvin
- Cycasin
- Safrole
- Betel nuts

Others
- Nitrosamine and amides
- Vinyl chloride, nickel, chromium
- Insecticides, fungicides
- Polychlorinated biphenyls

DIRECT ACTING CARCINOGENS

- Direct-acting agents require no metabolic conversion to become carcinogenic. have highly reactive eletrophile groups that directly damage DNA, leading to mutations and eventually cancer.
- Examples are : cancer chemotherapeutic drugs (e.g., alkylating agents)

  used as Rx of e.g., leukemia, lymphoma, Hodgkin lymphoma, and ovarian carcinoma, non-neoplastic disorders, such as rheumatoid arthritis or Wegener granulomatosis.
- may evoke later a second form of cancer, usually leukemia.

INDIRECT-ACTING CARCINOGEN

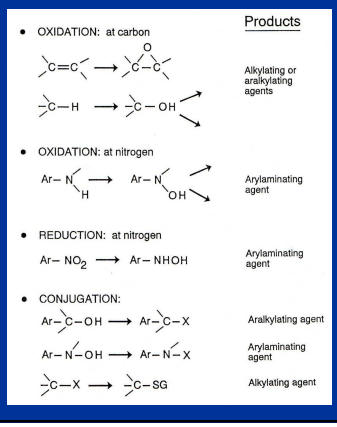
- The designation *indirect-acting agent* refers to chemicals that require metabolic activation & conversion to an *ultimate carcinogen* before they become active
- indirect-acting agents are not active until converted to an ultimate carcinogen by endogenous metabolic pathways e.g.. endogenous enzymes like cytochrome P-450 oxygenase.
- Examples :

  Benzopyrene,Polycyclichydrocarbons, aromatic amines and Azo dyes , Aflatoxin B1, insecticides, fungicides, nitrites used as food preservatives

Mechanisms of Action of Chemical Carcinogens

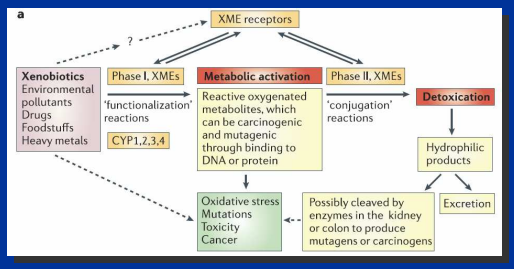
- Initiation
- Promotion
- the application of an initiator may cause the mutational activation of an oncogene such as *RAS*
- subsequent application of promoters leads to clonal expansion of initiated (mutated) cells.
- Forced to proliferate, the initiated clone of cells accumulates additional mutations, developing eventually into a malignant tumor.

Some modifications of pro-carcinogens leading to the formation of electrophilic groups



Induction of Metabolizing Enzymes

- May be a reason for tissue-, and/or species-selectivity of carcinogens
- Metabolites may be ligands for receptors
- Production of reactive oxygen species



Nebert & Dalton **Nat Rev Cancer** 2006

ACTIVATION

↓

FUNCTIONALIZATION
(epoxide formation)

↓

CONJUGATION (Glucuronic Acid; Acetyl group)

