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TOXICITY TEST

EXPOSURE

EXPOSURE SITE, RISK,
FREQUENCY & DOSE

Xenobiotic available to
absorb

TOXICOKINETICS

ABSORPTION DISTRIBUTION
BIOTRANSFORMATION END
EXCRETION

Delivery

TOXICODYNAMICS

REACTION OF THE ULTIMATE
TOXICANT WITH THE TARGET
MOLECULE

Alteration of biological
environment.

toxicity

Toxicant exposure characteristics

Exposure site

1. Gastrointestinal
2. Pulmonar
3. Dermal
4. Parenteral

Exposure time

Acute	<24 hr
Sub-acute	< 1 mes
Sub-chronic	1-3 meses
Chronic	> 3 meses

Length of time and
Frecuency

Examples of effects according to exposure lenght

Xenobiótico	Acute exposure (short lasting)	Chronic exposure (long-lasting)
benceno	Central nervous system depression	Leukemia
CCl ₄	Central nervous system depression	Hepatic and renal damage

Absorption route

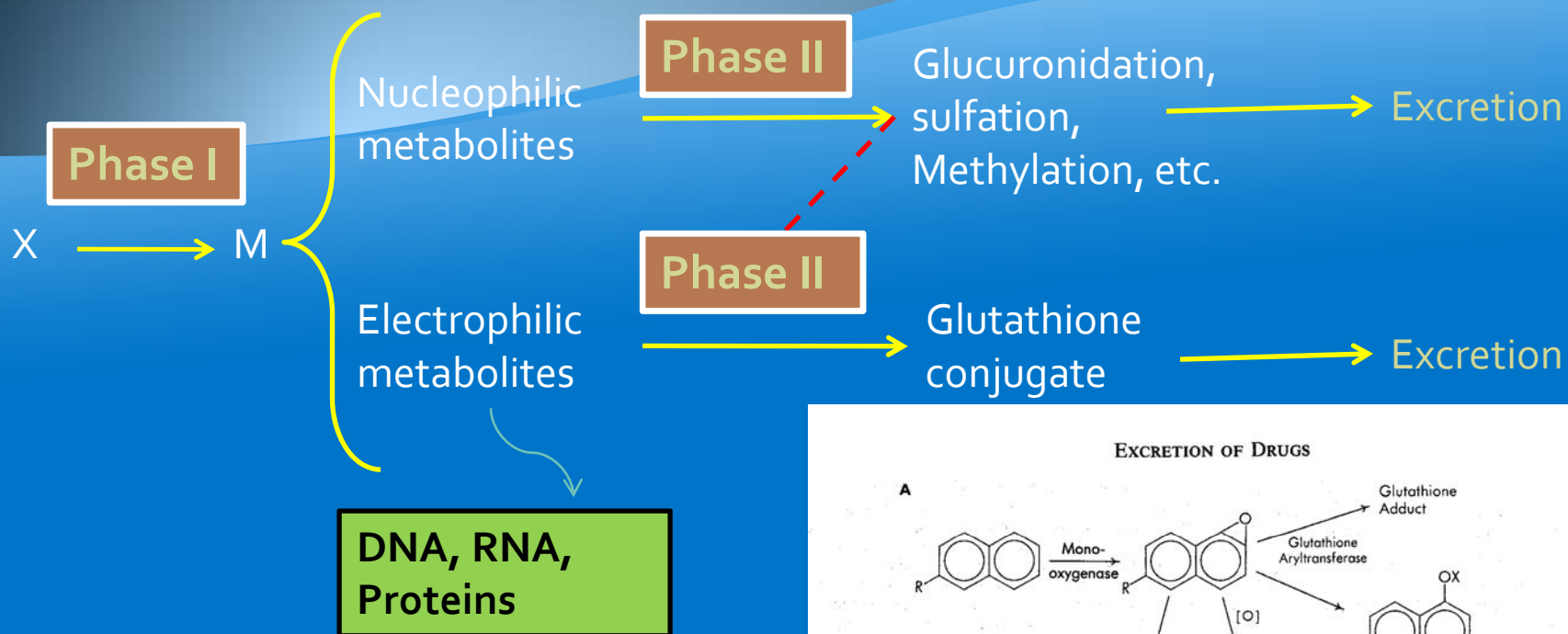
a. Gastrointestinal

b. Pulmonar

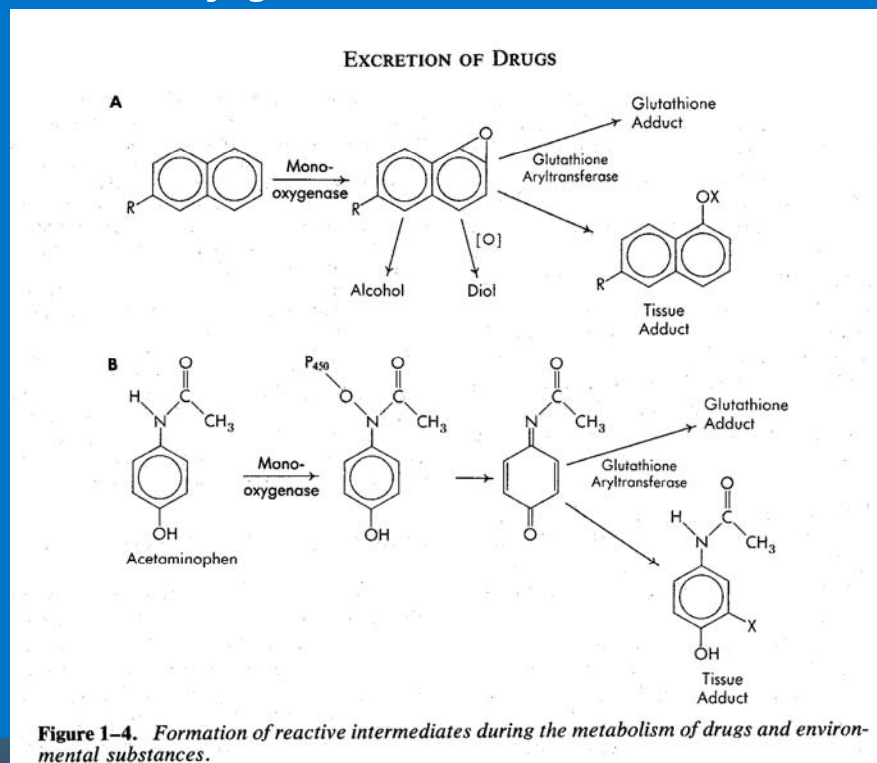
c. Dermal

Route of administration	LD ₅₀ (mg/kg wb)		
	Pentobarbital	Isoniazid	Procaine
Oral	280	142	500
Subcutaneous	130	160	800
Intramuscular	124	140	630
Intraperitoneal	130	132	230
Intravenous	80	153	45

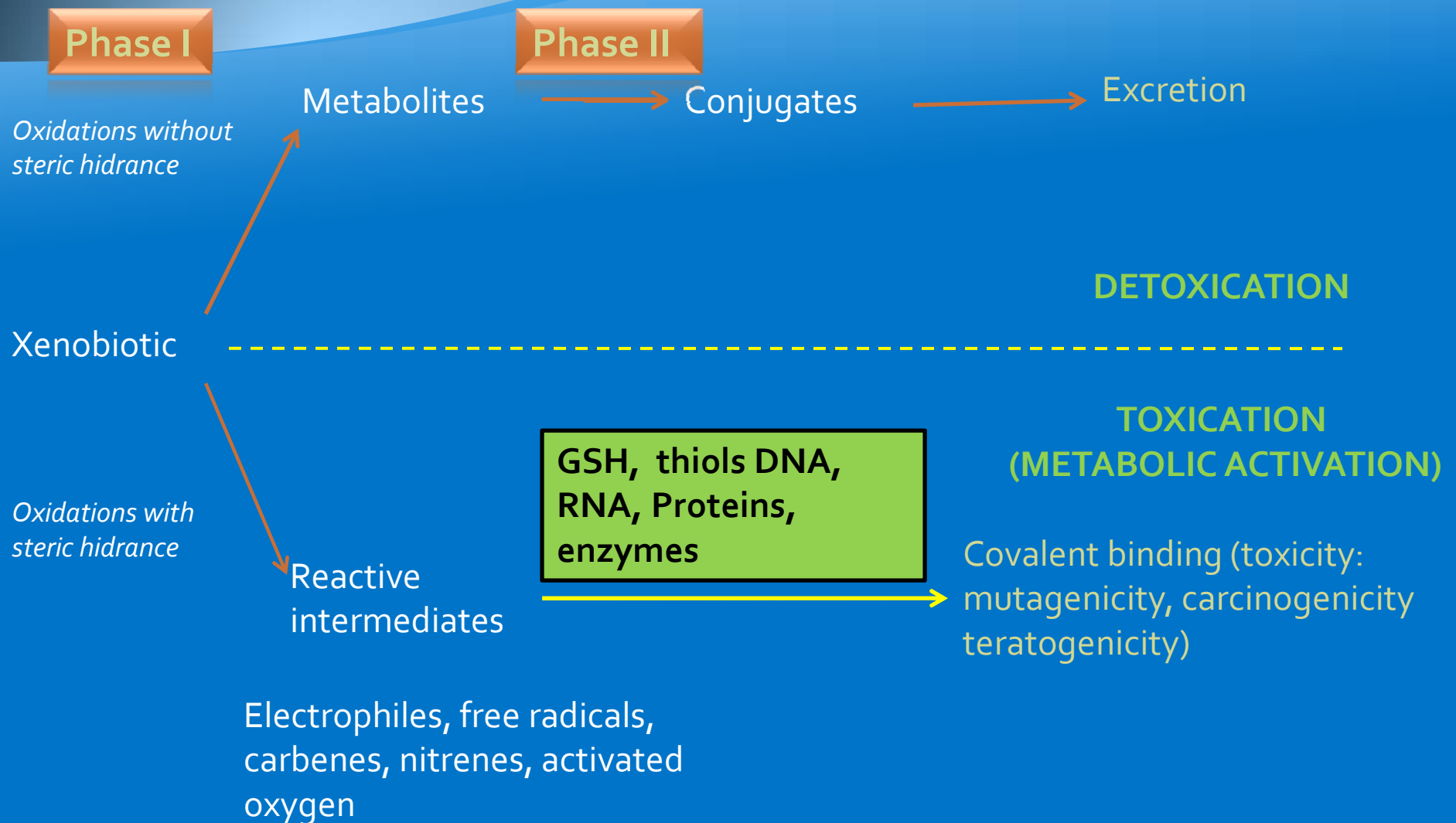
IMPORTANCE OF BIOTRANSFORMATION PATHWAYS



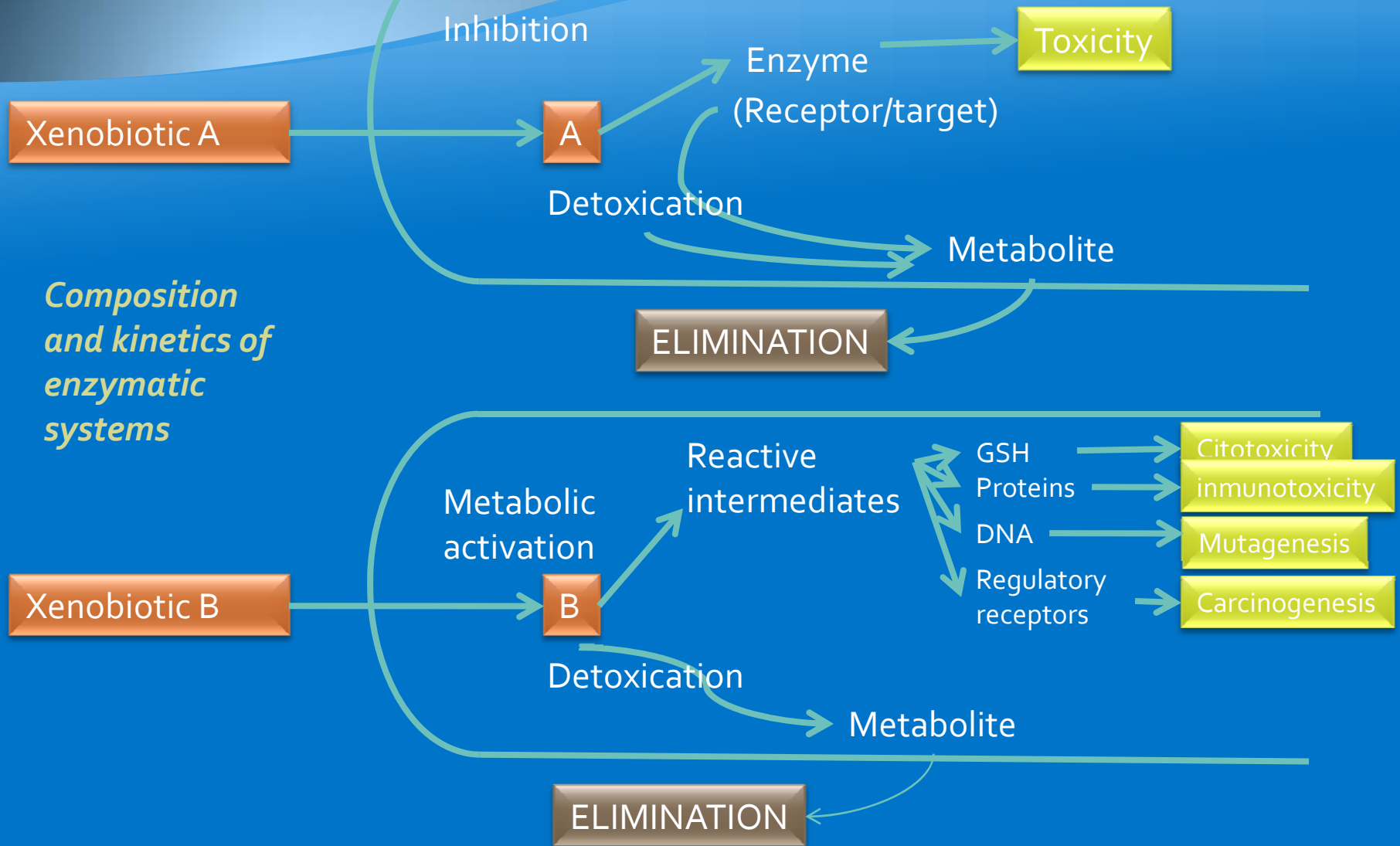
Electrophiles, free radicals, carbenes, nitrenes



