Ankara University
Faculty of Pharmacy

Analysis and evaluation of toxicity caused by drugs and toxic chemicals by cytogenetic tests

Assoc. Prof. İlker ATEŞ

What is Genotoxicity?

The fact that a chemical substance is genotoxic means that it can bind to the nucleophilic regions of the macromolecules (DNA…) due to its electrophilic property. Since DNA is a molecule that carries hereditary information; genotoxicity can be defined as a toxic effect that occurs in the genetic material of cells.

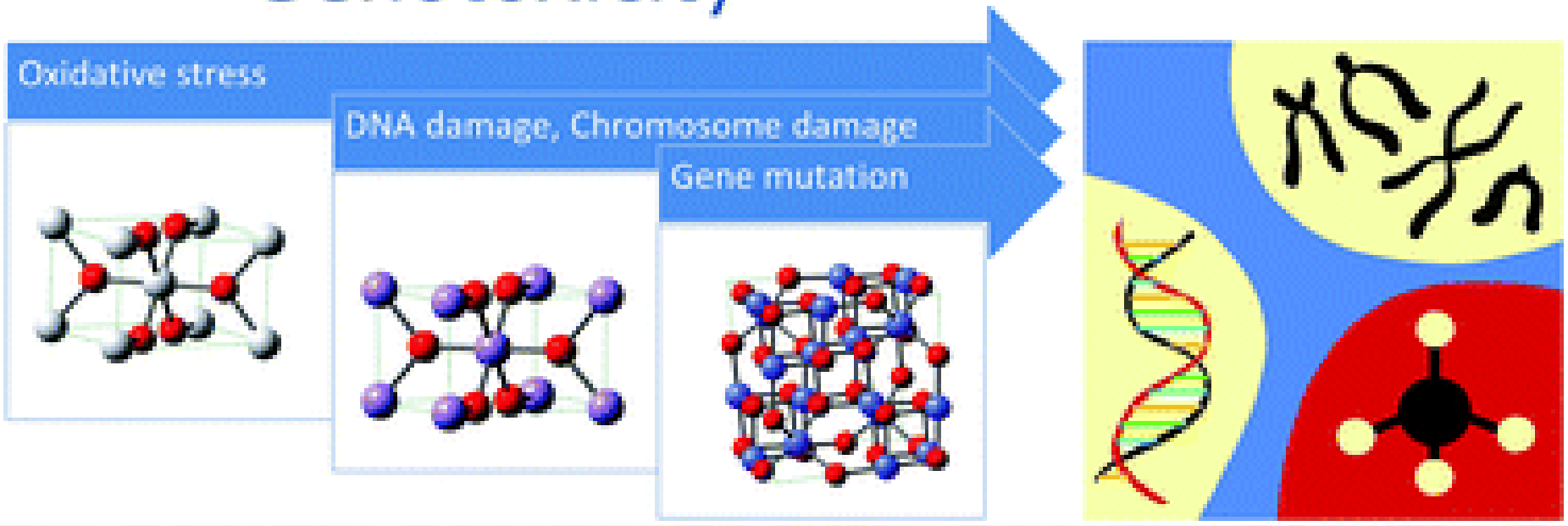
What is Genotoxicity?

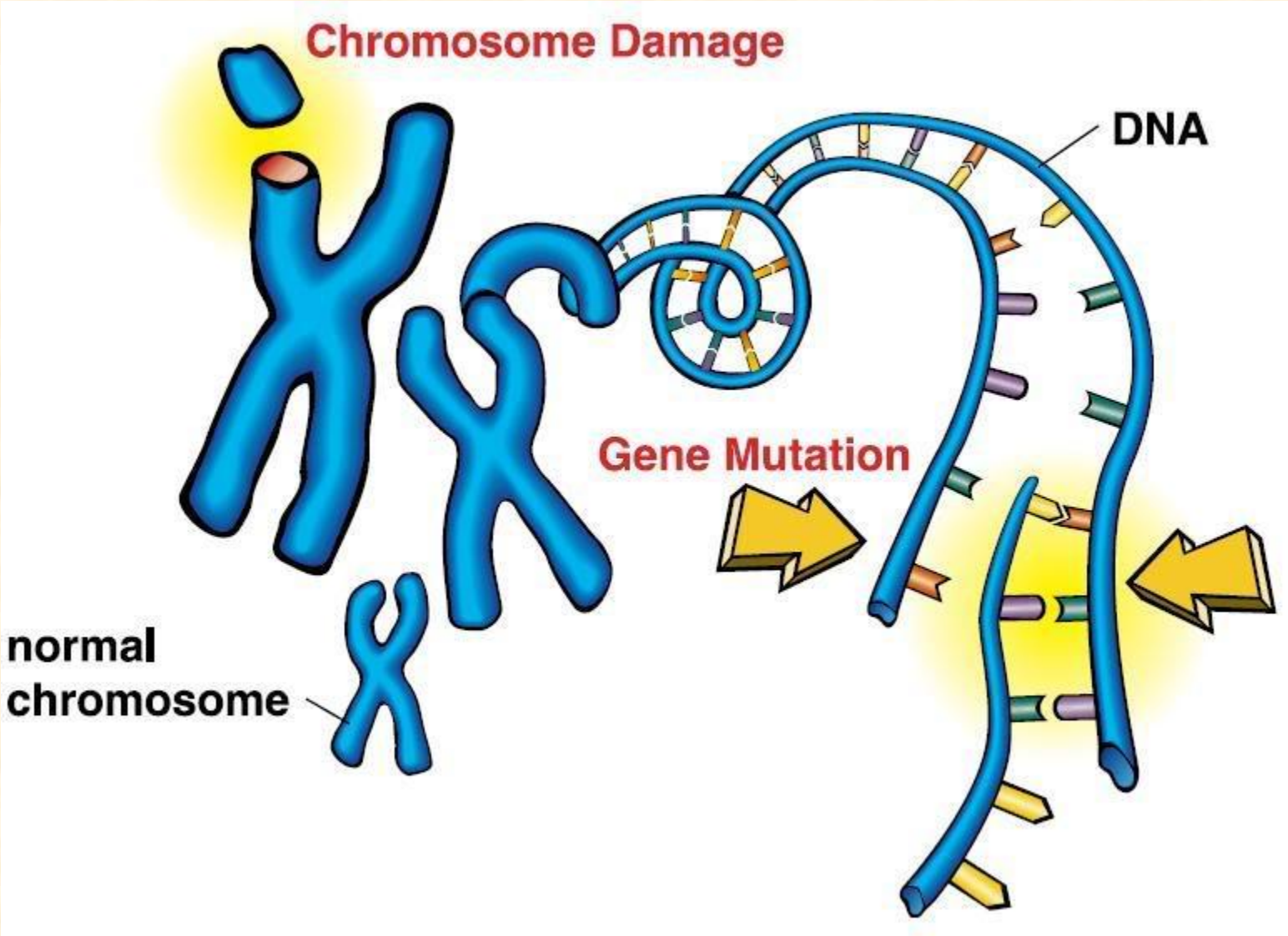
If a more detailed definition is made considering the direct and indirect effects that may occur in DNA:

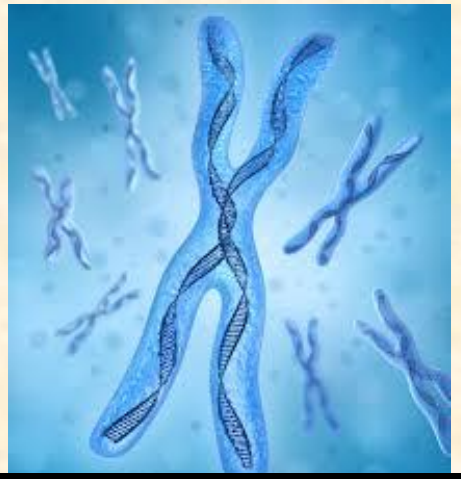
- Induction of mutation
- Observation of indirect events related with mutation (unplanned DNA synthesis etc.)
- Observation of DNA damage (adduct products formation etc.)

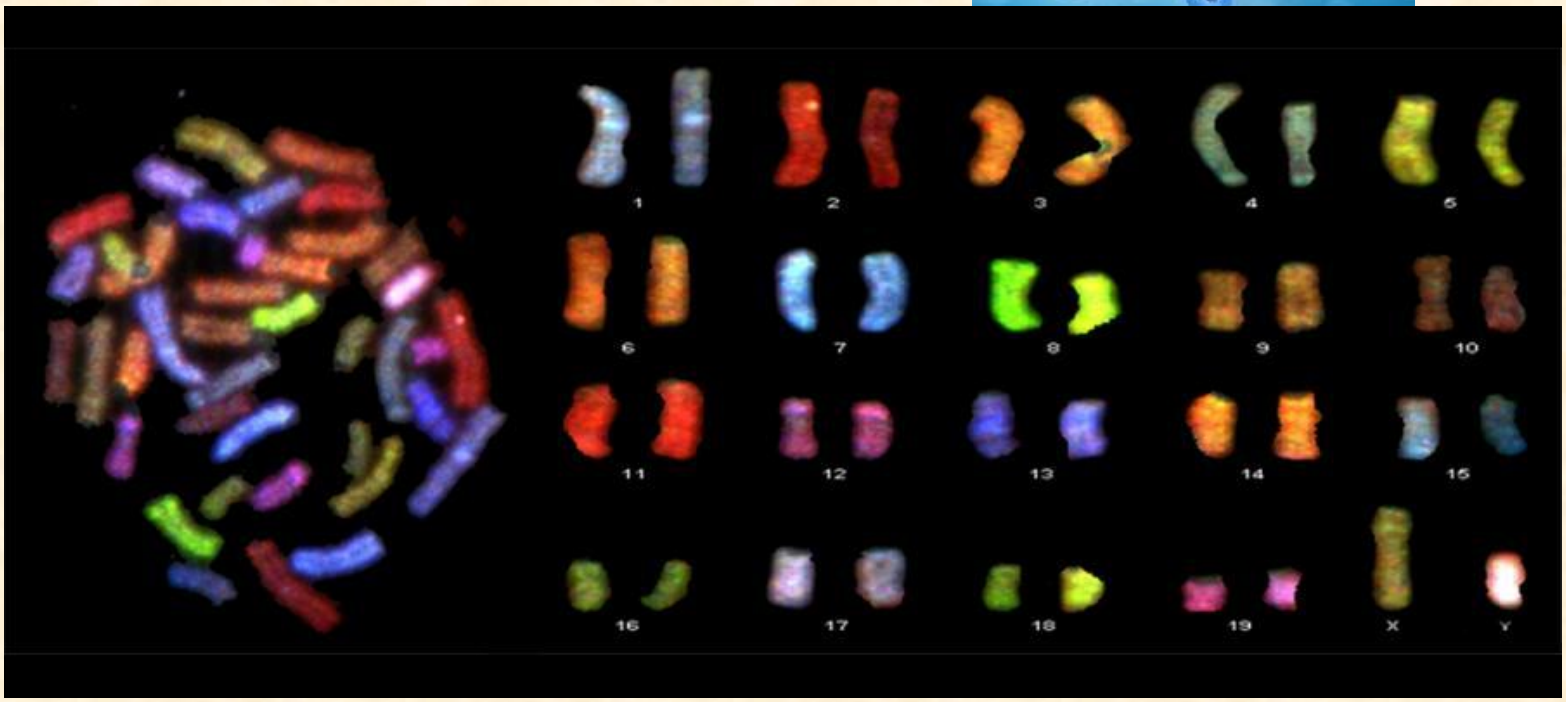
can be defined as a sequence of events (which can cause mutation).