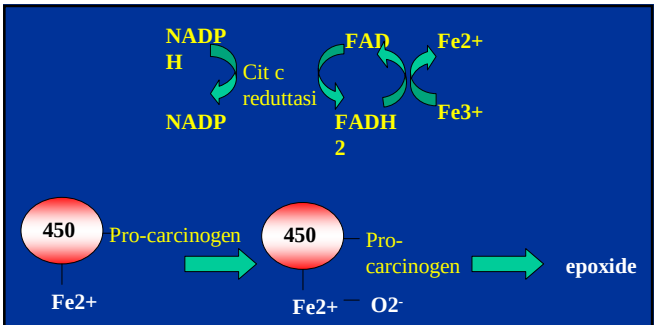
↓

INACTIVATION (e.g. by EPOXIDE hydrolase

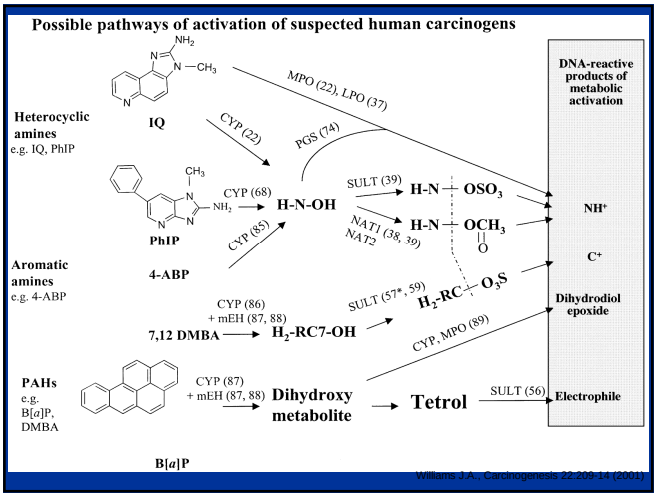
(in distant organ)

↓

RE-ACTIVATION
(in distant organ)

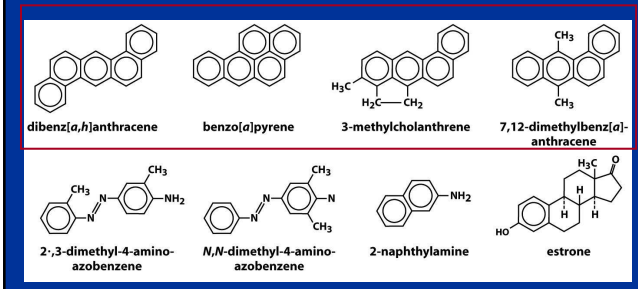


FUNCIONALIZATION



Carcinogenic hydrocarbons

PAH



Chemical carcinogens: chemicals that cause tumor formation

1. have a very broad range of structures with no obvious unifying features
2. are genotoxic and can be classified into two broad categories based on their action mechanisms:

   a. Direct-acting carcinogens
   - react with nitrogen and/or oxygen atoms in DNA
   example: ethylmethane sulfonate (EMS)

   b. Indirect-acting carcinogens
   - become reactive after metabolic activation
   examples: aflatoxin, benzo[a]pyrene

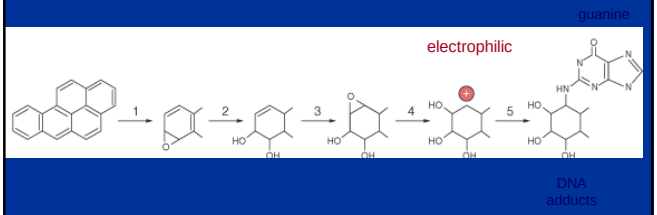
*genotoxic: an agent or process that interacts with cellular DNA, resulting in alteration of DNA structure

The direct-acting carcinogens interact with macromolecules through the covalent bond formation between an electrophilic form of the carcinogen and the nucleophilic sites in proteins (e.g., S, O, and N atoms in cysteine, tyrosine, and histidine, respectively) and nucleic acids (e.g., N and O atoms in purine or pyrimidine), such as *N*-methyl-*N*-nitrosourea, a chemically-reactive alkylating agent.

Some agents can intercalate (嵌入) into the DNA double helix by forming tight noncovalent bonds (e.g., daunorubicin).

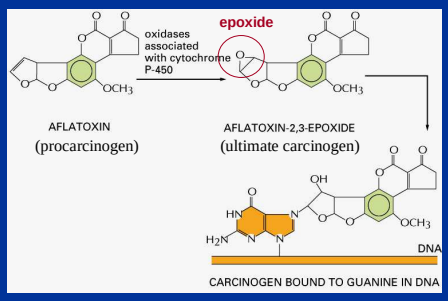
Most of carcinogens are indirectly-acting; they do not interact *in vitro* with macro-molecules until it has been incubated with liver homogenates or liver microsomal fractions. Thus, metabolic activation of certain carcinogenic agents is necessary to produce the "ultimate carcinogen" that actually reacts with crucial molecules in target cells.

