GENOTOXICITY

Branch of toxicology investigating the damaging effects of chemicals, radiations and biological agents on DNA and their consequences at non cytotoxic concentrations.

MAIN TYPES OF GENETIC DAMAGE
A) GENE MUTATIONS
B) CHROMOSOMAL ABERRATION
C) MODIFICATION OF CHROMOSOME NUMBER

A) GENE MUTATIONS
These are alterations in the sequnce of DNA within specific genes.

• BASE-PAIR SUBSTITUTIONS
Replacement of one nucleotide and its partner in the complementrary DNA strand with another pair of nucleotides, thus leading to the expression of a different codon (e.g. GAG specifies glutamate ⇒ AAG specifies lysin)

a) Transitions.
Change of a purine (A,G)/pyrimidine (T,C) base with another purine/pyrimidine base (e.g. A ⇒ G)

b) Transversion.
Change of a purine base with a pyrimidine base and viceversa.

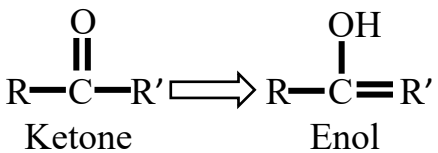
Point mutations can be:
• Silent
• Missense
• Nonsense

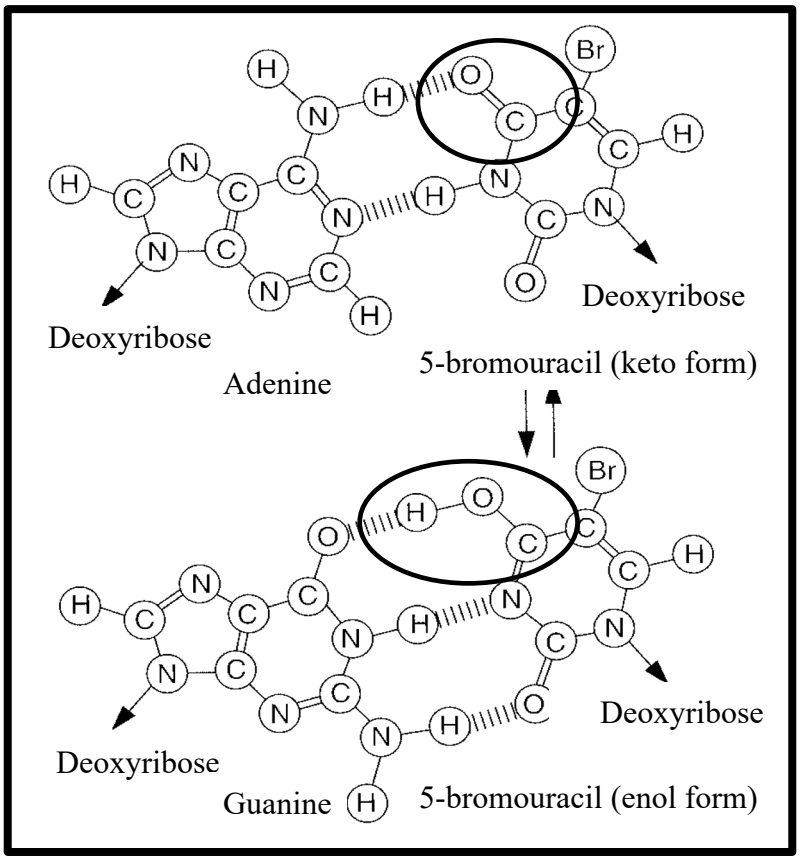
Mechanisms of gene mutations

1) BASE ANALOGUE INCORPORATION

e.g. 5-bromouracil (thymine analogue) is incorporated and is paired to adenine. When DNA replicates, 5-bromouracil can undergo keto-enol tautomerism and in its enol form it is read as cytosine and paired to guanine. Transition T-A ⇒ C-G.