IMPORTANCE OF BIOTRANSFORMATION PATHWAYS



Acute vs. Chronic toxicity



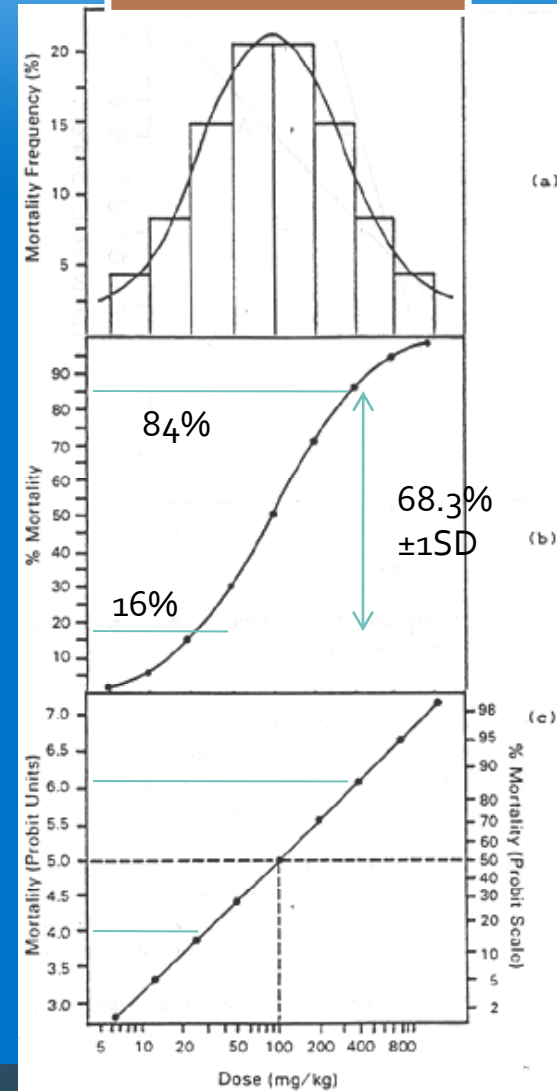
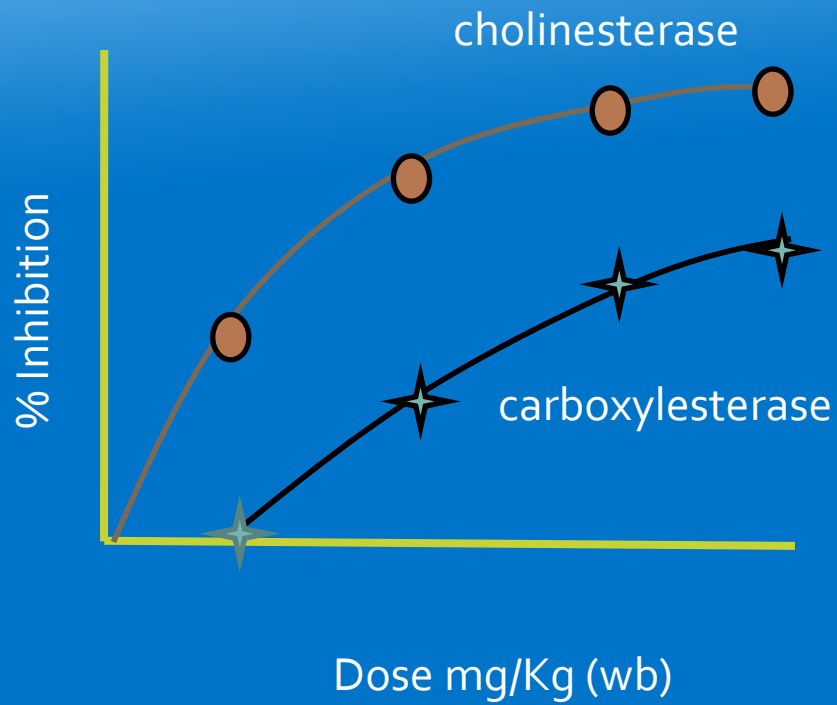
Toxicity test.

Examples of toxicity tests used for human health	
Genotoxicity	Ames test Micronucleus test Commet assay
Acute toxicity	Oral, dermal, inhalation Eye irritation Skin irritation Dermal sensitization
Metabolism and toxicokinetics	
Subchronic and chronic multidose studies	
Reproductive and developmental studies	
Carcinogenicity – lifetime studies	
Mechanistic studies and comparative toxicokinetics and metabolism to improve predictability	

Dose – Response Relationships

Individual (graded)

Population (Quantal)

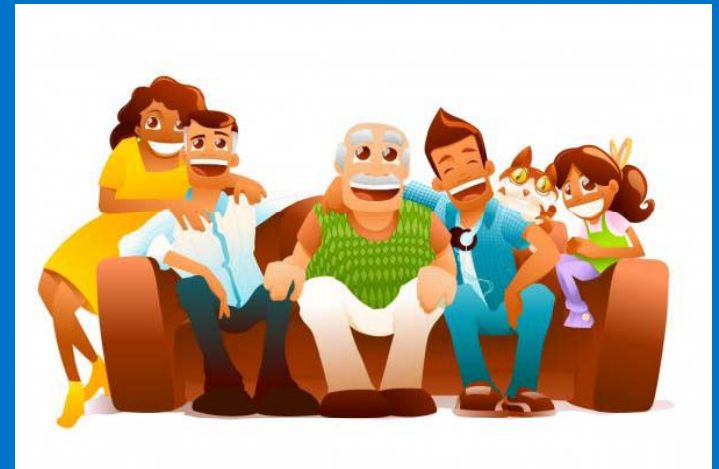


Factors involved in variation in toxicity

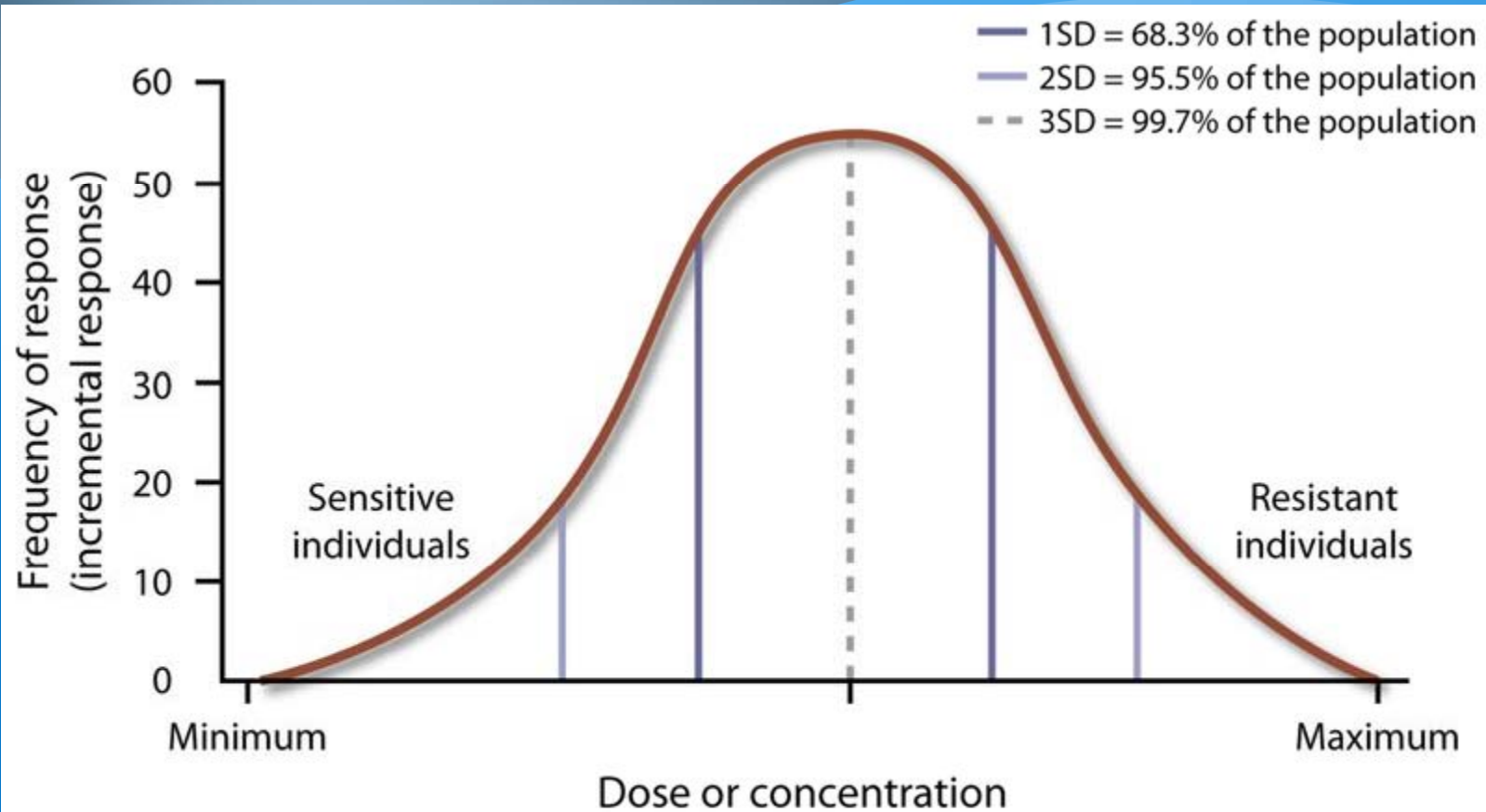
Biochemical heterogeneity among races



Species differences:
Biochemical heterogeneity
of animal and human



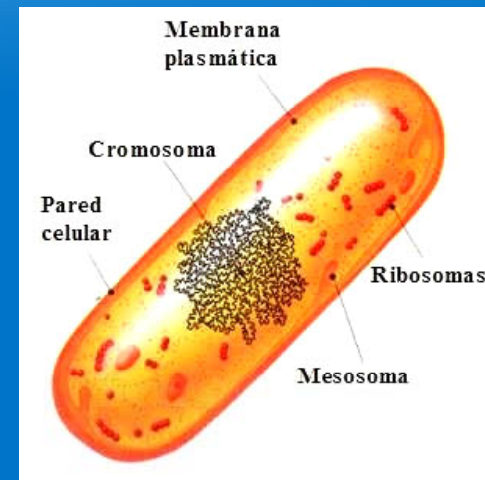
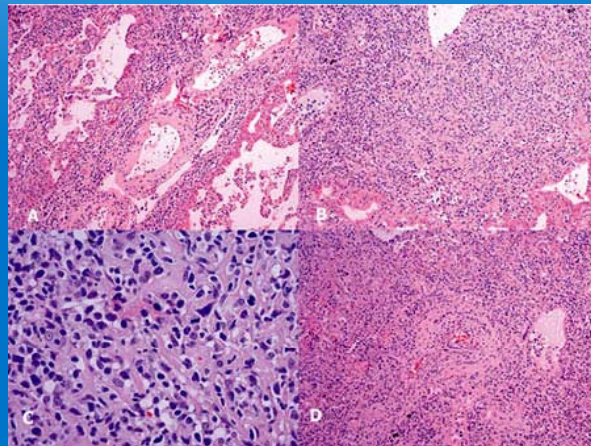
Individual differences in
response: heterogeneity
due to sex and age.



Adapted by CTLT from Loomis, Essentials of Toxicology, 4th ed., p. 20, Loomis, T.A. and Hayes, A.W., 1996, Academic Press.

Factors involved in variation in toxicity

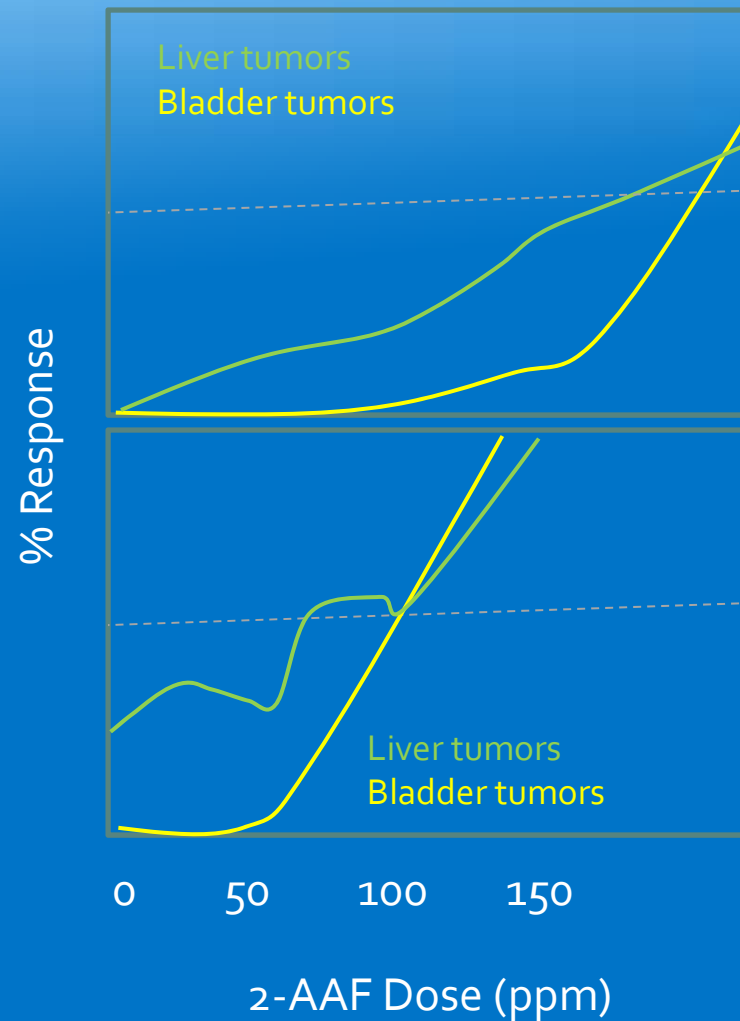
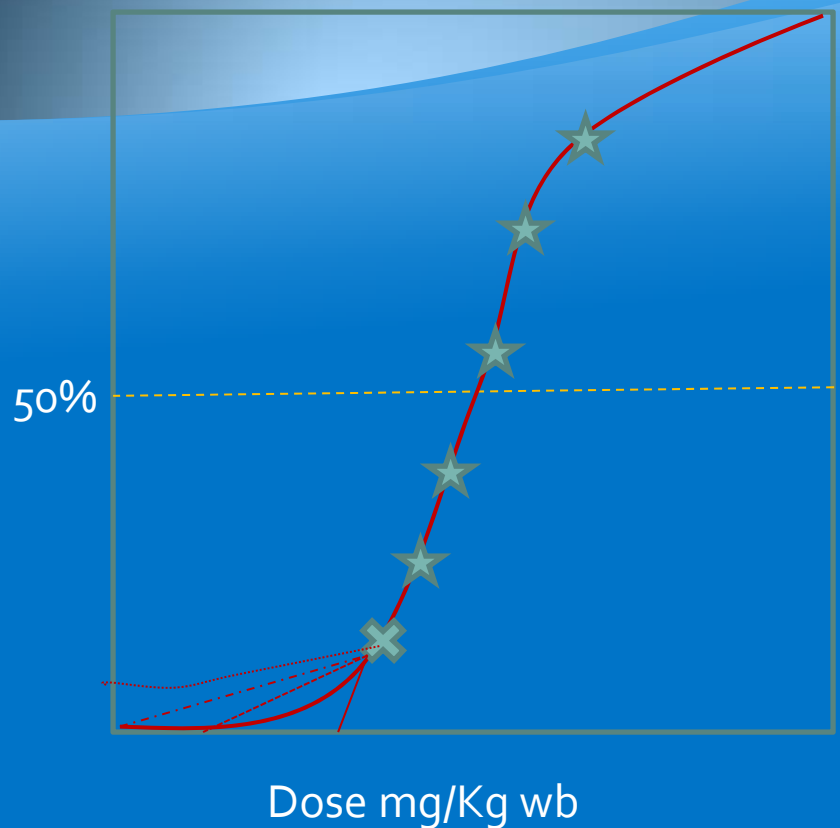
Selective Toxicity: Injury to one kind of living matter without harming another form of life even though the two may exist in intimate contact.