Metabolic activation of benzo[a]pyrene



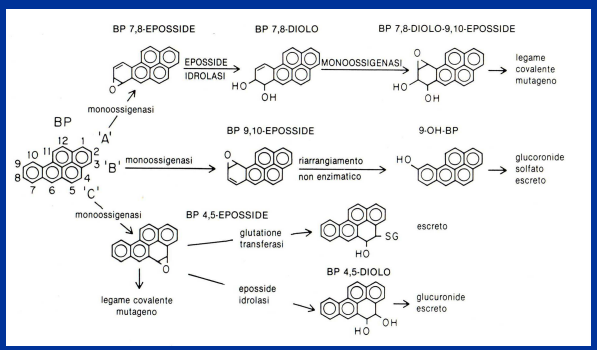
1. Cytochrome P450 catalyses initial epoxidation.
2. With the exception of the 1 - 2 and 2 - 3 oxides that convert to phenols, epoxide hydrolase may catalyze the formation of dihydrodiols.
3. Benzo[*a*]pyrene-7, 8-dihydrodiol is further metabolized at the olefinic double bond by cytochrome P450 to form a vicinal diol-epoxide (r7, t8-dihydroxy-c9, 10 epoxy-7,8,9,10-tetrahydroxybenzo[*a*]pyrene).
4. The highly unstable arene ring opens spontaneously to form a carbocation.
5. This electrophilic species forms a covalent bond between the 10 position of the hydrocarbon and the exocyclic amino group of deoxyguanosine.

Metabolic activation of aflatoxin



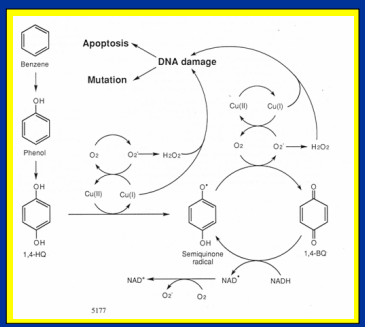
Aflatoxin B1, a toxin from a mold *(Aspergillus flavus oryzae)* that grows on grain and peanuts when they are stored under humid tropical conditions. It is thought to be a contributory cause of liver cancer in the tropics.

Indirect CARCINOGENS

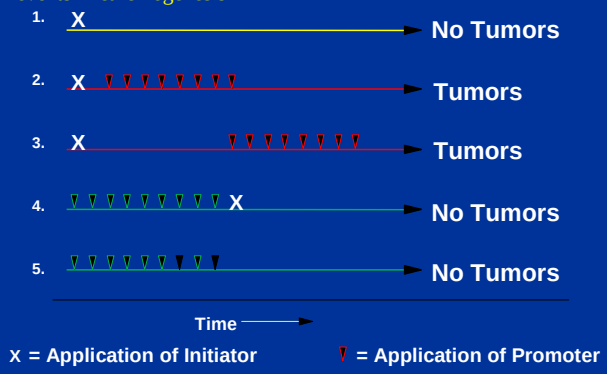


Metabolic activation of Benzo(a)pirene (PB)

Proposed mechanism of DNA damage, apoptosis, and carcinogenesis induced by benzene metabolites in the presence of NADH and Cu(II)



The experiment proving the need for a temporal sequence of events in carcinogenesis