Keto-enol tautomerism

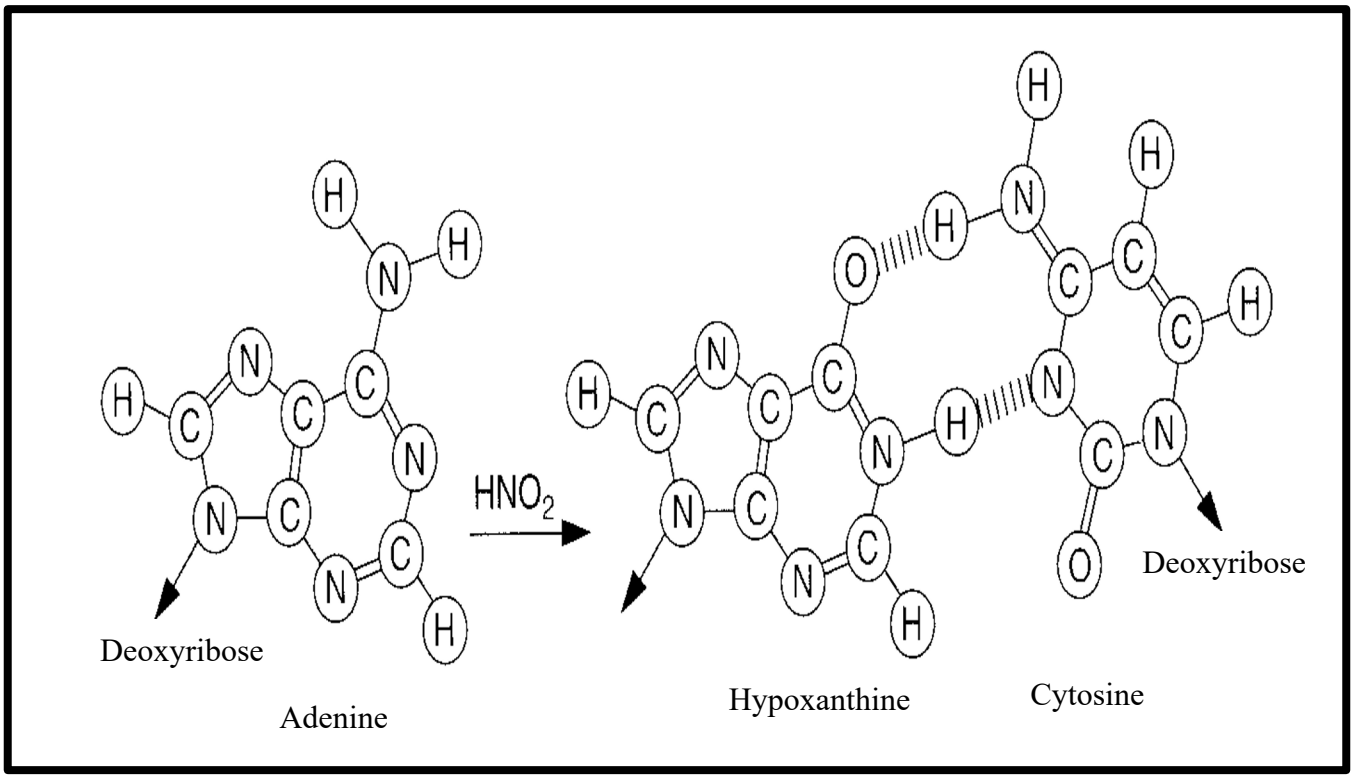




Adapted from: Cantelli Forte, Galli, Hrelia, Marinovich "Toosicologia molecolare e cellulare" Ed. UTET

2) BASE CHEMICAL MODIFICATION
e.g. oxidative deamination of adenine

Adenine ⇒ hypoxanthine ⇒ paired to cytosine
Transition A-T ⇒ G-C.