Genotoxicity




Chromosome Damage
DNA
Gene Mutation
normal
chromosome



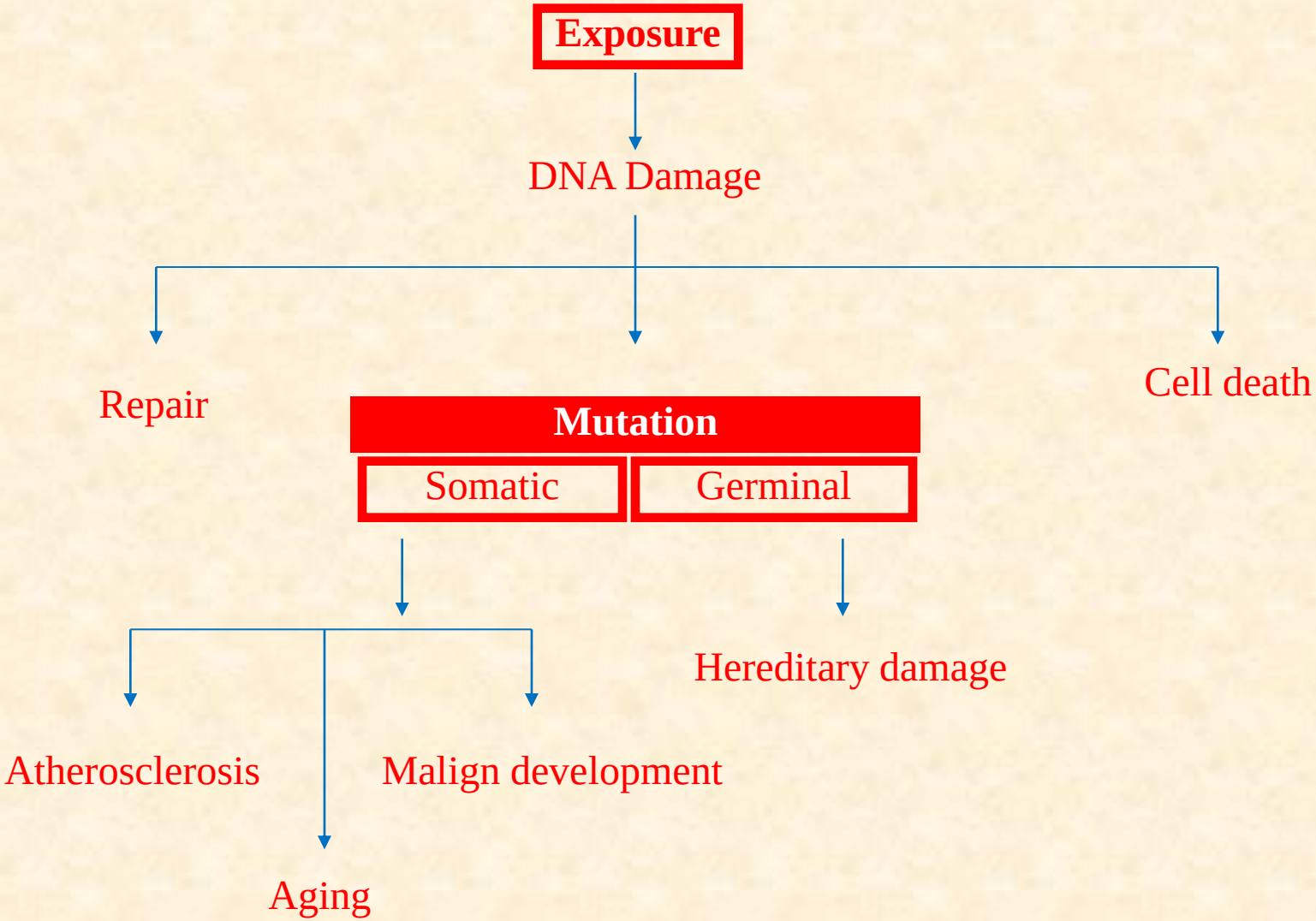

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
X
Y

Genotoxicity, Mutation and Cancer

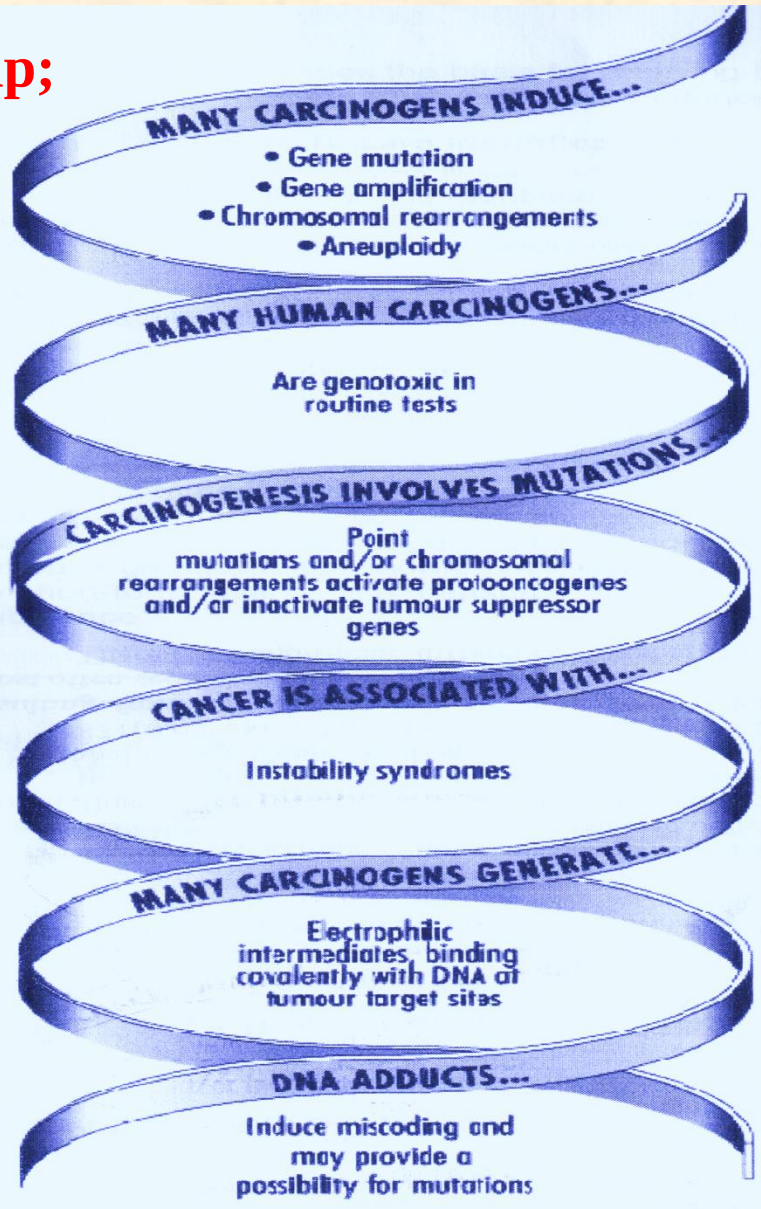
Mutation is permanent hereditary changes in somatic or germinal (sex) cells. Thus, the mutation can cause body cells to change and/or be transported to other generations by germinal cells. A genotoxic effect can often be repaired inside the cell and not cause mutations. However, genotoxic effects that cause irreparable damage are known to cause mutations.