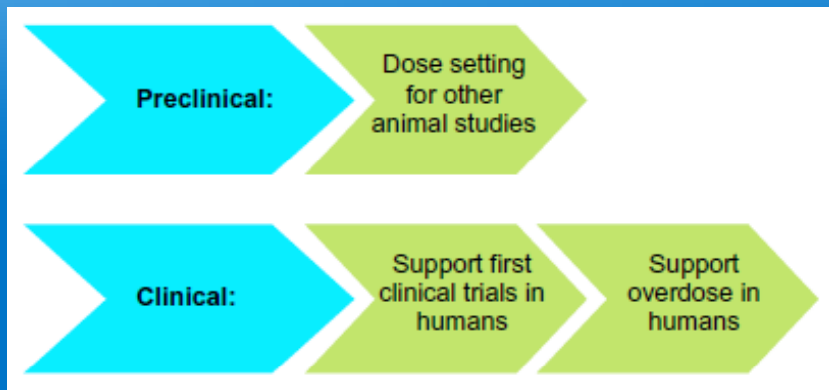
Species differences: both quantitative and qualitative differences in response to toxic substances may occur among different species.

Individual differences in response: interindividual differences in response to a chemical can occur because of subtle genetic polymorphism.
IDIOSYNCRATIC RESPONSES.

Carcinogens Dosis-Response



Claimed scientific drivers for acute toxicity studies with pharmaceuticals



Xenobiotic	LD ₅₀ (mg/Kg wb)
ethanol	10 000
NaCl	4 000
FeSO ₄	1 500
Morphine (sulphate)	900
Phenobarbital sodium	150
Strychnine	2
Nicotine	1
dioxin	0.001
C. Botulinium toxin	0.00001

Globally Harmonized System of Classification and Labelling of Chemicals.

Exposure Route	Category 1	Category 2	Category 3	Category 4	Category 5
Oral (mg/kg bw)	≤ 5	≤ 50	≤ 300	≤ 2000	≤ 5000
Dermal (mg/kg bw)	≤ 50	≤ 200	≤ 1000	≤ 2000	≤ 5000
Gases (ppm)	≤ 100	≤ 500	≤ 2500	≤ 20,000	Inhalation LC50 in the equivalent range of the oral and dermal LD50
Vapours (mg/l)	≤ 0.5	≤ 2	≤ 10	≤ 20	
Dusts and mists (mg/l)	≤ 0.05	≤ 0.5	≤ 1.0	≤ 5	

Testing Acute toxicity

1. Selection of species (rat is the preferred species)
2. Selection of route of administration

Conventional acute toxicity test - Principle of the test method.

The test substance is administered orally by gavage in graduated doses to several groups of experimental animals, one dose being used per group.

1. Selection of doses to test (based on the result of range finding test)
2. Selection of number of animals (lowest number of animals feasible)
3. Observations of effects and deaths are made
4. Animals that die during the test are necropsied
5. Surviving animals are sacrificed and necropsied

Considerations:

Animals showing severe and enduring signs of distress and pain may need to be humanely killed. Dosing test substances in a way known to cause marked pain and distress due to corrosive or irritating properties need not be carried out.

Substance to be tested
Vehicle: aqueous solution be considered first, followed by corn oil

Healthy Young adult 8-12 weeks old. Weight variation within 20%. Same sex.
The females should be nulliparous and nonpregnant



At least 5 experimentally naive rodents per dose level.

animals acclimatized for at least 5 days (22 ±3 °C / RH 30-70%, 12h light/12 h dark)

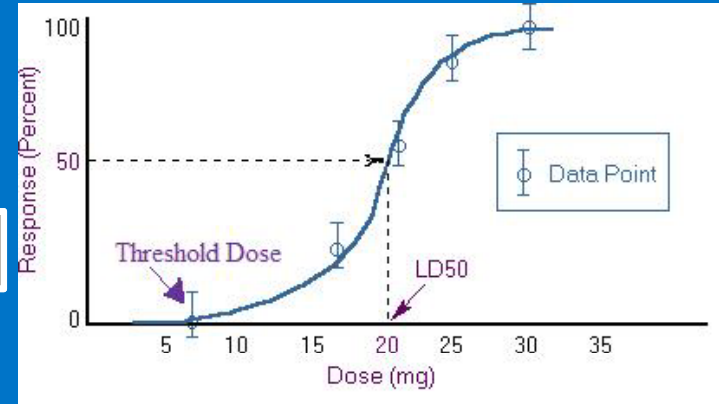
feed should be withheld overnight

Observation and register:

Daily weight

Time at which signs of toxicity appear, their duration
Evaluation of skin and fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, central nervous system effects, including tremors and convulsions, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypies or bizarre behavior (e.g., selfmutilation, walking backwards).

14 days



Health Effects Test Guidelines ,OPPTS 870.1100, Acute Oral Toxicity. EPA (712-C-98-190) August 1998

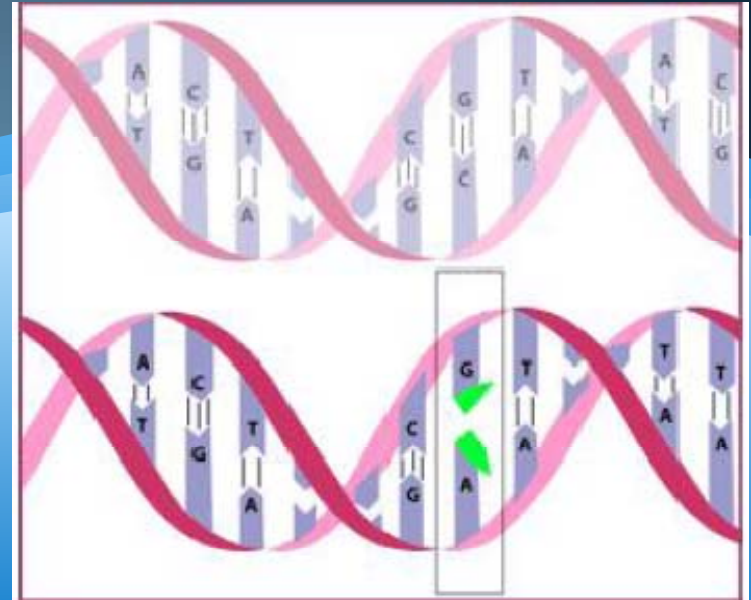
Time of death recorded as precisely as possible
Gross pathology of death and surviving animals

Genotoxicity test: prediction of mutagenic, carcinogenic and teratogenic effects.



Mutagénesis

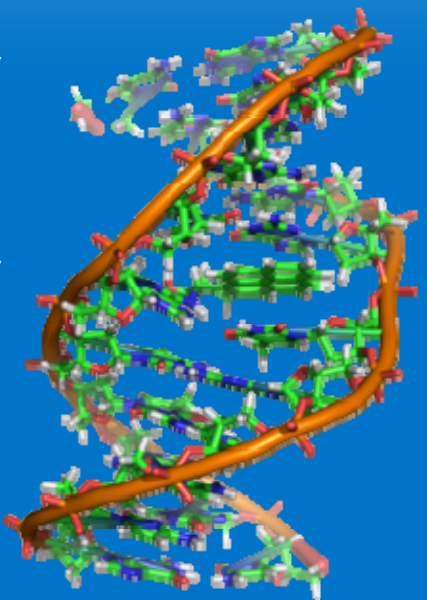
Hereditary changes produced on genetic information storage in DNA



Chemical and physical agents:

DNA can be damaged by many sorts of mutagens, which change the DNA sequence. Mutagens include oxidizing agents, alkylating agents and also high-energy electromagnetic radiation such as ultraviolet light and X-rays.

Examples: radiation, nitrogen mustards, epoxides, methyl sulphonates, benzo [α] pyrene diol epoxide, acridines, aflatoxin and ethidium bromide etc.

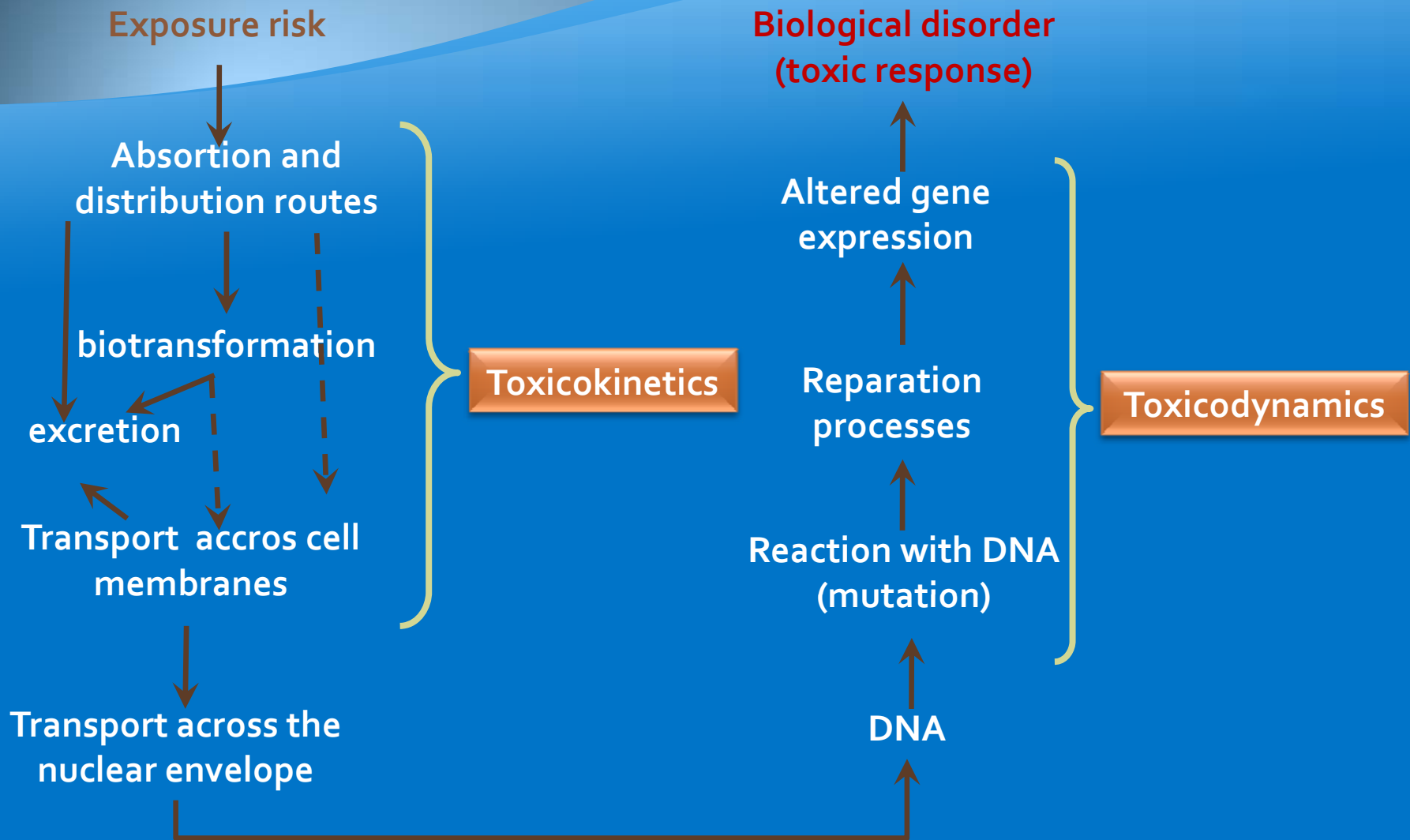


Toxic consequences of mutagenesis:

- Fertility disorders
- Embryonic and neonatal deaths
- Congenital malformation
- Hereditary diseases
- Cancer



Toxicological Paradigm



Mutation and Consequences

mutagen

Mutagenic event

Somatic cell

Germ cell

Cell death

cancer

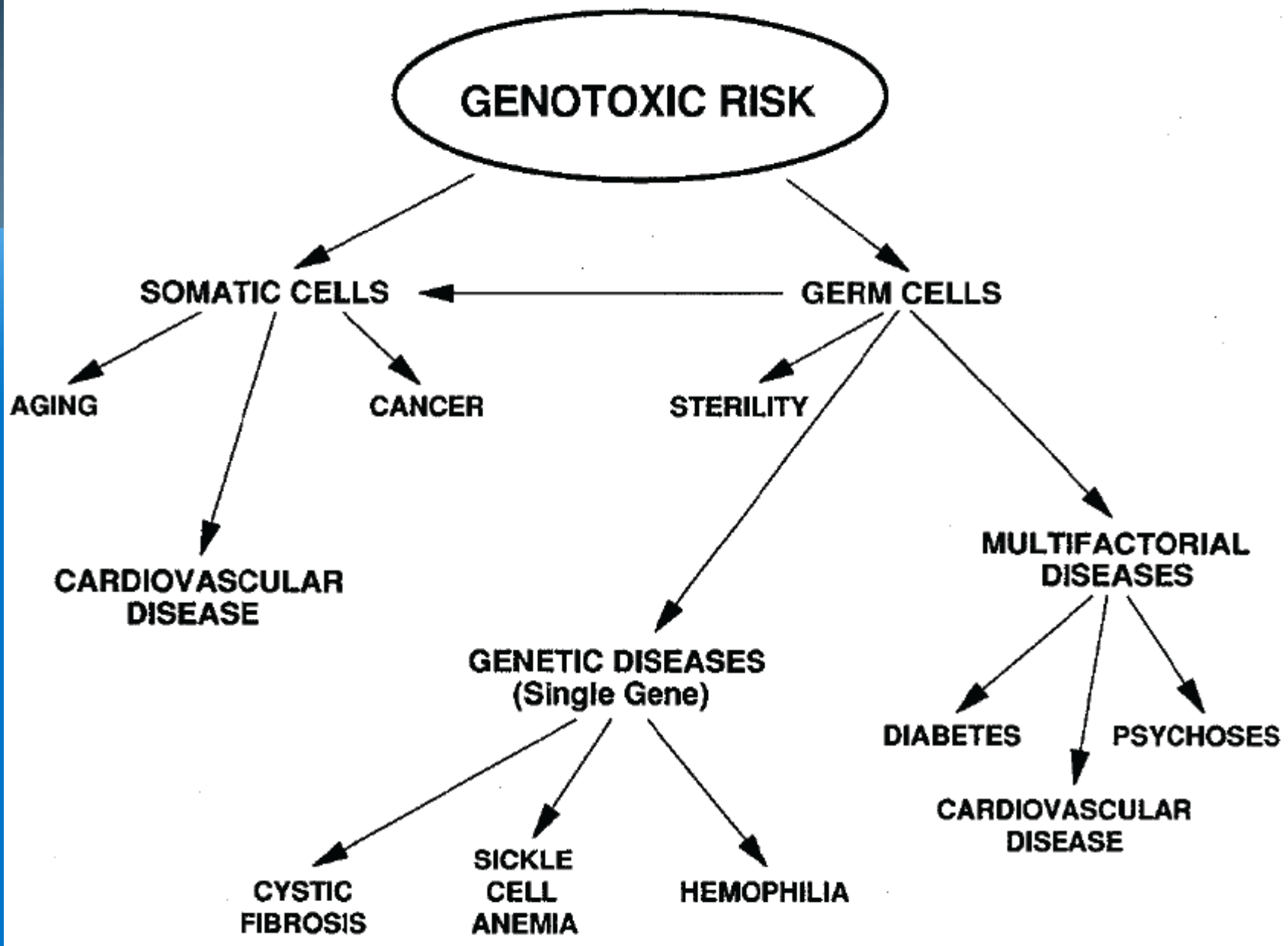
Foetal death

Recessive change

Recessive and dominant mutation

Dominant viable mutation

Damage transmissible to progeny



GENOTOXIC RISK

SOMATIC CELLS

GERM CELLS

AGING

CANCER

STERILITY

CARDIOVASCULAR DISEASE

GENETIC DISEASES (Single Gene)