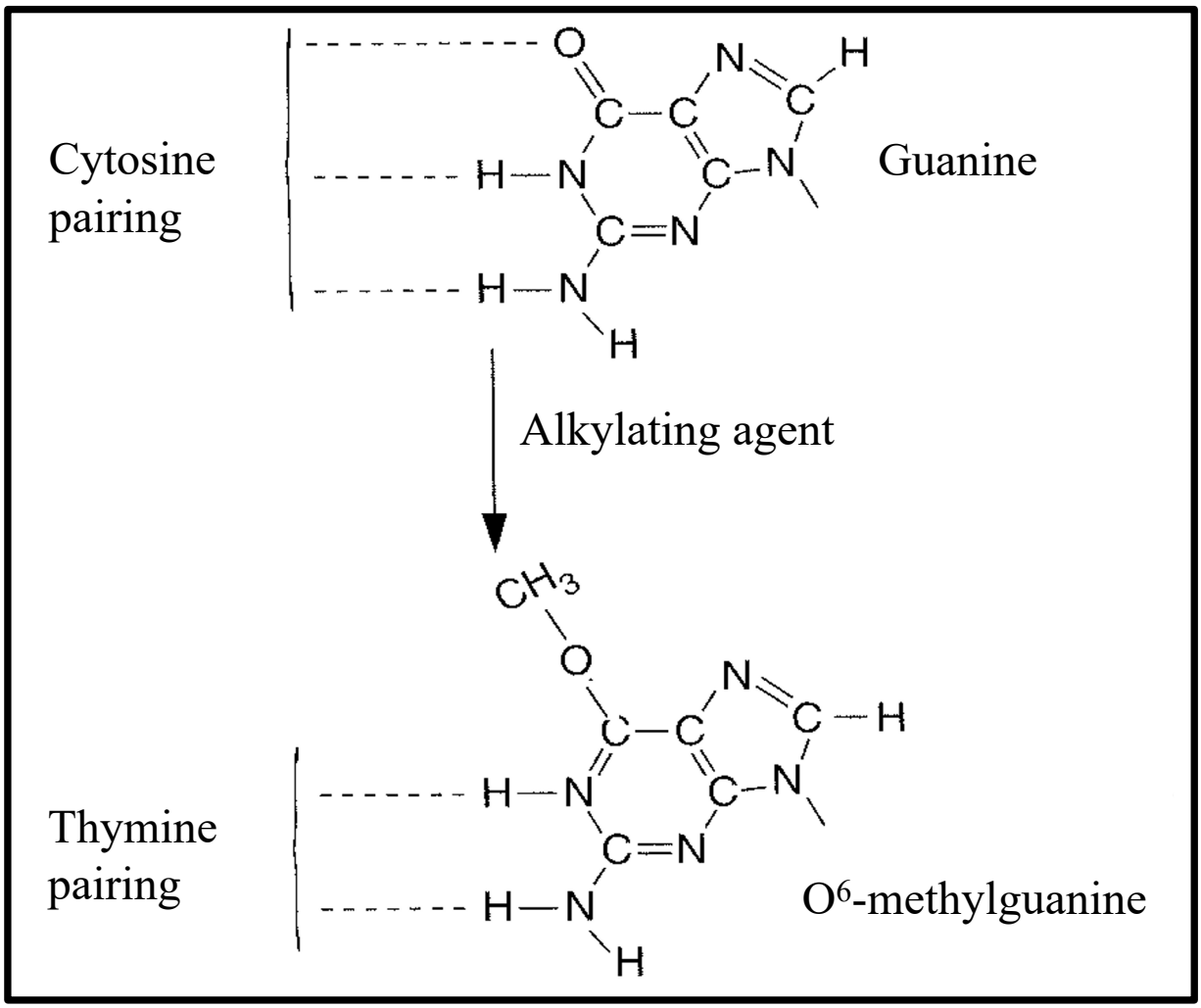
Adapted from: Cantelli Forte, Galli, Hrelia, Marinovich "Toosicologia molecolare e cellulare" Ed. UTET

3) BASE ALKYLATION

Binding of chemical to a purine/pyrimidine base with formation of base adducts (small or bulky).