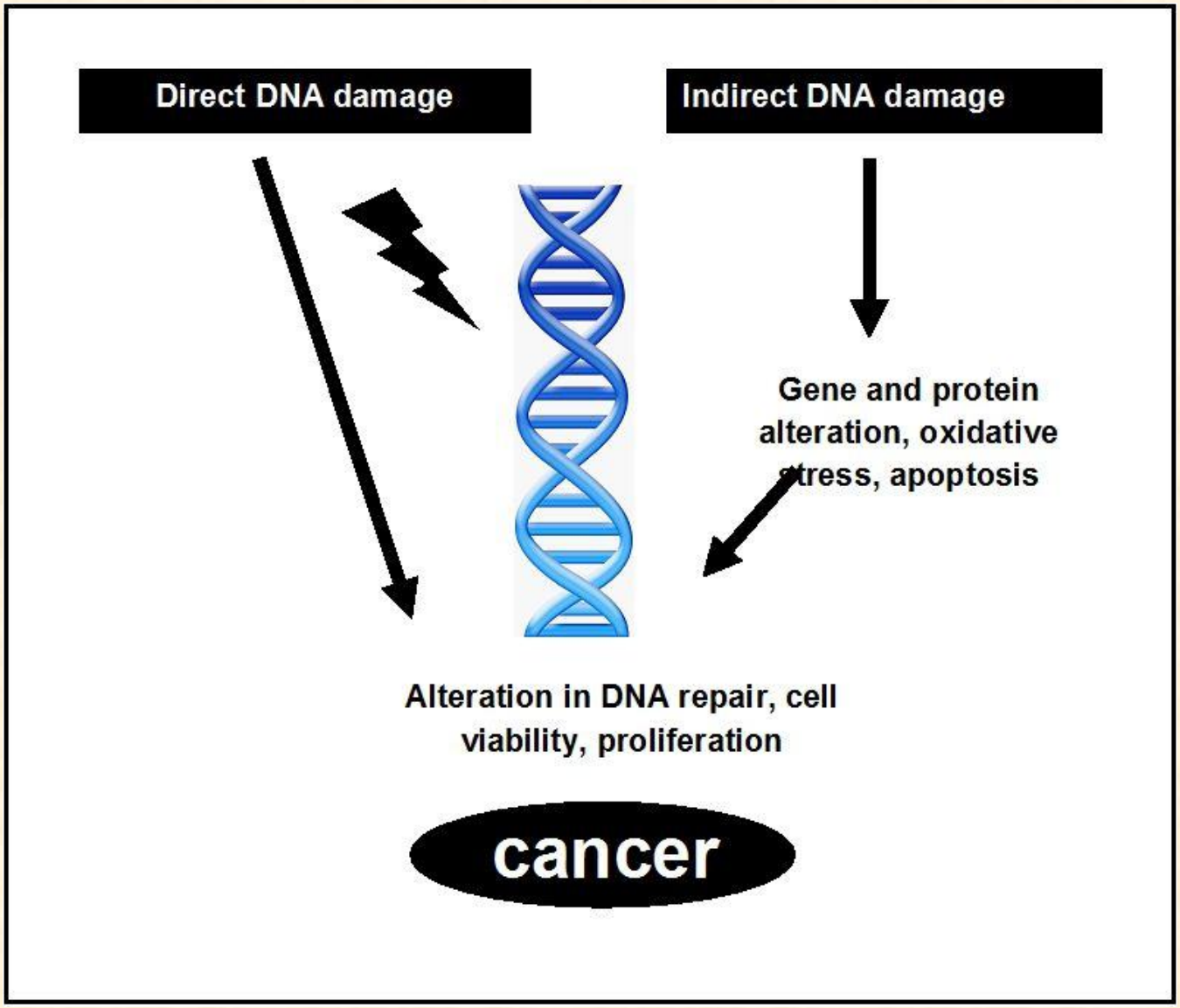
Many scientific studies support a significant relationship between genotoxicity and cancer.

This relationship is the basis for the use of genotoxicity biomarkers as an indicator in human monitoring studies against the risk of cancer formation.


Exposure
DNA Damage
Repair
Mutation
Somatic
Germinal
Cell death
Hereditary damage
Atherosclerosis
Malign development
Aging

Genotoxicity and cancer relationship;




Direct DNA damage
Indirect DNA damage
Gene and protein alteration, oxidative stress, apoptosis
Alteration in DNA repair, cell viability, proliferation
cancer

Genetic toxicity in danger or risk definition;

The role of genetic changes in cancer development has further increased the importance of genetic toxicity tests in identifying potential carcinogens. Accordingly, short-term test methods that can show many cytogenetic changes that are thought to be related to cancer development have been developed.

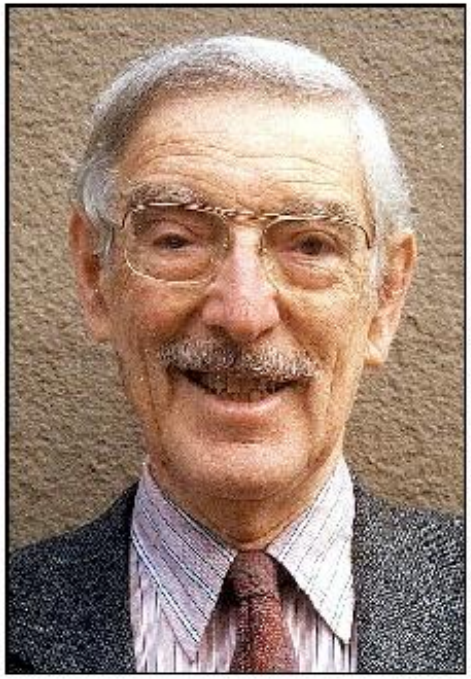
Genetic toxicity in danger or risk definition;

Many studies comparing the carcinogenic effects of chemical substances with these short-term tests have been conducted and are still continuing. At this point, no short-term test alone is sufficient to predict cancer. For this reason, more than one short-term test should be performed together in order to indicate that a chemical can cause cancer in humans.