MULTIFACTORIAL DISEASES

DIABETES

PSYCHOSES

CARDIOVASCULAR DISEASE

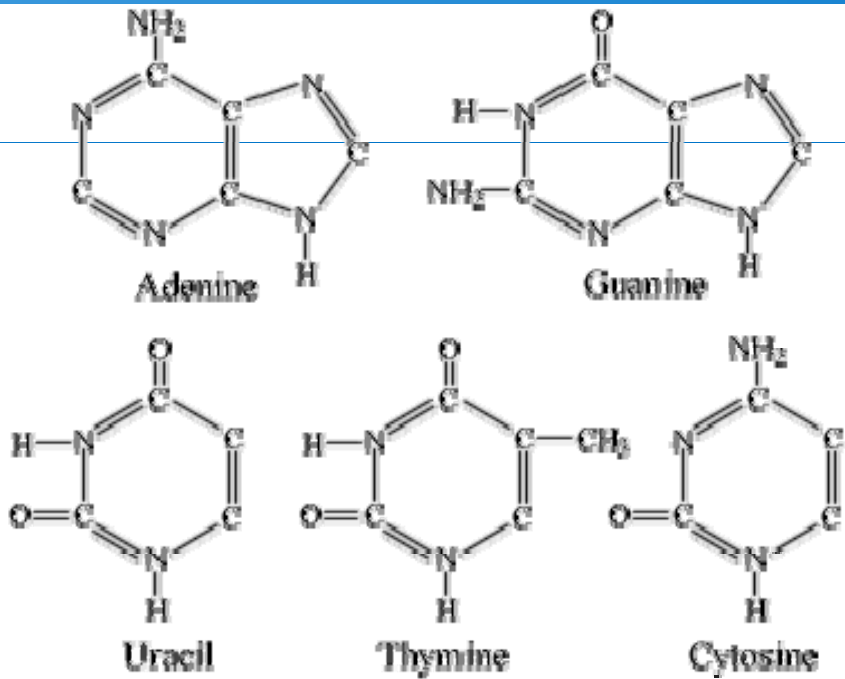
CYSTIC FIBROSIS

SICKLE CELL ANEMIA

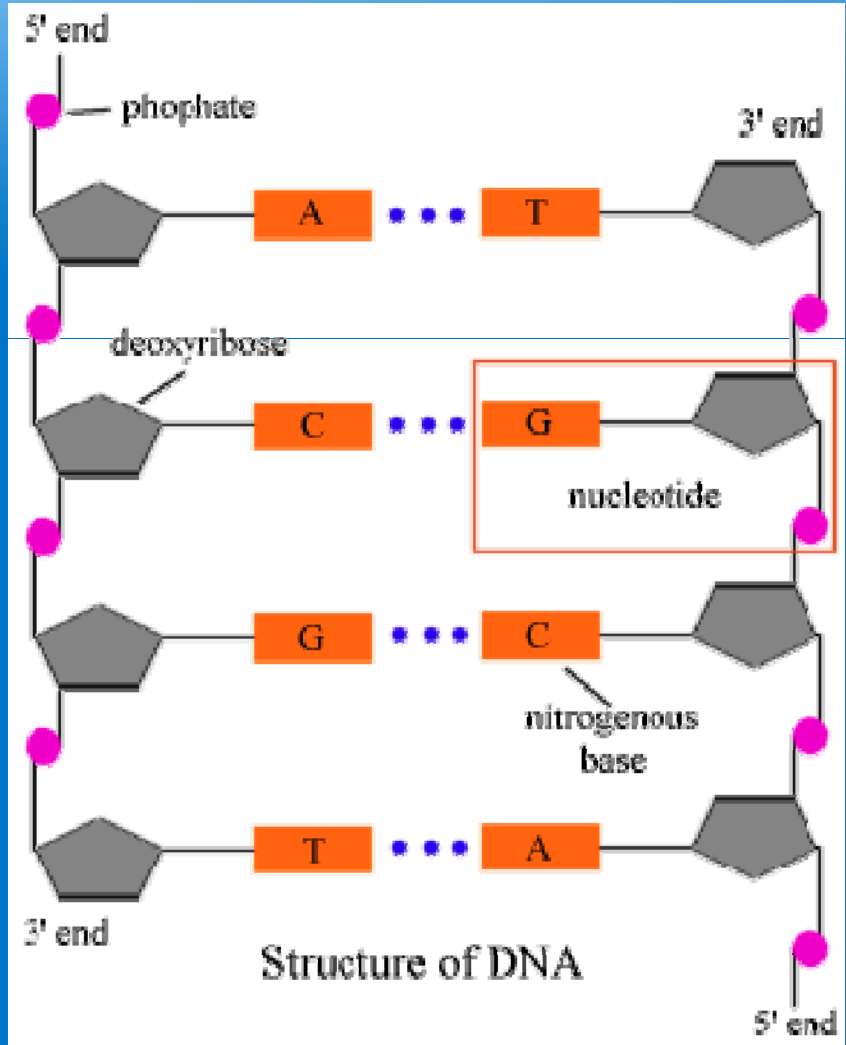
HEMOPHILIA

DNA's chemical composition & structure

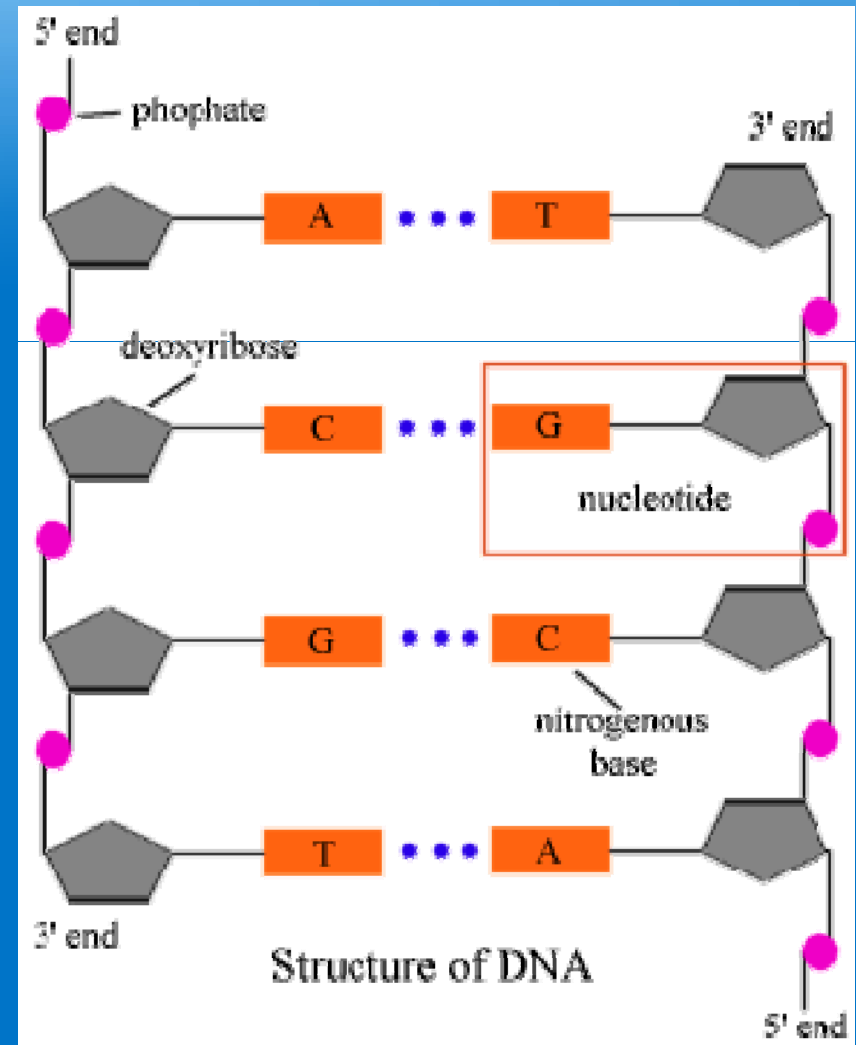
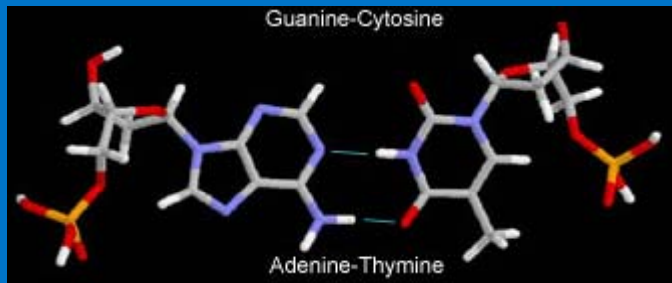
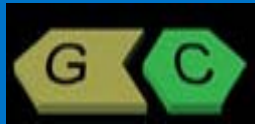
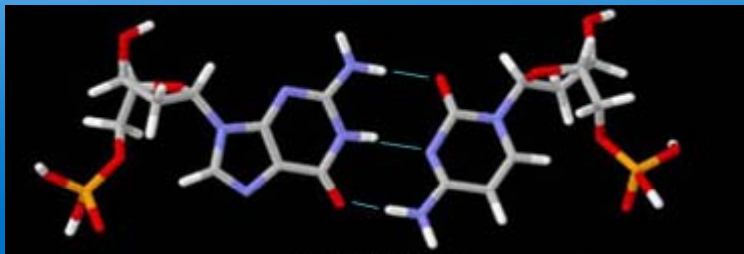
Purines



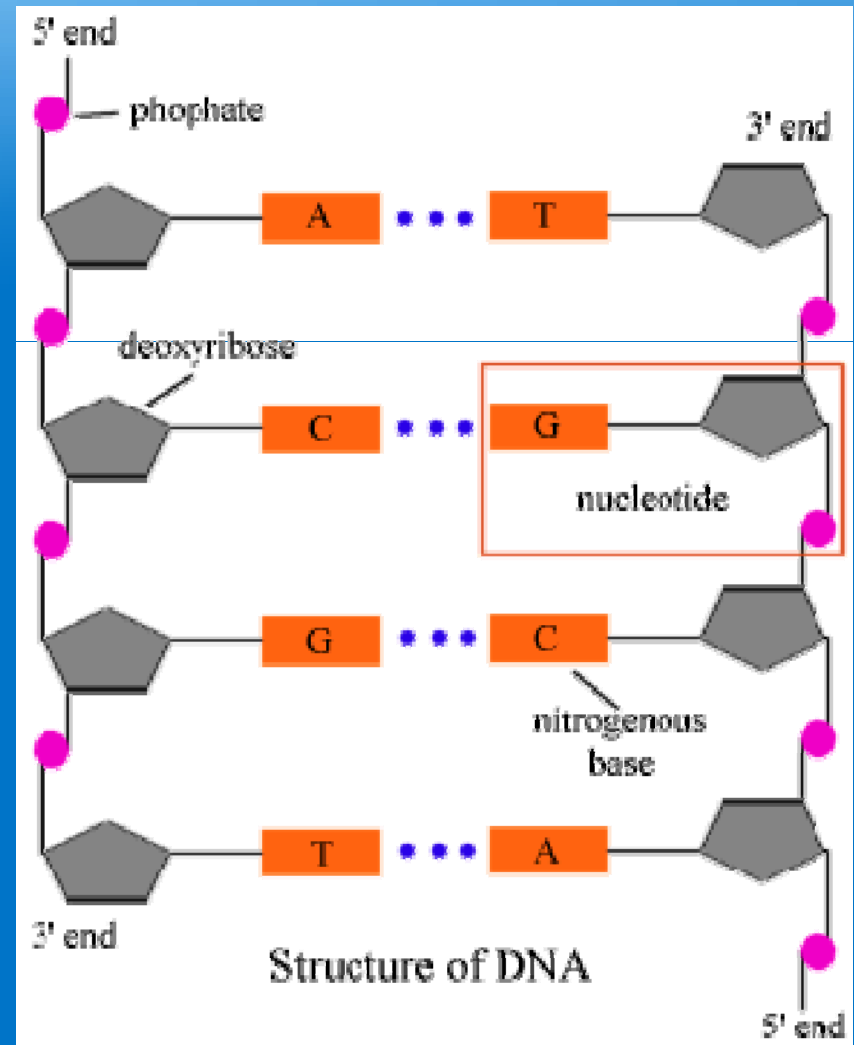
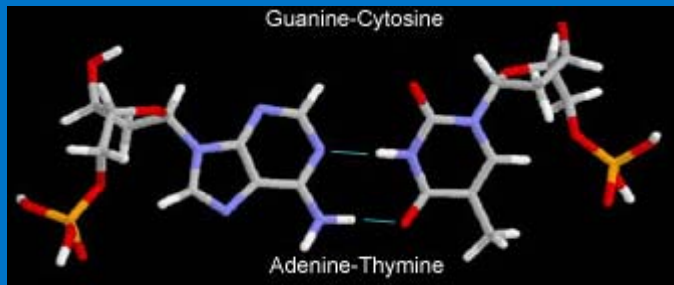
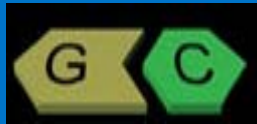
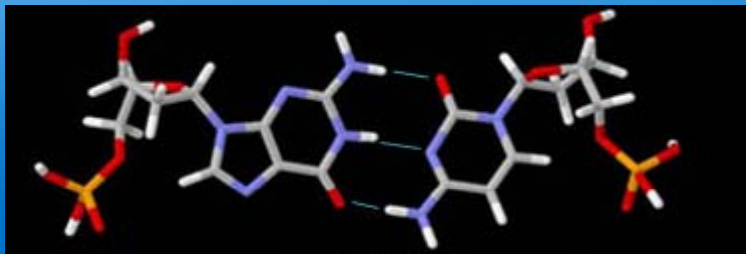
Pyrimidines