e.g. methylation of guanine

Transition GC ⇒AT

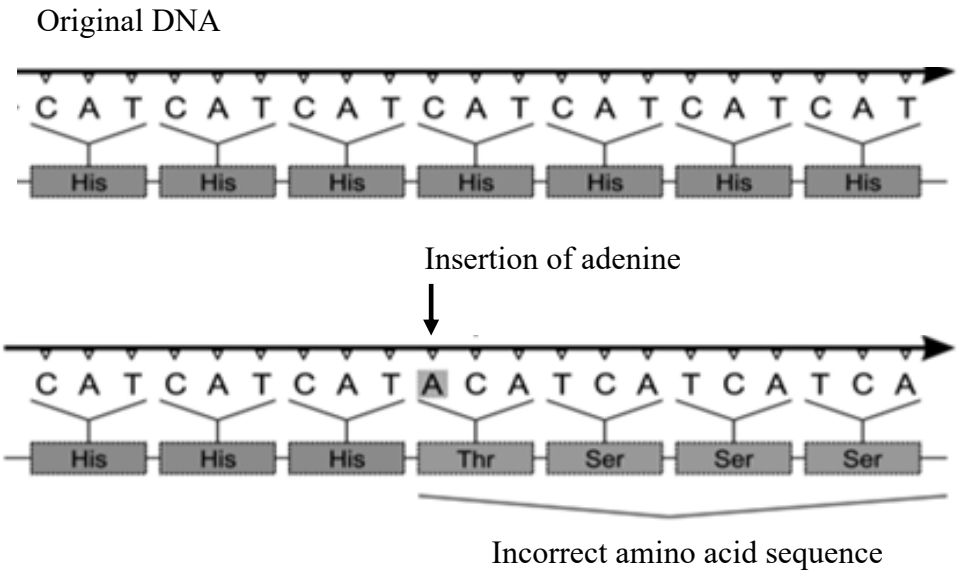


Adapted from: Cantelli Forte, Galli, Hrelia, Marinovich "Toosicologia molecolare e cellulare" Ed. UTET

In general, formation of bulky adducts leads to transversion (e.g. binding of PAH, bezopyrene)

• FRAME-SHIFT MUTATIONS

Due to addition/deletion of bases. More deleterious since all the reading frame after the mutation is altered.



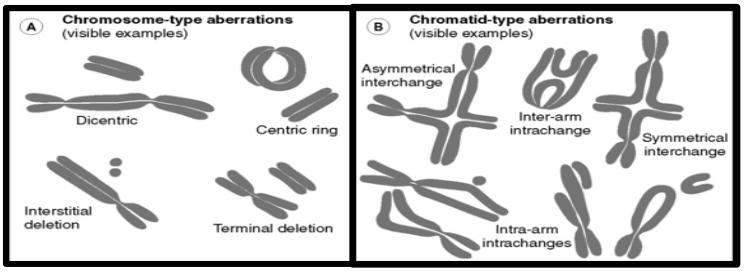
Adapted from: http://www.tankonyvtar.hu/hu/tartalom/tamop425/0011_1A_Molelkularis_diagnoszitka_en_book/ch03.html

In general, these mutations are induced by large molecules (e.g. acridine orange) intercalating in DNA double strands.

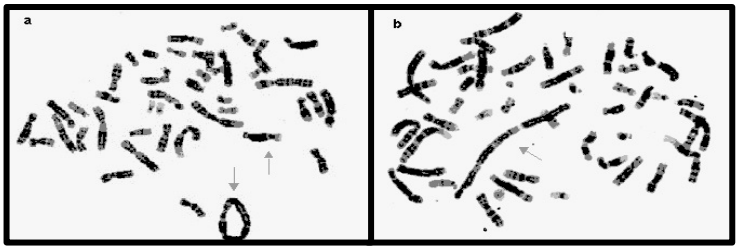
B) CHROMOSOMAL ABERRATIONS

Due to deletions, breaks or exchange of chromosomic material during cell cycle.

A) Chromosome-type aberrations involve both chromatids
B) Chromatid-type aberrations involve only one chromatid



Adapted from https://radiologykey.com/molecular-cellular-and-tissue-effects-of-radiotherapy/



Adapted from http://atlasgeneticsoncology.org/Deep/RingChromosID20030.html

C) MODIFICATIONS OF CHROMOSOME NUMBER

Aneuploidy

Increase or decrease of one or more chromosomes (e.g 2n-1; 2n+1)

Polyploidy

Alteration of the entire set of chromosomes (n aployd, 2n diployd, 3n triployd etc).

DNA REPAIR

- Direct base repair

There are specific enzymes able to directly repair bases (e.g. O^6-alkylguanine DNA alkyl transferase, DNA photolyase)

- Excision repair

A) Single base excision

In this case, the altered base is removed. DNA glycosiylases ⇒ cleavage of N-glycosilic bond ⇒ base removal ⇒ AP site ⇒ endonucleases ⇒DNA polymerases (synthesis of deoxyribonucleotide) ⇒ DNA ligase.