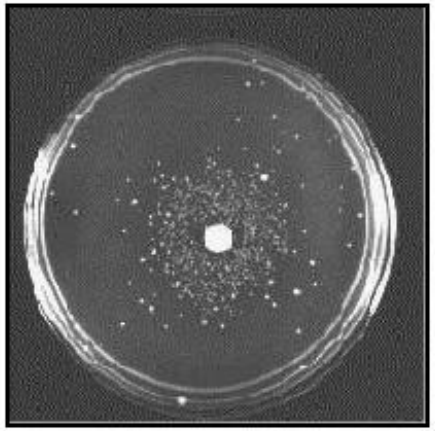
Genetic toxicity in danger or risk definition;

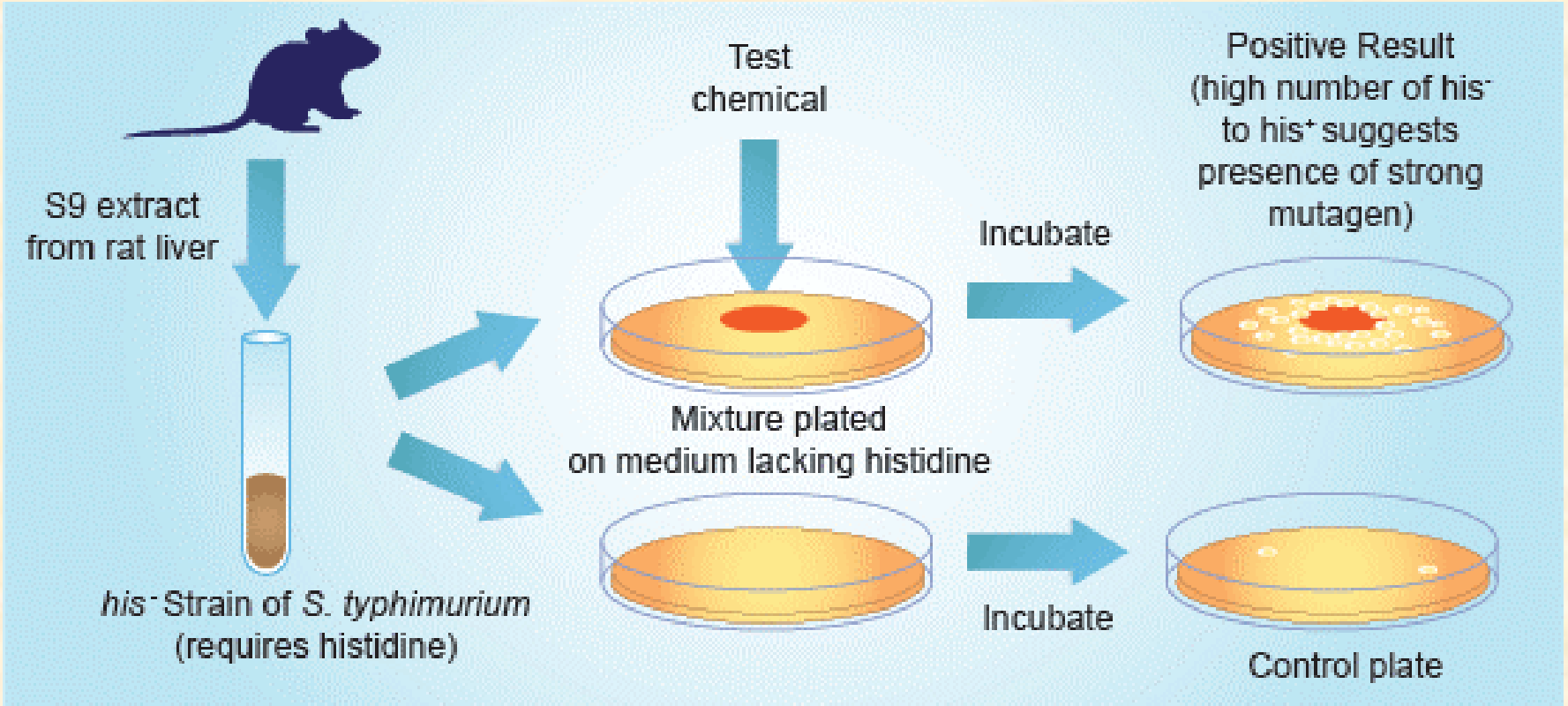
International Agency for Research on Cancer (IARC) reports that the vast majority of currently detected human carcinogens respond positively to the short-term tests Salmonella (Ames test) and chromosomal damage tests currently in use. However, it is not possible to detect that non-genotoxic (such as hormones) epigenetic carcinogens are carcinogenic by these short-term tests.

Ames test

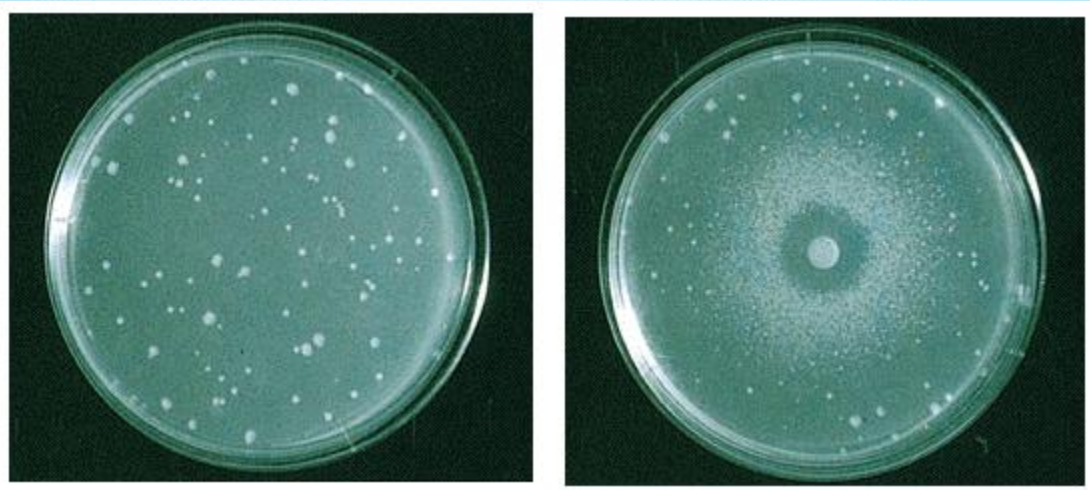


Bruce Ames (born 1928)




Test chemical
Positive Result (high number of his⁻ to his⁺ suggests presence of strong mutagen)
S9 extract from rat liver
Incubate
Mixture plated on medium lacking histidine
his⁻ Strain of S. typhimurium (requires histidine)
Incubate
Control plate

Ames Test



IARC CLASSIFICATION

Classification of carcinogens	Number of chemicals	Genotoxic/Carcinogenic ratio (%)
1: Carcinogenic to humans	120	83
2A: Probably carcinogenic to humans	81	72
2B: Possibly carcinogenic to humans	294	60
3: Not classifiable as to its carcinogenecity to humans	505	27
4: Probably not carcinogenic to humans	1*	

* Caprolactam.(common synthetic polymer)

http://monographs.iarc.fr/ENG/Classification/

International Agency for Research on Cancer
World Health Organization

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

English | Français

NEWS | MEETINGS | CLASSIFICATIONS | PUBLICATIONS | PREAMBLE | STAFF

You are here: Home / Classifications / **List of Classifications**

CLASSIFICATIONS

- List of Classifications
  - Volumes 1-118
  - Alphabetical order
  - CAS® Registry Number order
  - Cancer site

AGENTS CLASSIFIED BY THE *IARC MONOGRAPHS*, VOLUMES 1–118

Group 1	*Carcinogenic to humans*	120 agents
Group 2A	*Probably carcinogenic to humans*	81
Group 2B	*Possibly carcinogenic to humans*	294
Group 3	*Not classifiable as to its carcinogenicity to humans*	505
Group 4	*Probably not carcinogenic to humans*	1

For definitions of these groups, please see the Preamble.