DNA's chemical composition & structure

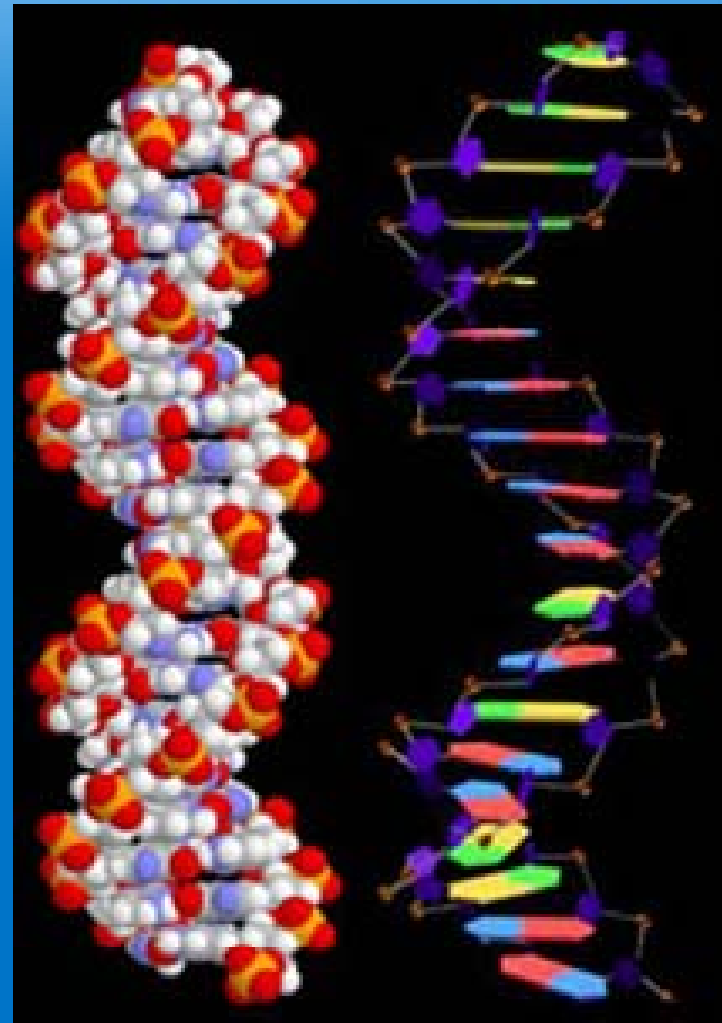
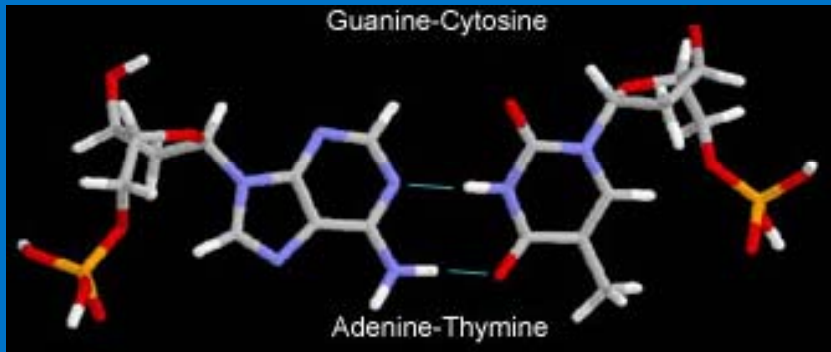
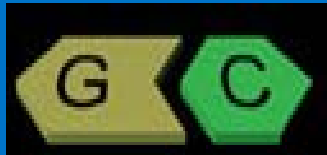
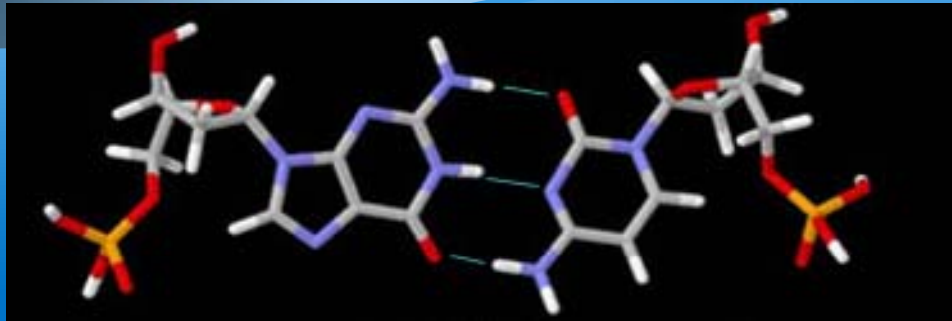


DNA's chemical composition & structure



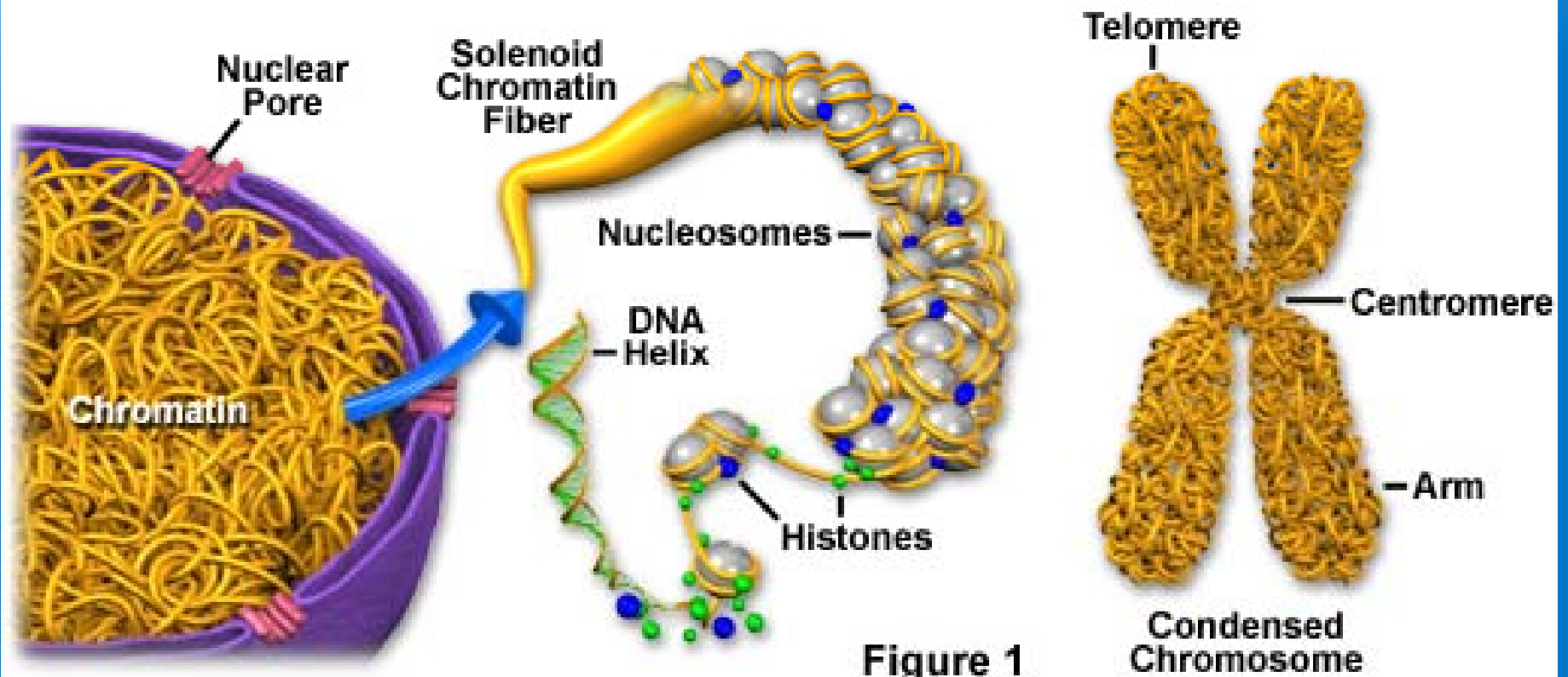
Structure of DNA

DNA's chemical composition & structure

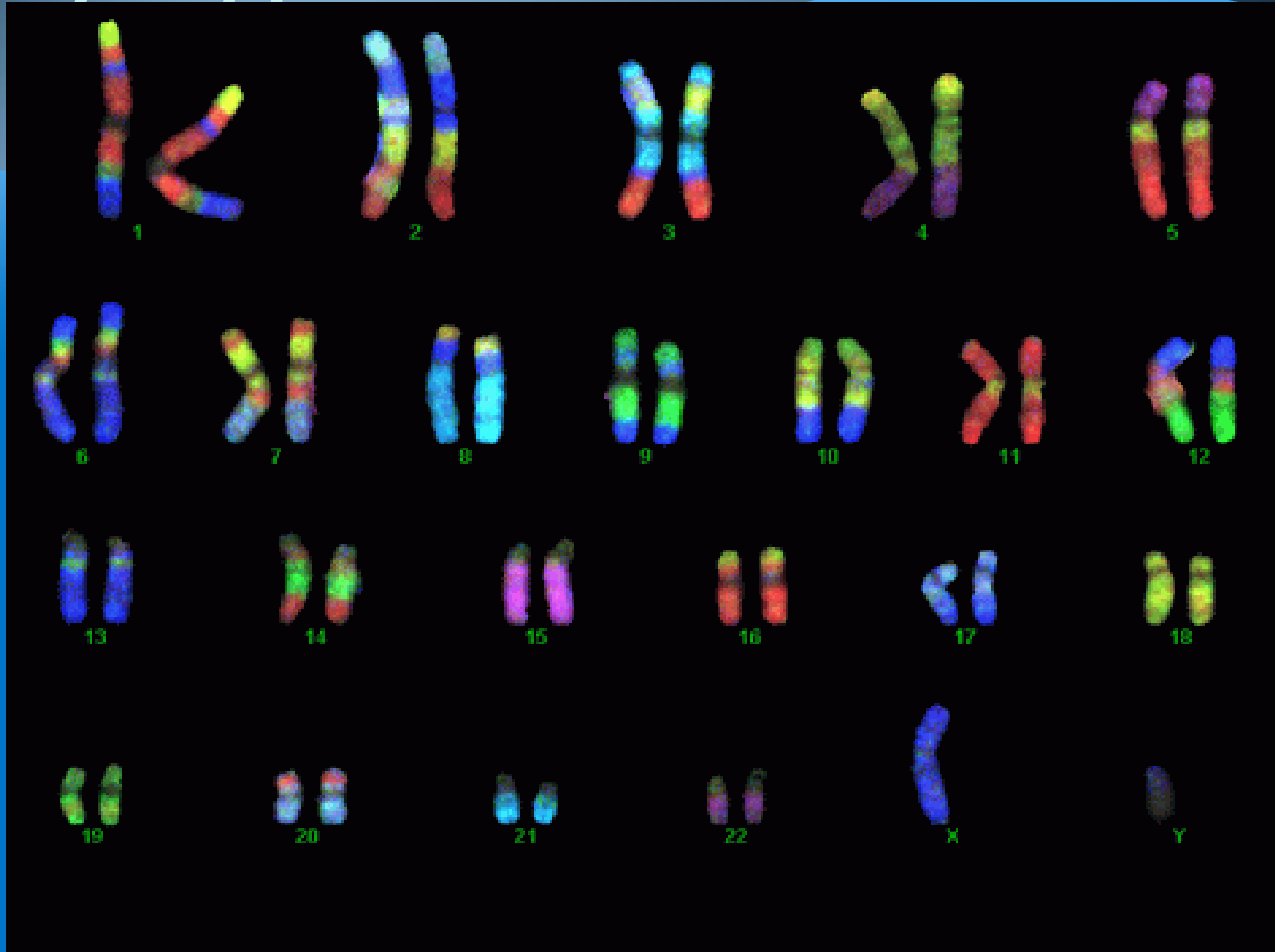


Nucleosomes & chromosomes

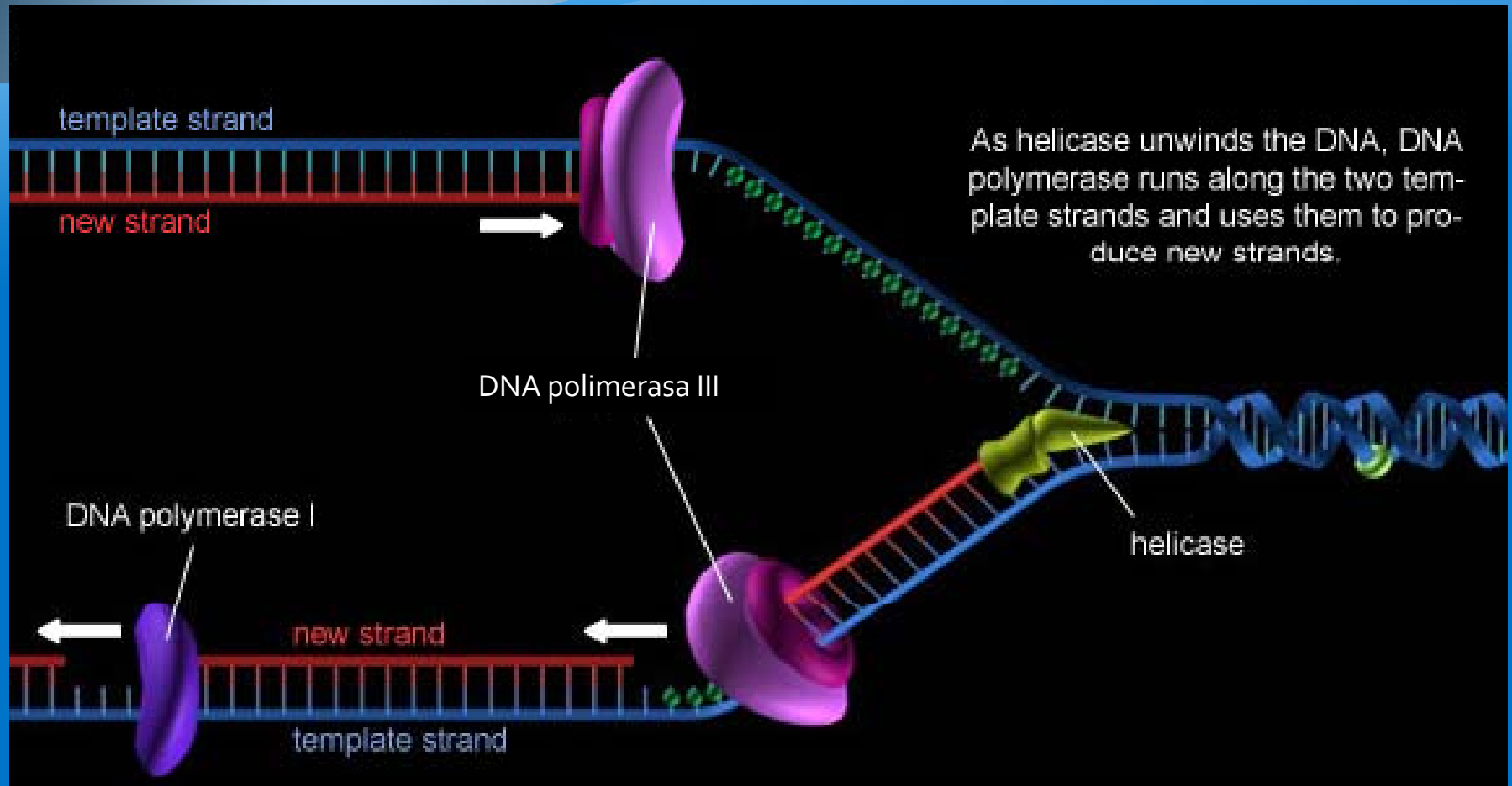
Chromatin and Condensed Chromosome Structure