B) Nucleotide excision

It operates in case of bulky adduct lesions to DNA.

ATP-dependent nucleases cleave some nucleotides from from both sides of the damage ⇒ the 23-32 nucleotide excised DNA fragment is removed ⇒ DNA polymerases ⇒ DNA ligase

- Post-replication repair

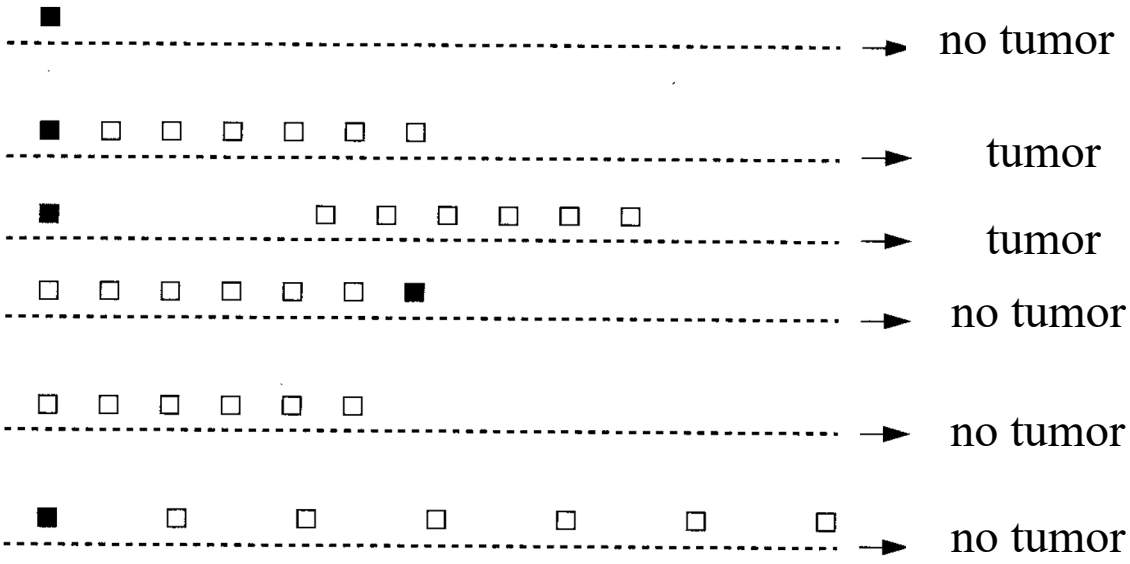
Series of concerted mechanims repairing DNA damage after replication.

DNA REPAIR SYSTEMS

Constitutive	Repairing enzymes do not increase with the increase of DNA damage
Inducible	Repairing enzymes can increase in parallel with increasing DNA damage
Error free	Repairing process progress with an accurate substitution of bases
Error prone	Repairing process is not accurate and DNA aberrant sequences can be produced by polymerases, thus causing new mutations

CHEMICAL CARCINOGENESIS

Chemical carcinogenesis is a multistep process with different molecular modifications. By studying skin cancer development in mice following tar (bezopyrene) and croton oil exposure (TPA, 12-O-tetradecanoyl-phorbol-13-acetate), Beremblum (1941) identified two different phases.



Adapated from: Cantelli Forte, Galli, Hrelia, Marinovich "Tossicologia molecolare e cel

• INITIATION. It is the first phase characterized by an irreversible damage (mutation) to DNA by chemical or physical agents. There is no clear dose-effect relationship (no threshold dose). It requires one or more rounds of cell replication for the "fixation" of the mutation.
The targets of mutations are genes involved in the different mechanisms of cell cycle regulation.

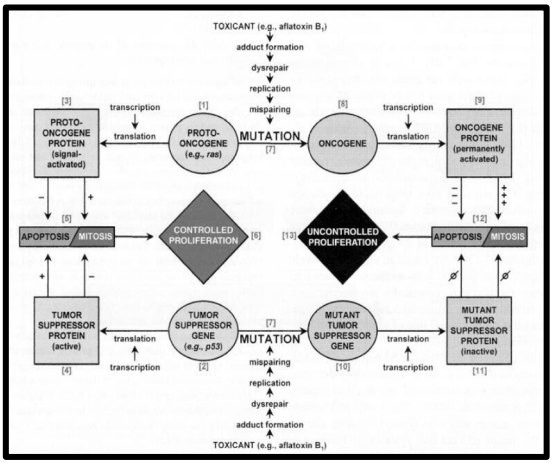
PROTO-ONCOGENES
These are genes whose expression products play a key role in activating cell replication (growth factors, receptors, protein kinases, transcription factors, etc.)