It is strongly recommended to consult the complete *Monographs* on these agents, the publication date, and the list of studies considered. Significant new information might support a different classification.
For agents that have not been classified, no determination of non-carcinogenicity or overall safety should be inferred.

- List of classifications, Volumes 1-118 (*embedded spreadsheet*)
- List of classifications by cancer site (*PDF file*)
- French version of the List of classifications by cancer site, as hosted by Centre Léon Bérard

See Preventable Exposures Associated With Human Cancers (Cogliano *et al.*, 2011)
Although care was taken in preparing these lists, mistakes may be present.
If you find an error, please notify us at imo@iarc.fr.

Last update: 13 April 2017

IARC, 150 Cours Albert Thomas, 69372 Lyon CEDEX 08, France - Tel: +33 (0)4 72 73 84 85

https://monographs.iarc.fr/agents-classified-by-the-iarc/

International Agency for Research on Cancer
World Health Organization

IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

Agents Classified by the IARC Monographs, Volumes 1–125

Group 1	Carcinogenic to humans	120 agents
Group 2A	Probably carcinogenic to humans	83 agents
Group 2B	Possibly carcinogenic to humans	314 agents
Group 3	Not classifiable as to its carcinogenicity to humans	500 agents

For definitions of these groups, please see the Preamble.

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For agents that have not been classified, no determination of non-carcinogenicity or overall safety should be inferred.

List of Classifications (optimized for the latest versions of the browsers Chrome and Mozilla Firefox)

List of Classifications by cancer site (PDF file)

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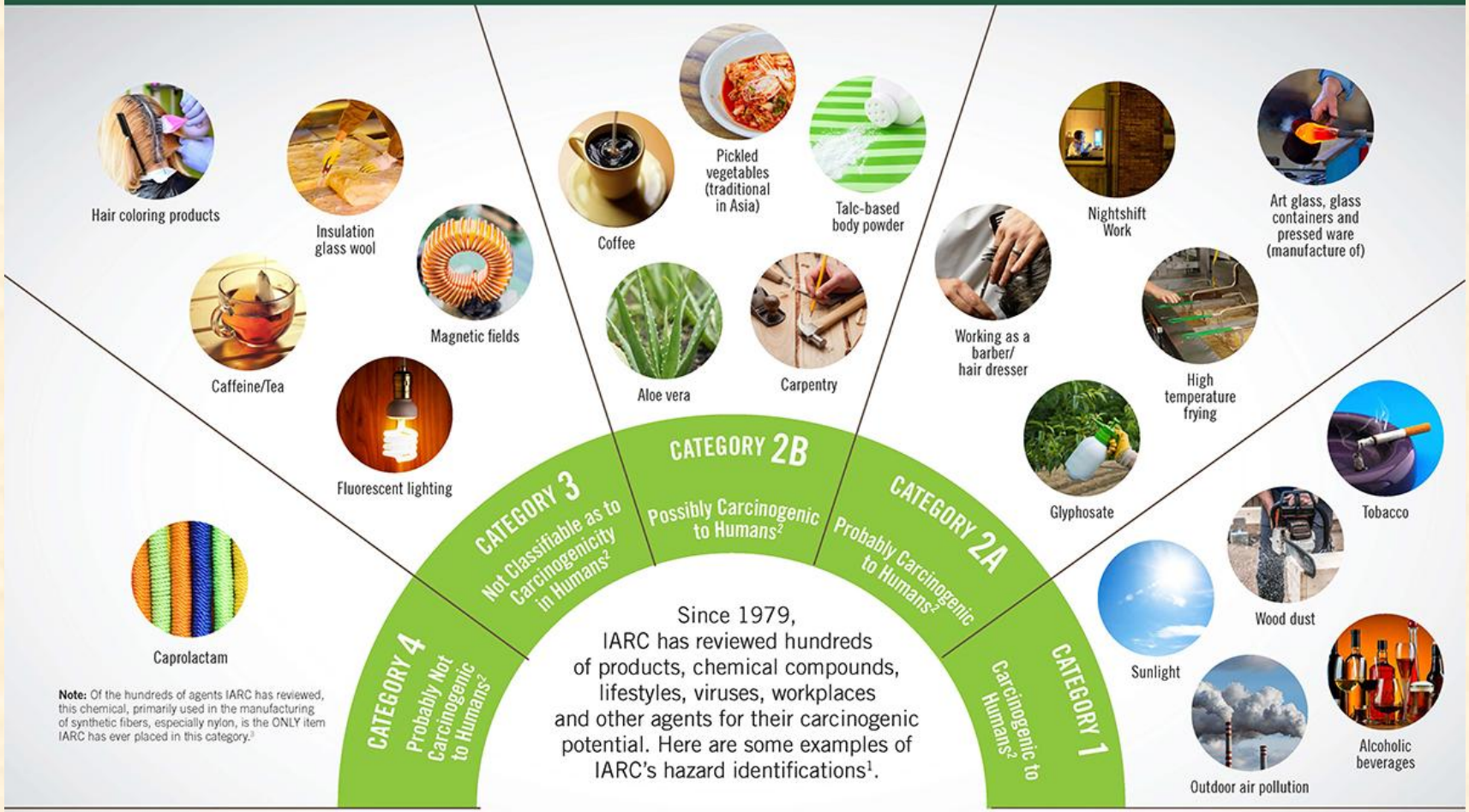
Last update: 18 February 2020

Examples of International Agency for Research on Cancer (IARC) Carcinogenic Classifications

gmo answers



Note: Of the hundreds of agents IARC has reviewed, this chemical, primarily used in the manufacturing of synthetic fibers, especially nylon, is the ONLY item IARC has ever placed in this category.[3]

Since 1979, IARC has reviewed hundreds of products, chemical compounds, lifestyles, viruses, workplaces and other agents for their carcinogenic potential. Here are some examples of IARC's hazard identifications[1].