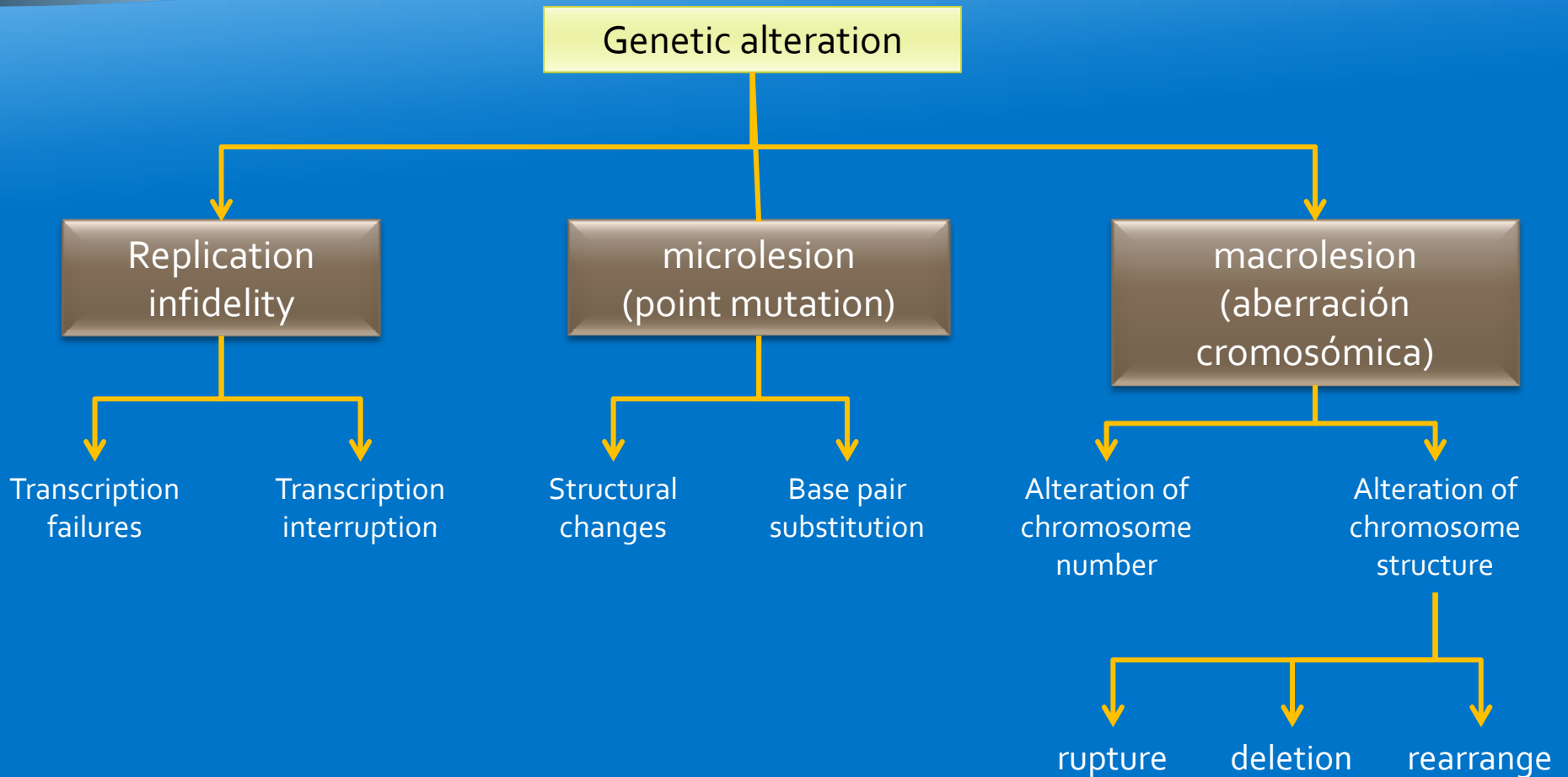
Karyotype



DNA replication



Principal types of genetic alterations



Testing mutagenic activity

Pruebas en procariontes

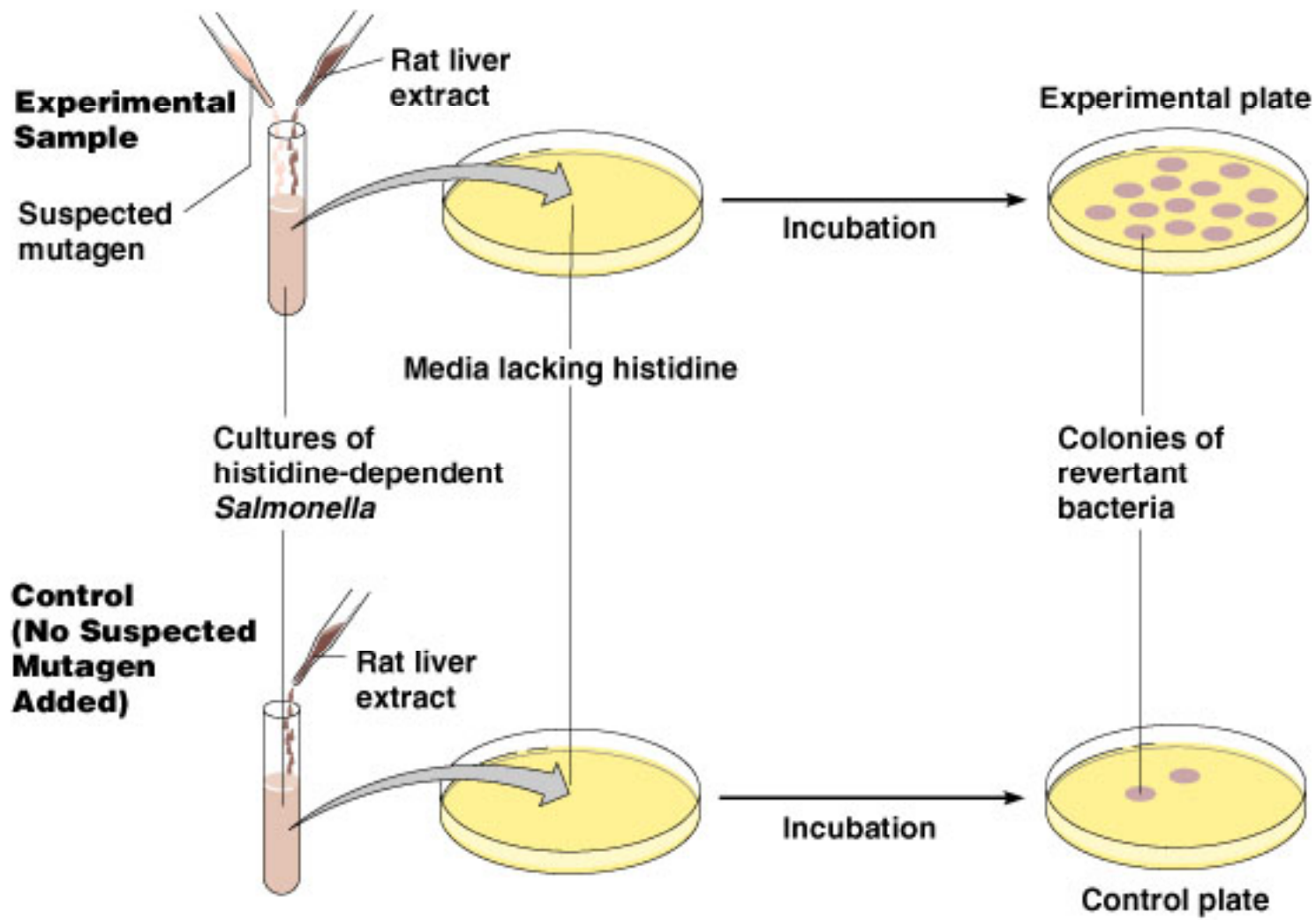
Ames Test: *Salmonella thypimurium*

Pruebas en eucariontes

In vitro: Sister chromatid exchange, comet assay, micronuclei.

In vivo: *Drosophila melanogaster*, micronuclei

Ames Test: Bacterial Reverse Mutation Assay



Example.

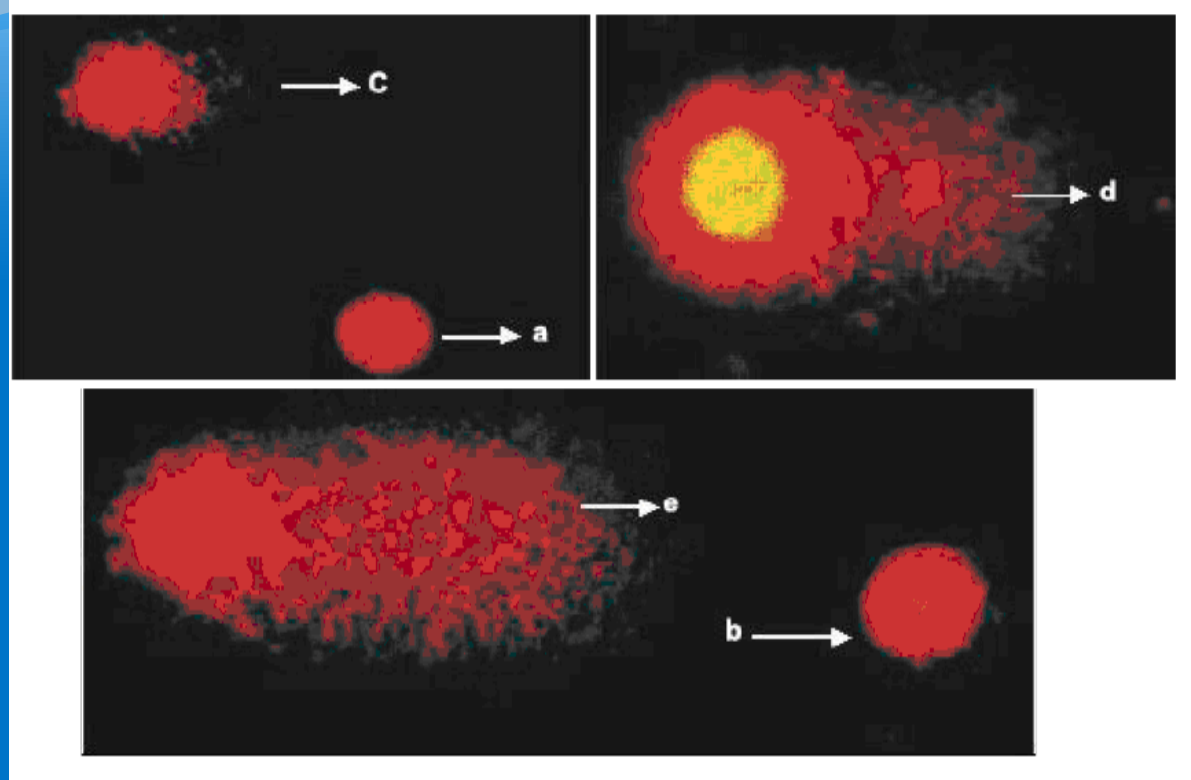
strain	Immediate incorporation		Pre-incubation	
		S9 mix		S9 mix
TA 98	1.92 ± 0.91	negativo	9.15 ± 0.90	2.43 ± 0.24
TA 100	5.02 ± 0.70	negativo	28.11 ± 2.85	6.66 ± 0.94
TA 102	15.05 ± 0.61	9.83 ± 0.57	30.38 ± 2.87	26.70 ± 2.87
TA 104	negativo	negativo	34.10 ± 2.28	negativo
YG 1024	negativo	negativo	14.47 ± 3.77	negativo

Cepas de Salmonella typhimurium	Tipos de mutaciones detectadas
TA 1535	Base pair substitution
TA 100	Similar to TA 1535, contains plasmid pKM101 which increases the sensitivity to DNA repair failure.
TA 102, TA 104	Base pair substitution
TA 1537	Base pair insertion, frameshit
TA 1538	Base pair insertion, frameshit..
TA 98	Contains plasmid pKM101.
TA 1535	Single base pair deletion
YG 1024	Highly sensitive to heterocyclic aromatic amines

Comet assay

Nuclei morphology in comet assay

- a) without damage
- b) small damage
- c) medium damage
- d) severe damage
- e) total damage

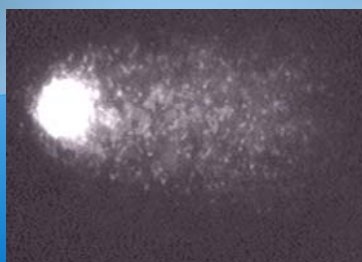


- The **Single Cell Gel Electrophoresis** assay (also known as **comet assay**) is an uncomplicated and sensitive technique for the detection of DNA damage at the level of the individual eukaryotic cell.