Proto-oncogenes	Function
c-fos	Nuclear protein, Transcription factor
c-myc	Transcription factor
c-erb	EGF
c-sis	PDGF
c-raf	Ser/Thr PK
c-src	Tyr PK

CHEMICAL CARCINOGENESIS

Once mutated, they become oncogenes and can lead to:
a) alteration of gene coding sequence ⇒ production of altered protein
b) alteration of gene expression (e.g. promoter or regulatory region) ⇒ overproduction of normal protein

TUMOR (ONCO-) SUPPRESSOR GENES
Genes expressing proteins that inhibit progression of cell division.
One of the best known is p53 that has many functions:
a) it activates genes whose products stop cell cycle (e.g. p21) or promote apoptosis (e.g. fas receptors, bax);
b) it represses genes that express anti-apoptotic proteins (e.g. bcl-2)



Adapted from: Casarett & Doull's Toxicology. The Basic Science of Poisons. Ed. Klaassen, Curtis D.

• PROMOTION
It is the phase in which there is expansion of the cell progeny of initiated cell population. It leads to the formation of pre-neoplastic cells with the same mutation(s) (mutated phenotype). Promoting xenobiotics directly or indirectly influence gene expression/cell proliferation. This phase can be triggered also by endogenous hormones or by citotoxic chemicals. There is a clear dose-effect relationship with a defined threshold dose. It can be reversible. There is no DNA damage.

• PROGRESSION
It follows promotion and it is characterized by complex genetic alterations leading to karyotypic/genomic instability (chromosomal translocations, deletions, gene amplification, etc.). Progression is an irreversible phase.

Cancer cells show:
- loss of growth inhibition
- autonomy of proliferation
- apoptosis resistance
- immuno escape mechanisms (immunoediting ⇒ loss of antigenicity/immunogenicity; e.g. alteration of MHC, expression of PD-L1, secretion of suppressive cytokines)
- induction of angiogenesis
- invasion/metastasis

CHEMICAL CARCINOGENESIS

Carcinogens can be:

- Initiating agents: incomplete carcinogens, genotoxic carcinogens
- Promoting agents: co-carcinogens, epigenetic carcinogens
- Progressor agents
- Complete carcinogens

Genotoxic carcinogens (direct or procarcinogens)

- Alkylating agents (e.g. cyclophosphamide, cisplatin, alogenated hydrocarbons)
- Nitrosamine (e.g. dimethylnitrosamine, N-nitrosonornicotine)

They are formed by reaction of secondary/tertiary amines with a nitrosating agent. Nitrosamine are used in the manufacture of different products (e.g. pesticides, rubber products). They can be also formed by nitrosation of amines in food due to nitrite (food preservative) conversion into nitrous anhydride (N_2O_3) in acidic environment (stomach). They are also present in tobacco smoke (e-cigarettes??).

- Primary aromatic amines (PAAs) and heterocyclic aromatic amines (HAAs)

PAAs are found in many industrial products. They can be found in food packaging. HAAs can be formed during cooking of meat and fish (pyrolysis; highest with charcoal grilling/barbecuing and pan-frying). Also pizza toppings.

- Polycyclic Aromatic Hydrocarbons (PAH, anthracene, benzopyrene).

Primarily formed by incomplete combustion of organic fuels (coal, oil, gas, wood) or pyrolysis of organic matter (e.g. food grilling/barbecuing, roasting and smoking, pizza). Present in petroleum products, vehicle exhaust fumes, etc

- Heterocyclic Aromatic Hydrocarbons (Hetero-PHAs; quinoline,).

Industrial products, combustion product (also food).