[1] http://www.24d.reviews/IARC-and-24D.php
[2] http://monographs.iarc.fr/ENG/Classification/ClassificationsAlphaOrder.pdf
[3] http://www.epa.gov/ttnatw01/hlthef/caprolac.html

CHEMICAL COMPOUNDS IN CIGARETTE SMOKE

THIS GRAPHIC OFFERS A SUMMARY OF A SELECTION OF HAZARDOUS COMPOUNDS IN CIGARETTE SMOKE & THEIR EFFECTS

ESTIMATED NUMBER OF CHEMICAL COMPOUNDS IN CIGARETTE SMOKE

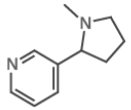
7,357

70

NUMBER OF THESE COMPOUNDS WITH CONFIRMED CARCINOGENIC ACTIVITY

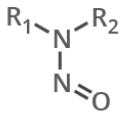
The compounds shown below are all found in cigarette smoke. The mass figures, given in µg, take into account both mainstream (inhaled) and sidestream smoke. 1 µg is equal to 1 millionth of a gram. Amounts of these compounds vary in different brands of cigarettes - these figures are approximate.

NICOTINE



- Approx. 919µg per cigarette
- Addictive
- Increases heart rate
- Increases blood pressure
- Increases blood glucose
- Lethal dose: around 500-1000mg

N-NITROSAMINES

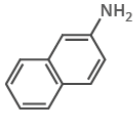


- Large class of compounds
- Several are tobacco-specific
- **Known human carcinogens**
- Most carcinogenic: NNK & NNN
- NNK: approx. 0.3µg per cigarette
- NNN: approx. 2-50µg per cigarette
- May cause reproductive damage

BENZENE

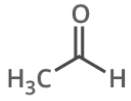
- Approx. 46-272µg per cigarette
- **Known human carcinogen**
- Damages bone marrow
- Lowers red blood cell count
- May harm reproductive organs

AROMATIC AMINES



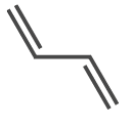
- Large class of compounds
- Includes 2-aminonaphthalene:
  - **Known human carcinogen**
  - Linked with bladder cancer
  - Approx. 0.04µg per cigarette

ACETALDEHYDE



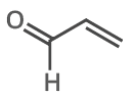
- Approx. 680-1571µg per cigarette
- **Known animal carcinogen**
- **Probable human carcinogen**
- Irritant to skin & eyes
- Irritant to respiratory tract

1,3-BUTADIENE



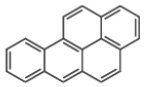
- Approx. 36-191µg per cigarette
- **Known human carcinogen**
- **Suspected human teratogen**
- Irritant to eyes & skin
- Irritant to upper respiratory tract

ACROLEIN



- Approx. 69-306µg per cigarette
- **Possible human carcinogen**
- **Known DNA mutagen**
- Irritant to skin & nasal passages
- May contribute to heart disease

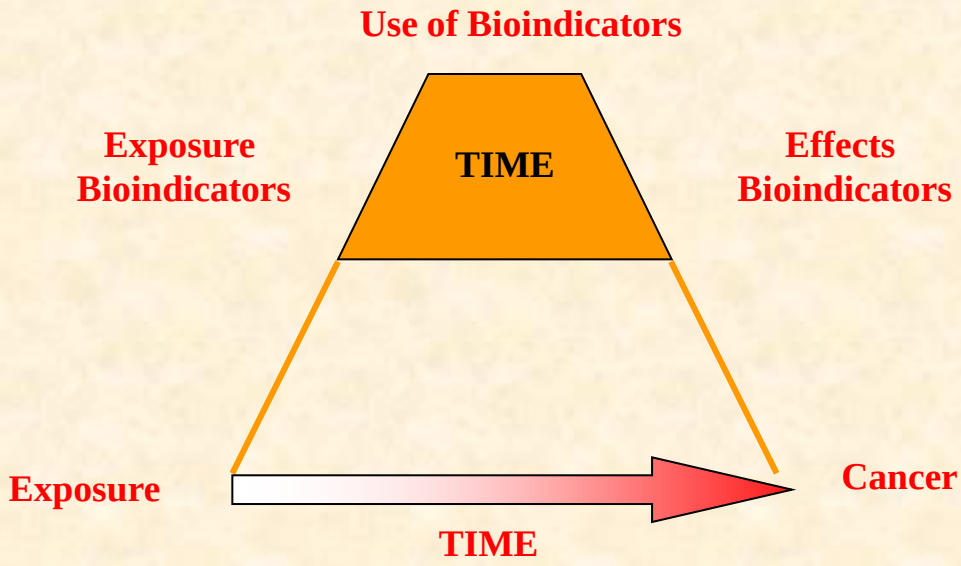
POLYAROMATICS



- Large class of compounds
- Includes benzo[a]pyrene:
  - **Known human carcinogen**
  - **Known DNA mutagen**
  - Affects reproductive capacity
  - Up to 0.14µg per cigarette

Genetic Biomonitoring;

Genetic biomonitoring (occupational or environmental monitoring of genotoxic effects that may occur in a population that is exposed to a chemical) use genetic toxicology methods. Thus, genotoxic exposure in a certain population can be identified earlier. In addition, individuals at high risk can be identified and intervention priorities can be determined. The use of bioindicators in a group exposed to a factor both saves time and prevents the occurrence of undesirable effects (such as cancer).