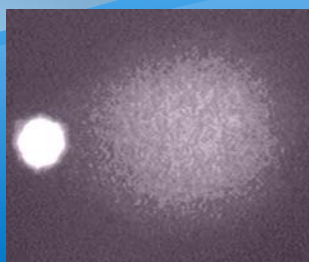
Daño oxidante al ADN



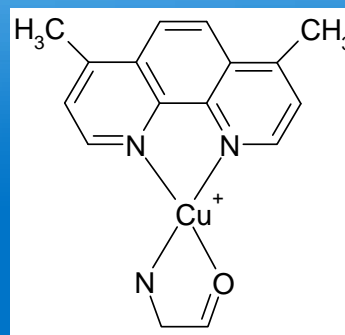
Tipo 1
Nuclioid



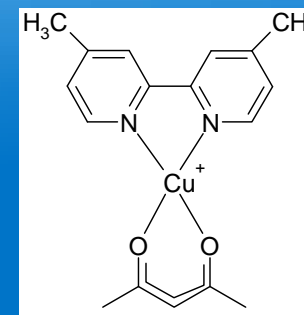
Tipo 2
clasico comet



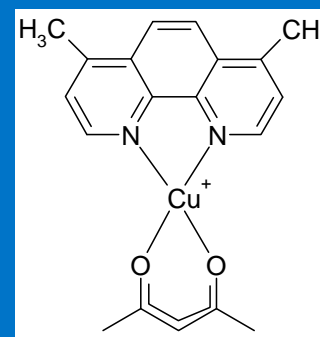
Tipo 3
apoptotic comet



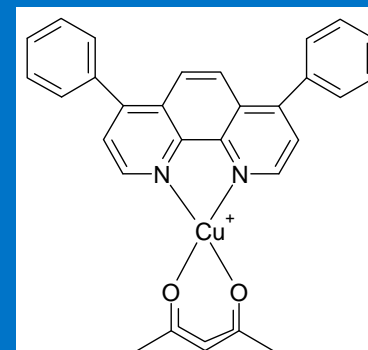
Cas II-gly



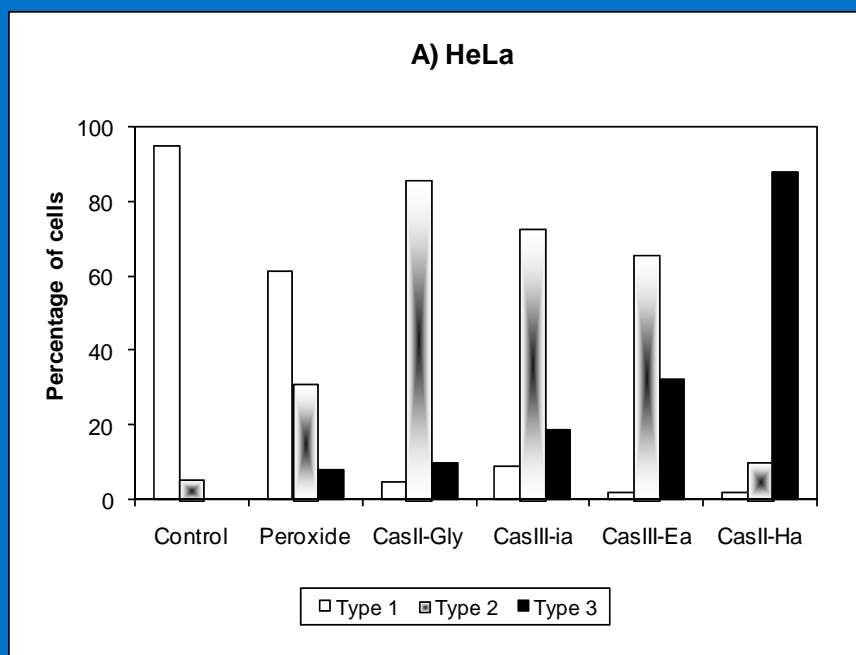
Cas III-ia

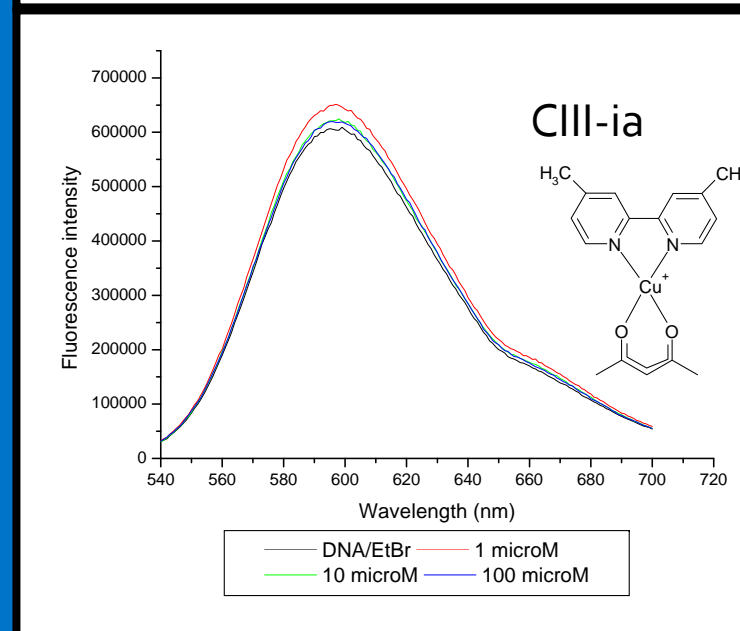
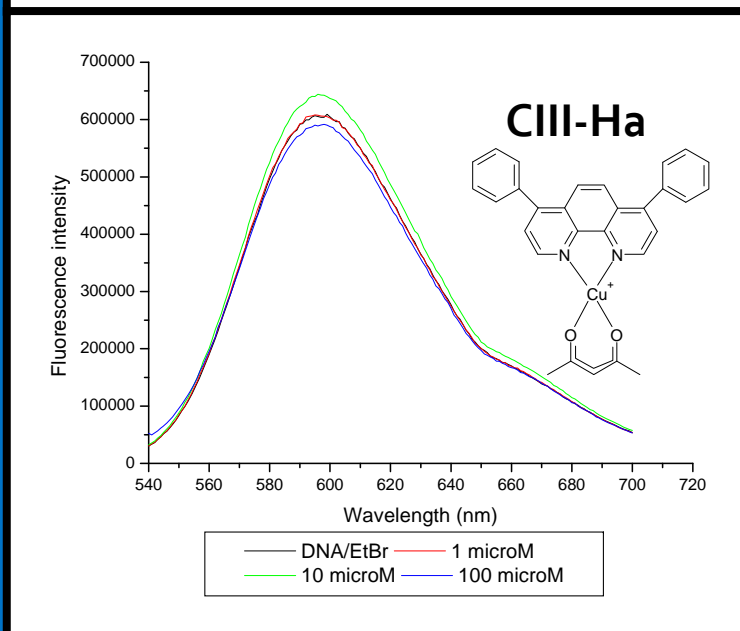
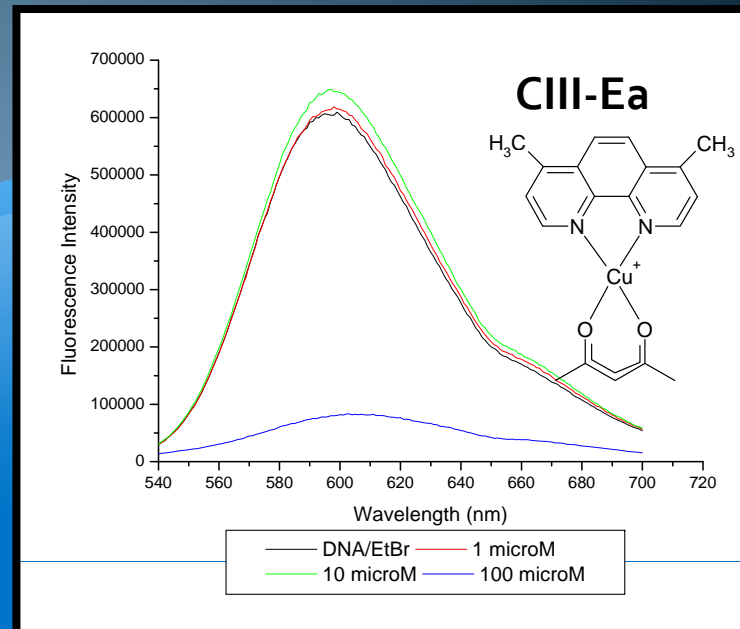
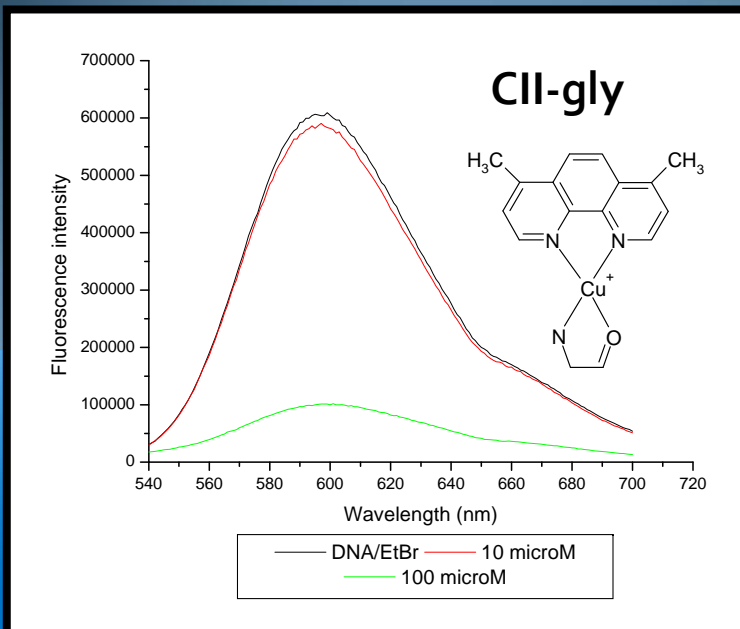