- Halogenated hydrocarbons (e.g. $CHCl_3$)
- Metals (Ni, Cd, Cr, Pb)

Epigenetic carcinogens

- Mitogens and agents influencing gene expression (hormones, phorbol esters, phenobarbital, dichlorodiphenyltricholoroethane DDT, tetrachlorodibenzo-p-dioxin TCDD)
- Cytotoxic agents
- Immunosuppressants

Progressor agents (asbestos fibers, arsenic salts)

Chemicals that show clastogenic and carcinogenic activity but may not be capable of initiation (asbestos fibers, arsenic salts).

Complete carcinogens

Chemicals having the capability of inducing initiation, promotion and progression

DEVELOPMENTAL TOXICOLOGY

It is a modern branch of Toxicology investigating pharmacokinetics, mechanisms, pathogenesis and outcome of exposure to agents causing an abnormal development.
Teratology (study of birth malformation) is very old (6500-5000 B.C.)

STADI DEL CONCEPIMENTO

Fertilization (oocyte+ sperm)

Zygote

Preimplantation phase and blastocyst formation (4-6 days)

Implantation phase (5-12 days), ecto/meso/endoderm formation (6-13 days)

Neural crest formation (18-20 days)

Organogenesis phase (21-56 days)

Fetal phase (56-270 days)

Birth

DEVELOPMENTAL TOXICOLOGY

Main causes

Physical agents: radiations (X rays)
hyperthermia

Chemicals: drugs, pesticides, solvents, food additives, heavy metals

Biological agents: viral infections (rubella, cytomegalovirus); protozoan infections (toxoplasmosis)

Metabolic alterations: diabetes mellitus, phenylketonuria

Types of toxic effects

Direct effects. Direct toxic insults on embryo/fetus

Indirect effects (maternal toxicity). Toxic effects in the mother (anemia, reduction of blood flow, hypoxia, acid-base imbalance) or to placenta functions (anchoring, nutrition, gas exchange, removal of fetal waste products, hormone synthesis)

The Wilson's six general principles of teratology (1959)

1) Susceptibility to teratogenesis depends on the genotype and on environmental factors (e.g. maternal characteristics, nutrition)

2) Susceptibility to teratogenesis varies with the developmental stage at the time of exposure

3) Teratogenic agents act in specific ways (mechanisms) on developing cells and tissues to initiate sequences of abnormal developmental events (pathogenesis)

4) The access of adverse influences to the developing tissues depends on the nature of the influence (agent)

5) The four manifestations of deviant development are:
   a) Death (embryonic, perinatal, infant)
   b) Malformation
   c) Growth retardation
   d) Functional deficit

6) Manifestations of deviant development increase in frequency and degree as dosage increases, from no effect to totally lethal level.

DEVELOPMENTAL TOXICOLOGY

MECHANISMS

a) Interference with nucleic acids. Many embryotoxic agents cause DNA damage (mutations, breaks, chromosomal aberrations) that can lead to cell death. If damaged cells exceed a certain threshold level (depending on the organ and developmental state), the effect is irreversible and can cause embryo death or congenital anomalies.

b) Apoptosis induction/inhibition. In both cases, malformations can occur (e.g. syndactily, webbing of fingers or toes)

c) Interactions with receptors and/or enzymes. Folate metabolism, RXR and RAR receptors, $GABA_A$ receptors. etc.

d) Interference with migration and differentiation. Developmental processes orchestrated by a variety of signals that are critical for morphogenesis.

MAIN PHASE-RELATED EFFECTS

Preimplantation

Exposure during this phase can cause embyionic death if the number of damaged cells is excessive. In other cases, effective repair can occur leading to survival.