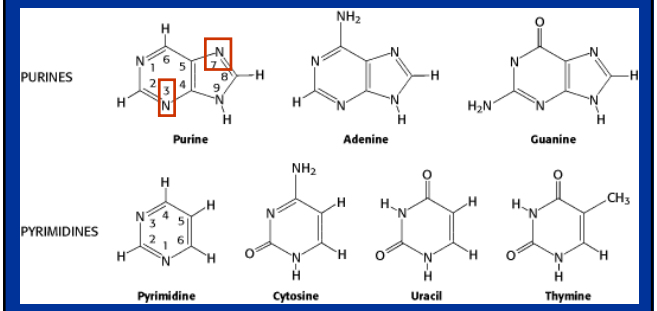


DNA adduct formation

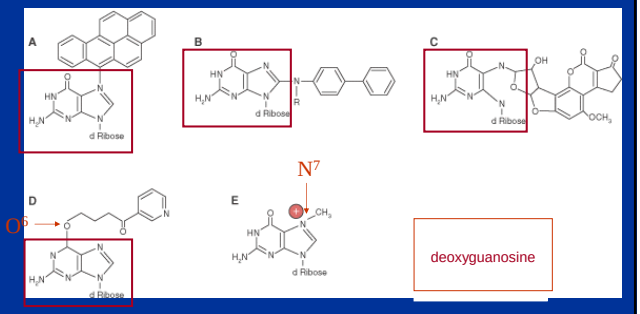
Since most chemical carcinogens react with DNA and are mutagenic, interactions with DNA have been viewed as the most important reactions of these agents.

The principal reaction products of the nitrosamines and similar alkylating agents with DNA are N^7 and O^6 guanine derivatives. Reactions also occur with other DNA bases.

Purines and Pyrimidines



Examples of carcinogen- DNA adducts



A. N-7 (benzo[a]pyren-6-yl)guanine **B.** *N*-(deoxyguanosin-8-yl)-{acetyl}aminobiphenyl
C. 8,9-dihydro-8-(N5-formyl- 2 , 5 , 6 -triamino-4 -oxo-N5-pyrimidyl)-9-hydroxy-aflatoxin B1
D. O^6-[4-Oxo-4(3-pyridyl)butyl]guanine, a mutagenic lesion formed by the metabolism of the tobacco-specific nitrosamine, NNK **E.** N-7-methyldeoxyguanosine

Potential biological consequences of DNA-adduct formation

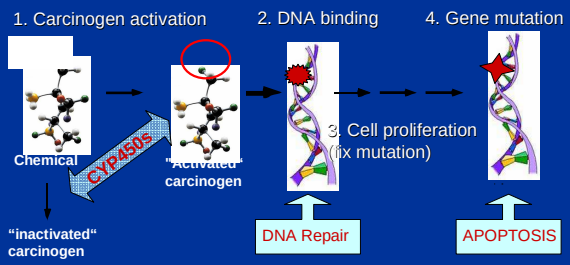
a. An insertion of the flat planar rings of a polycyclic hydrocarbon between the stacked bases of double-helical DNA may distort the helix, leading to a frame-shift mutation during DNA replication past the point of the intercalation.

b. Alkylated bases in DNA can mispair with the wrong base during DNA replication – for example, O^6 methyguanine pairs with thymine instead of cytosine during DNA replication, leading to a base transition (i.e., GC→AT) type of mutation during the next round of DNA replication.

c. Many of the base adducts formed by carcinogens involve modifications of N-3 or N-7 positions on purines that induce an instability in the glycosidic bond between the purine base and deoxyribose. This destabilized structure can then undergo cleavage by DNA glycosylase, resulting in loss of the base.

d. Interaction with some carcinogens has been shown to favor a conformational transition of DNA from its usual double-helical B form to a Z-DNA form. This could alter the transcribability of certain genes, since B→Z conformational transitions are thought to be involved in regulating chromatin structure.

Mechanism of Carcinogenesis: Genotoxic Carcinogens

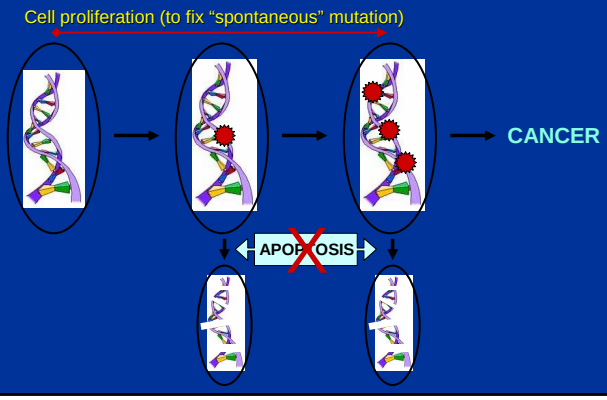


Non-Genotoxic Carcinogens

1) Mitogens:
- stimulation of proliferation
- mutations may occur secondarily to cell proliferation
- may cause preferential growth of preneoplastic cells