Cas III-Ea



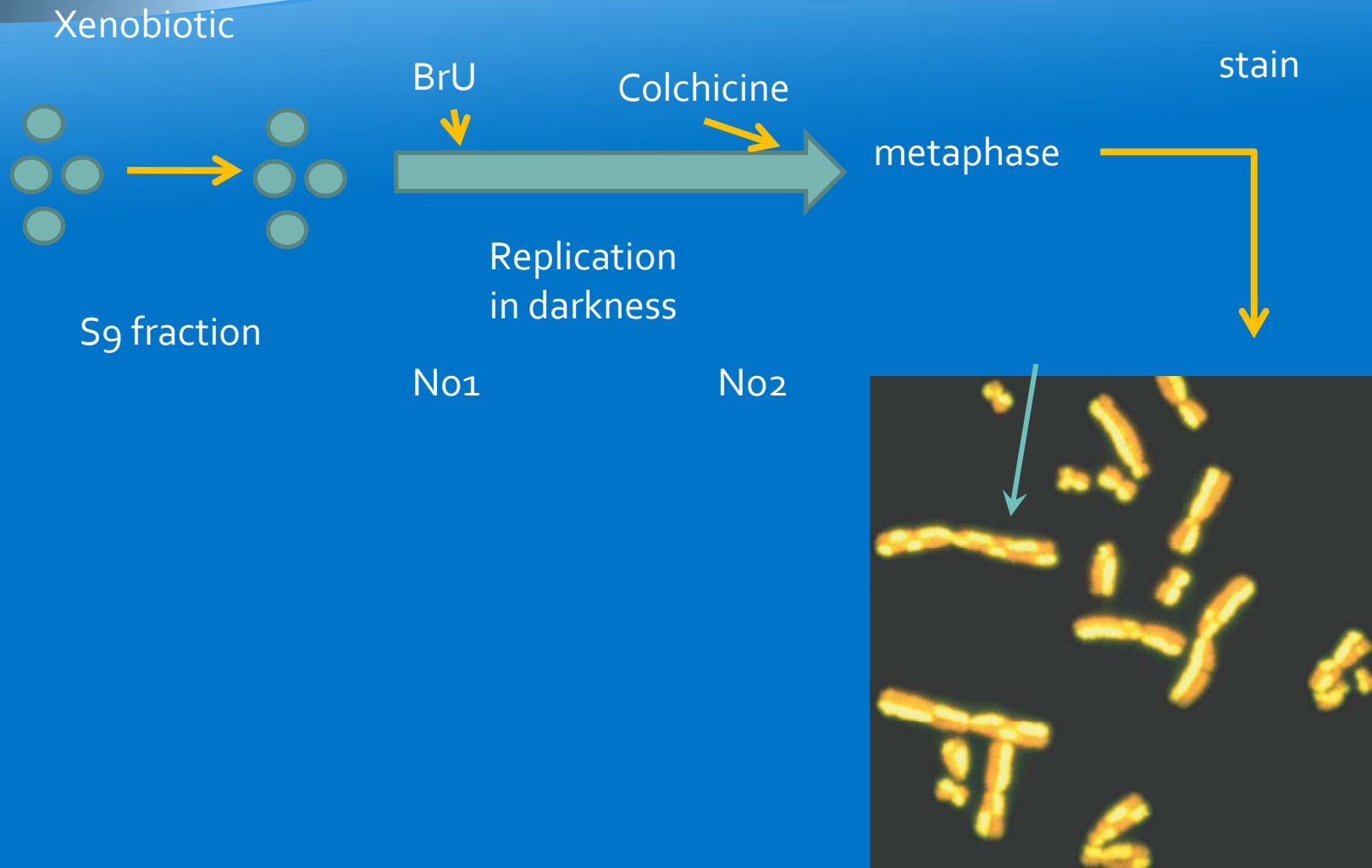
Cas III-Ha



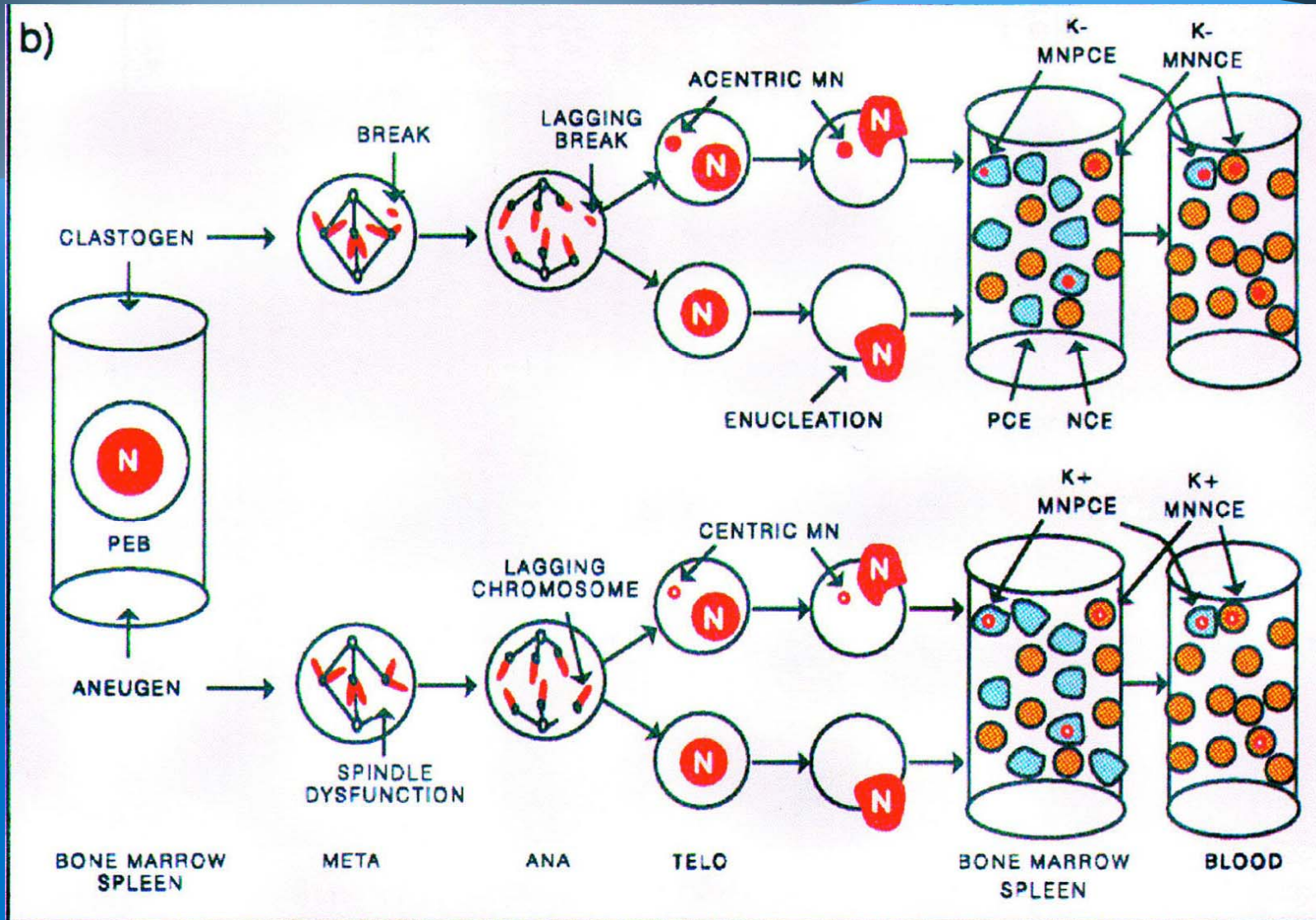


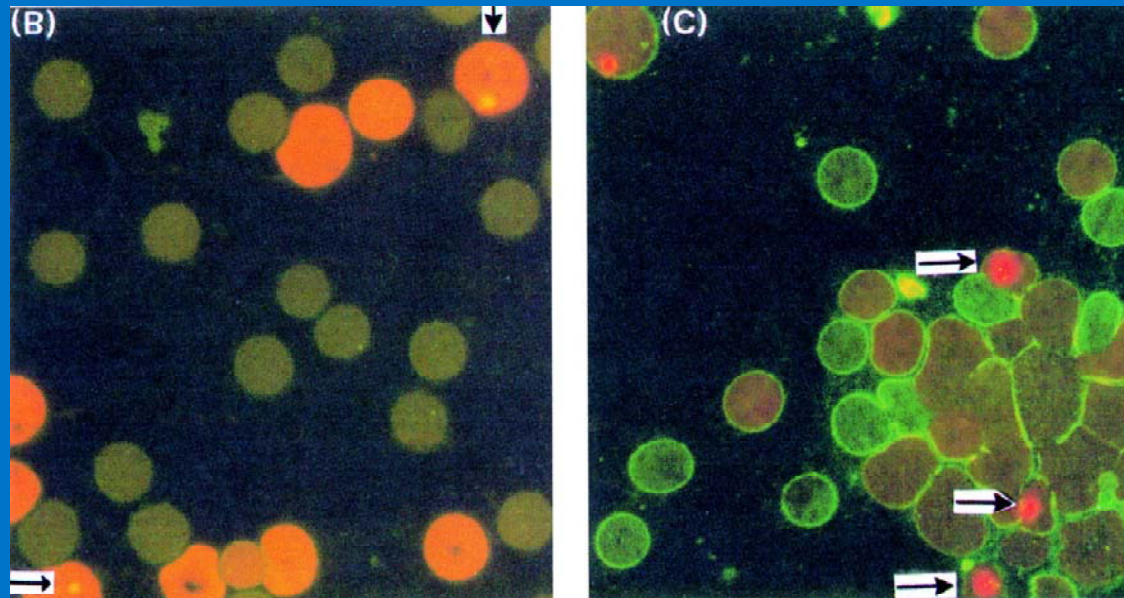
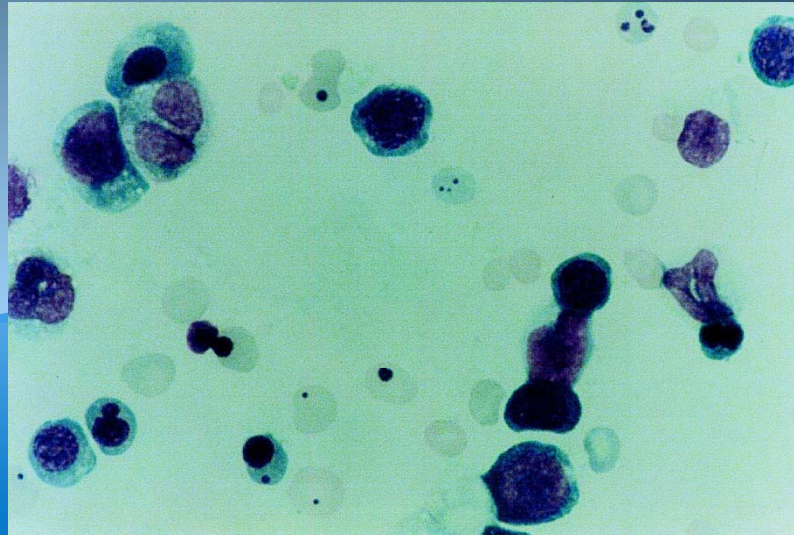
ADN 20 microg/ml; Et Br 1 microM; Excitation 526; detection 540-700;
 Dr. Jorge H. Serment Guerrero.

Sister chromatids exchange.



Micronucleus test





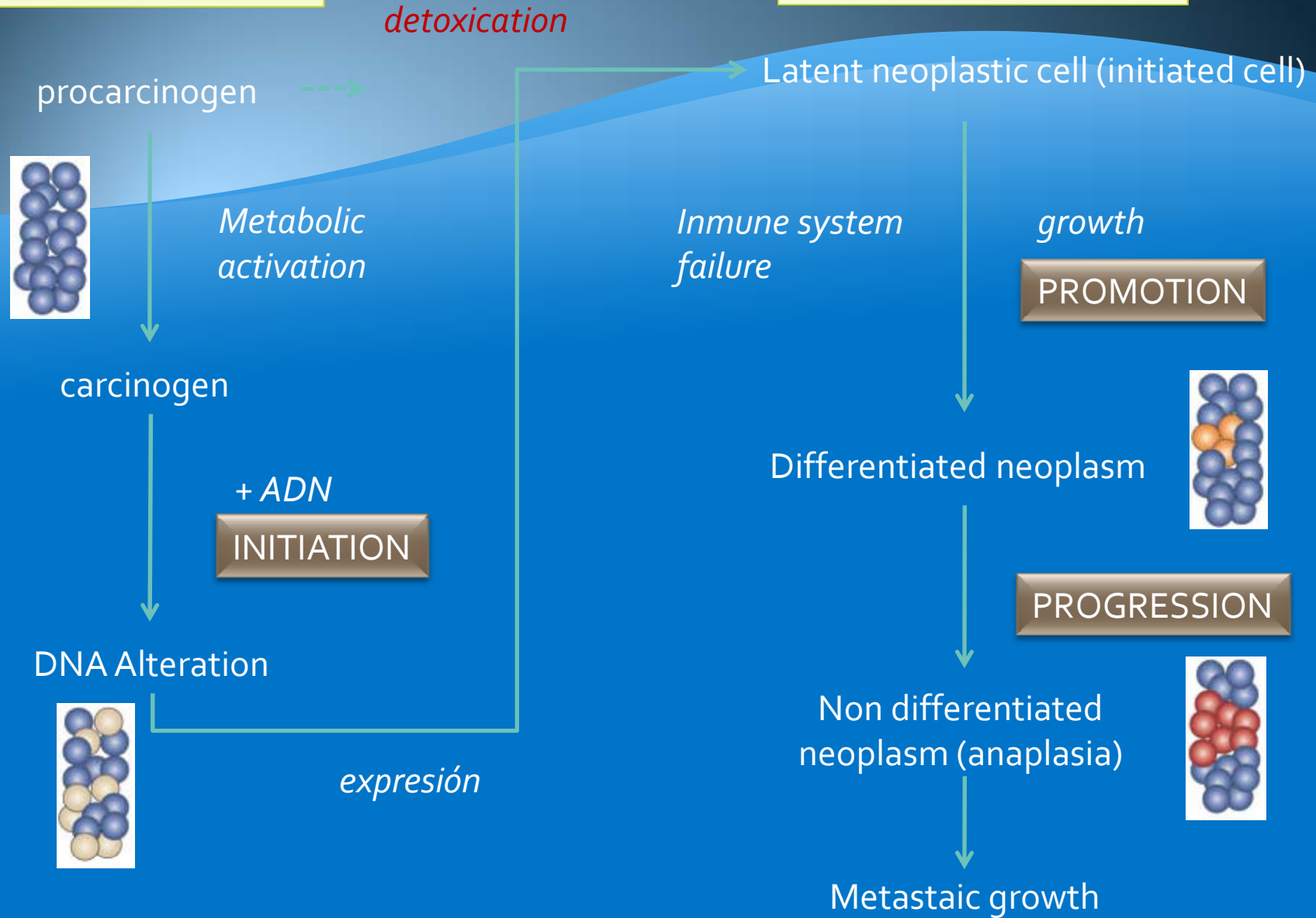
G. Krishna, M. Hayashi / Mutation Research 455 (2000) 155–166

Drosophila melanogaster.



Neoplastic conversion

Neoplastic development



Classification of Chemical Carcinogens in Relation to Their Action on One or More Stages of Carcinogenesis

Initiating agent (incomplete carcinogen): a chemical capable only of initiating cells

Promoting agent: a chemical capable of causing the expansion of initiated cell clones

Progressor agent: a chemical capable of converting an initiated cell or a cell in the stage of promotion to a potentially malignant cell

Complete carcinogen: a chemical possessing the capability of inducing cancer from normal cells, usually possessing properties of initiating, promoting, and progressor agents.

Bioensayos

Short term (1-3 m)	Medium term (2-8 m)	Long term (18-24 m)
Gene mutation assays in vitro: Ames , mouse lymphoma thymidine kinase (TK), Chinese hamster ovary (CHO)	Qualitative and quantitative analysis of preneoplasia	Two years chronic bioassays in animals for potential carcinogens

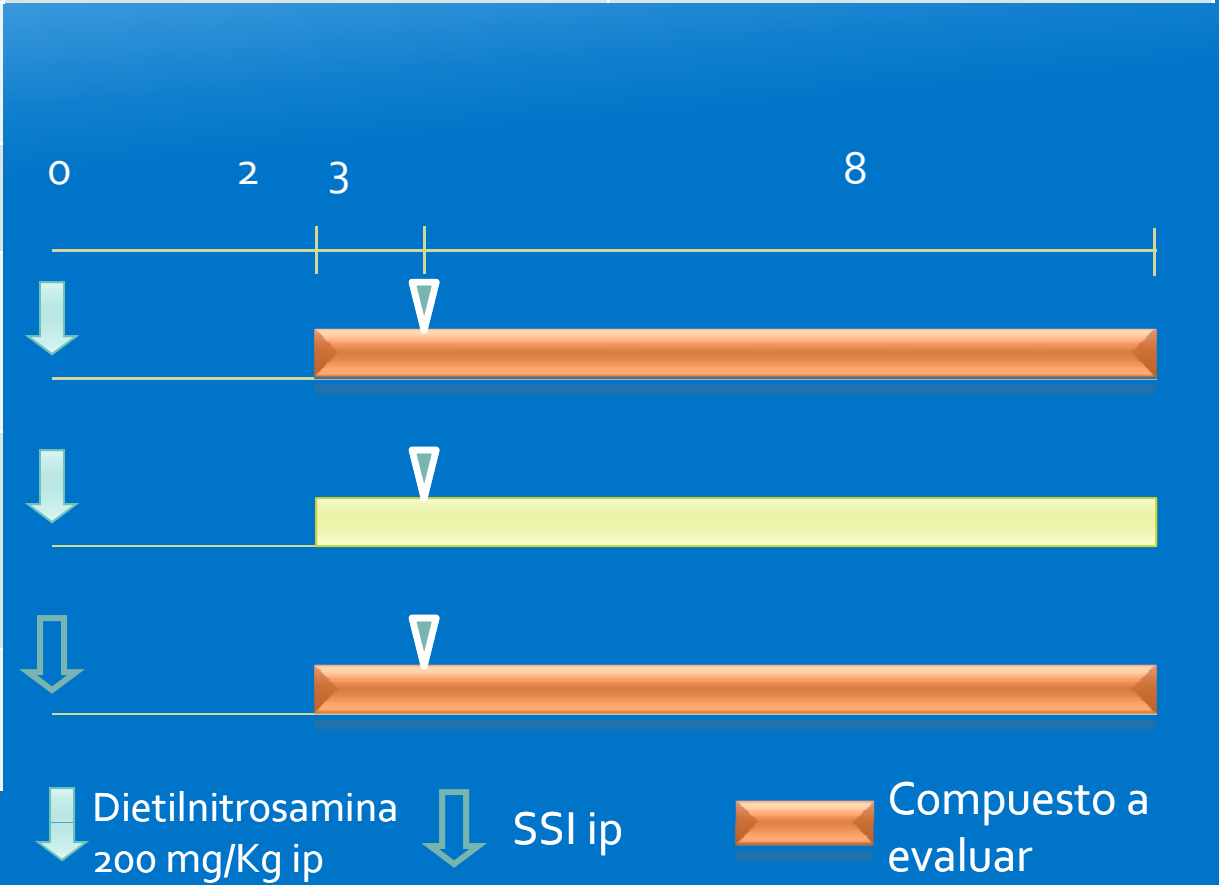
Gene mutation assays in vivo:
 Dominant lethal assay, Sperm abnormality induction.

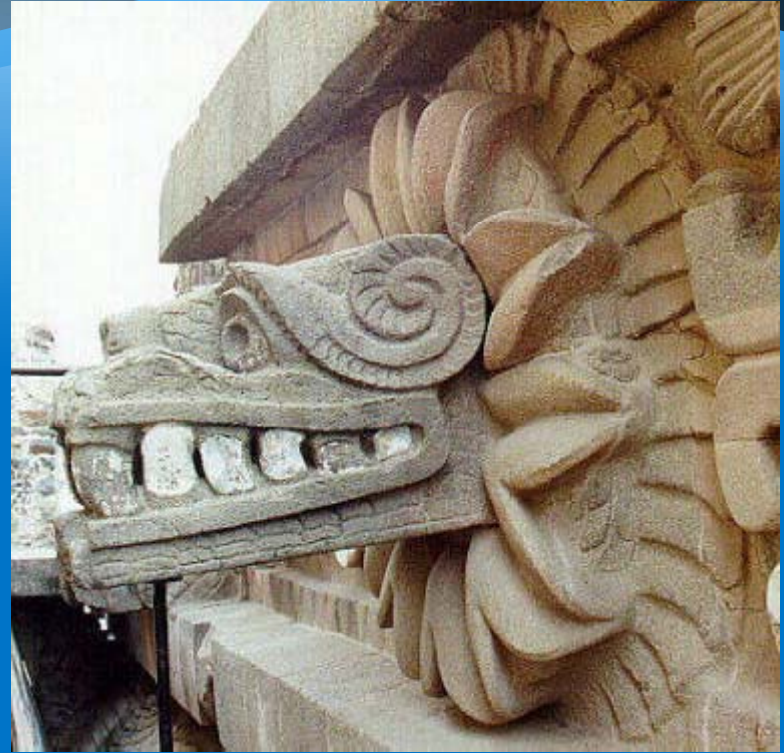
Mutation induction in transgenes in vivo

Chromosomal alterations in vivo:
 Heritable translocation test (mice), Rat bone marrow clastogenesis in vivo, micronuclei test

Chromosomal alterations in vitro:
 Mitotic recombination, mitotic crossing over, or mitotic gene conversion in yeast, Induced chromosomal aberrations in cell lines, **Sister chromatid exchange**

Primary DNA damage: DNA repair in vivo or in vitro, rodent liver unscheduled DNA synthesis induction.





THANK YOU!!!!!!