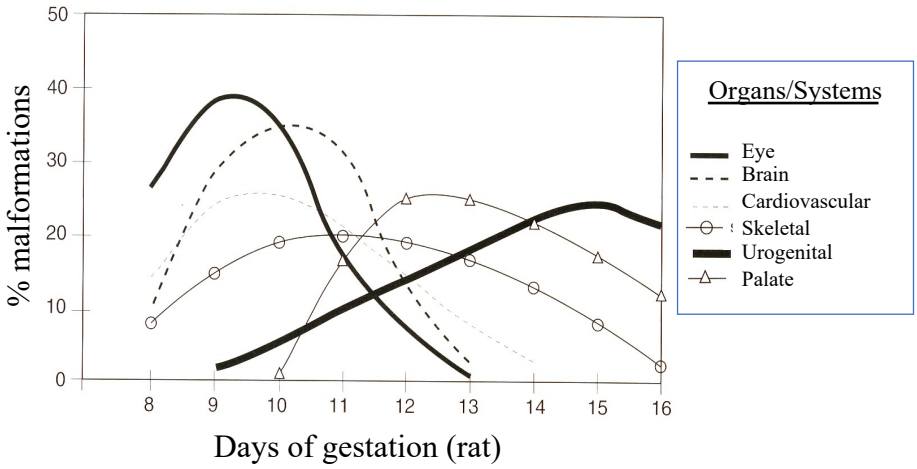
Recent evidence has shown that toxic effects during this phase could also result in malformations.

Implantation

This period is susceptible to teratogenic effects especially to eyes, face and brain.

Organogenesis

It is the period of maximum susceptibility to deformations and each forming structure has a peak susceptibility coinciding with the time key developmental events that occur in that structure.



Adapted from: Cantelli Forte, Galli, Hrelia, Marinovich "Tossicologia molecolare e cellulare" Ed. UTET

DEVELOPMENTAL TOXICOLOGY

Fetal period

Toxicity usually manifests as growth anomalies and/or functional deficits, including behavioral, mental and motors ones. These manifestations are not apparent prenatally and can be detected by careful postnatal observation and testing.

THALIDOMIDE (1960)

Its teratogenic properties are sadly known. Used to reduce morning sickness (e.g nausea and vomiting in pregnant It has caused severe birth defects in more than 10000 children. Effects include damage to limbs (phocomelia, amelia), ears, eyes and internal organs (heart, kidney, GI tract), as well as to vertebral column)

Teratogenic beteween 20 a 36 after fertilization. Damage mechanisms: angiogenesis inhibition, oxidative stress cell death, growth factor antagonism, alteration of GSH redox system.

DIETHYILSTILBESTROL (DES; 1971)

Synthetic nonsteroidal estrogen used bteween 1940 and 1970 to prevent threatened miscarriage by stimulating placental synthesis of estrogen and progesteron. It caused clear cell adenocarcinoma (CCA) of the vagina in young women (15-22 years) borne from mothers exposed to DES during the first trimester. In males, it caused a high incidence of epididymal cystis, hypothrophic testes, sperm damage.

ETHANOL (1973)

High ethanol consumption during pregnancy is the cause of the Fetal Alcohol Syndrome (FAS) that is characterized by carnio-facial alterations, intrauterine and postnatal growth retardation, retarded psychomotor and intellectual development (IQ 68) and other alterations.

FAS associted ethanol consumption: 80-100 g/day.

It has quite high incidence in alcoholic mothers. incidenza nelle madri alcolizzate (2.5%).

Lower consumption has been associated to less severe, though dangerous effects (neurological/behavioral disorders) termed Fetal Alcohol Effects (FAE).

TOBACCO SMOKE

Tobacco smoking during pregnancy can lead to: spontaneous abortion, perinatal death, increased risk of sudden infant death syndrome (SIDS), increased risk of learning and attention disorders, morphogenesis and maturation of lungs.

Passive smoking, also called secon-hand smoking (SHS) or environmental tobacco smoke (ETS) is known to represent a significant risk to pregnant non smokers.

DEVELOPMENTAL TOXICOLOGY

COCAINE

Alkaloid with local anesthetic and vasoconstrictor properties that has become an illegal substance of abuse during the 80's. It can cause damage to placenta, premature labor and delivery, microcephaly, altered development of brain regions, decreased birth weight, neonatal neurologic syndrome of abnormal sleep, tremor, seizures, SIDS.

RETINOIDS

The teratogenic effects of vitamin A (retinol) excess have been long known (1954) and retinoic acid was later found to induce similar effects (1967). They cause malformations of the face, limbs, heart, CNS and skeleton. By acting on RAR and RXR receptors, retinoids regulate expression of genes playing key roles in the embryo development. 13-cis-retinoic acid has been marketd (1982) for the treatment of severe recalcitrant cystic acne. Used by pregnant women, it has caused many cases of teratogenic effects in infants.