2) Cytotoxicants:
- cytolethal
- induce regenerative growth
- mutations may occur secondarily to cell proliferation

Mechanism of Carcinogenesis: Non-Genotoxic Carcinogens



Classification of Carcinogens According to the Mode of Action

NON-GENOTOXIC:
- Do not directly cause DNA mutation
- Mechanism of action is not completely understood
- Difficult to detect - requires rodent carcinogen bioassay

Mechanisms of Non-Genotoxic Carcinogenesis (what's in a "black box" ?)

- Increased cell proliferation
- Decreased apoptosis
- Changes in gene expression
- Induction of metabolizing enzymes
- Activation of receptors (signaling)
- Oxidative stress
- ???

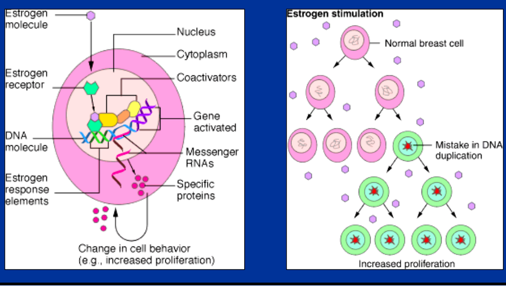
Cell Replication is Essential for Multistage Carcinogenesis

- Decreases time available for DNA repair
- Converts repairable DNA damage into non-repairable mutations
- Necessary for chromosomal aberrations, insertions, deletions and gene amplification
- Clonally expands existing cell populations

Cellular and Molecular Mechanisms in Multistage Carcinogenesis: PROMOTION

Reversible enhancement/repression of gene expression:
- increased cell proliferation
- inhibition of apoptosis

No direct structural alteration in DNA by agent or its metabolites



From http://newscenter.cancer.gov/sciencebehind/

NON GENOTOXIC CARCINOGENESIS

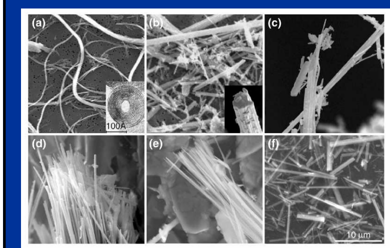
Table 1 Infection and inflammation associated with cancers

Cause	Site
Chewing tobacco/oral irritation	Oral cancers
Smoking/chronic bronchitis	Lung
Asbestos	Mesothelioma
Reflux disease	Barretts' adenocarcinoma of the esophagus
Chronic Helicobacter infection	Gastric adenocarcinoma and lymphoma
Chronic pancreatitis	Pancreatic cancer
Opisthorchis sinensis infection (liver fluke)	Cholangiocarcinoma
Viral hepatitis	Hepatocellular carcinoma
Ulcerative colitis and Crohn's disease	Colorectal carcinoma
Human papilloma virus	Anogenital carcinomas
Schistosomiasis	Bladder cancer
Pelvic inflammatory disease	Ovarian cancer
Chronic osteomyelitis	Osteosarcoma
Chronic scar tissue	"Scar" cancer arising in the lung, skin and other areas of scarring

Asbestos

- Widely used in construction, insulation, and manufacturing
- Family of related fibrous silicates
- Chrysotile
- Crocidolite

ASBESTOS



It is a silica-based natural fiber widely employed as termo-acustic-insulator and fireproof.

Fibers with lenght >5µm and diameter <3 µm are the most dangerous (as they reach the alveoli)

SERPENTINO: Chrysotile (a);
ANFIBOLI: Crocidolite (b); Antofillite (c); Ricterite (d); Tremolite (e); Amosite (f)

Malignant Mesothelioma

- Mainly occurs in pleural and peritoneal cavities
- Rare in general population
- Latent period of ≥20 years