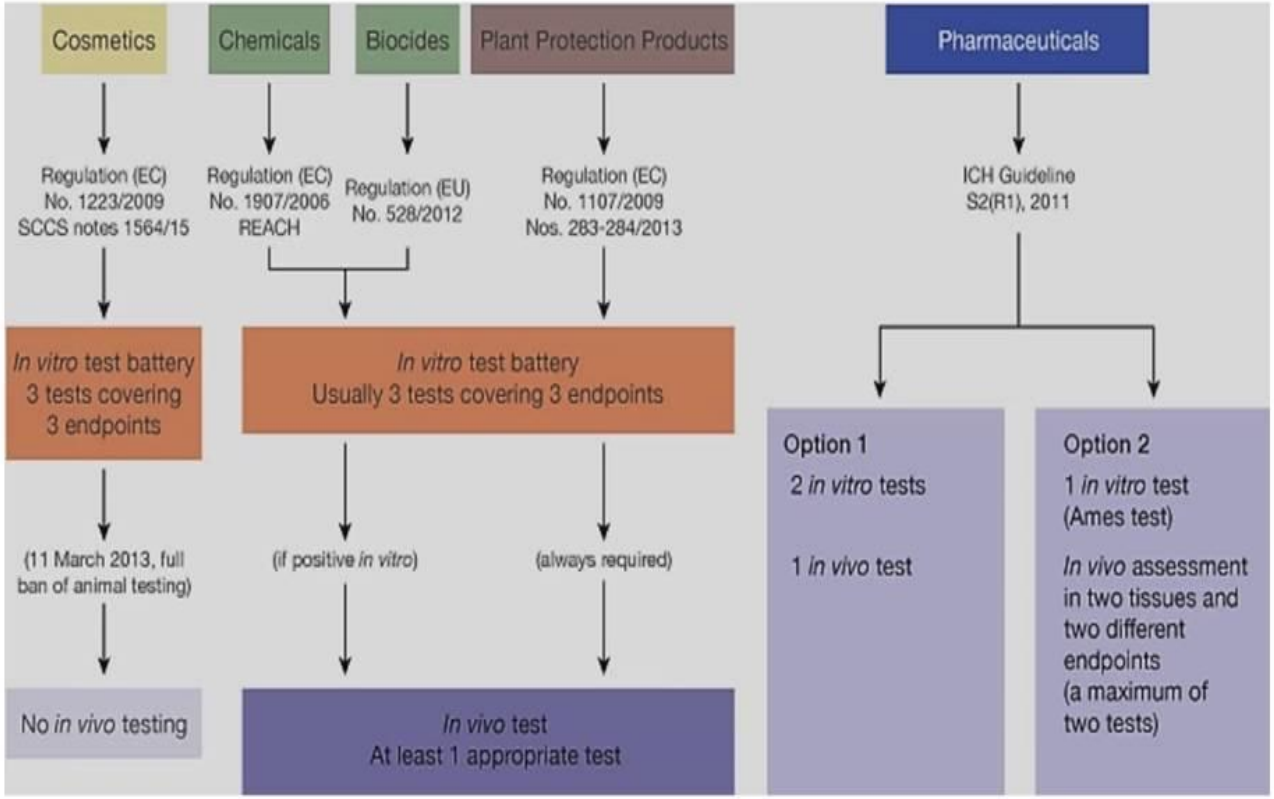


Samples taken or used in biomonitoring must meet many criteria, such as easy availability and representation of the target tissue.

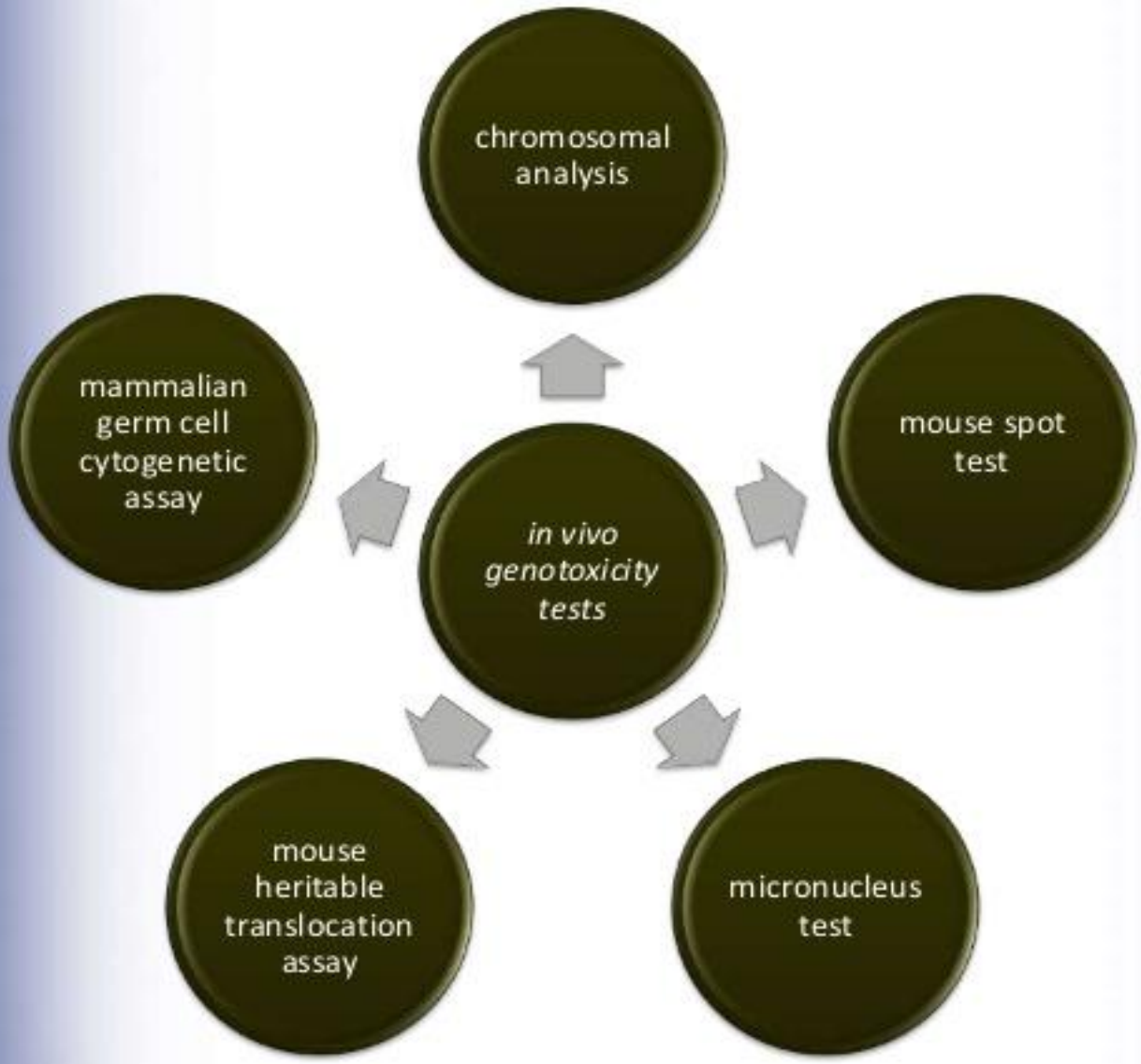
Below are the bioindicators used in genetic biomonitoring of genotoxic exposures and cell and tissue samples used for this purpose.

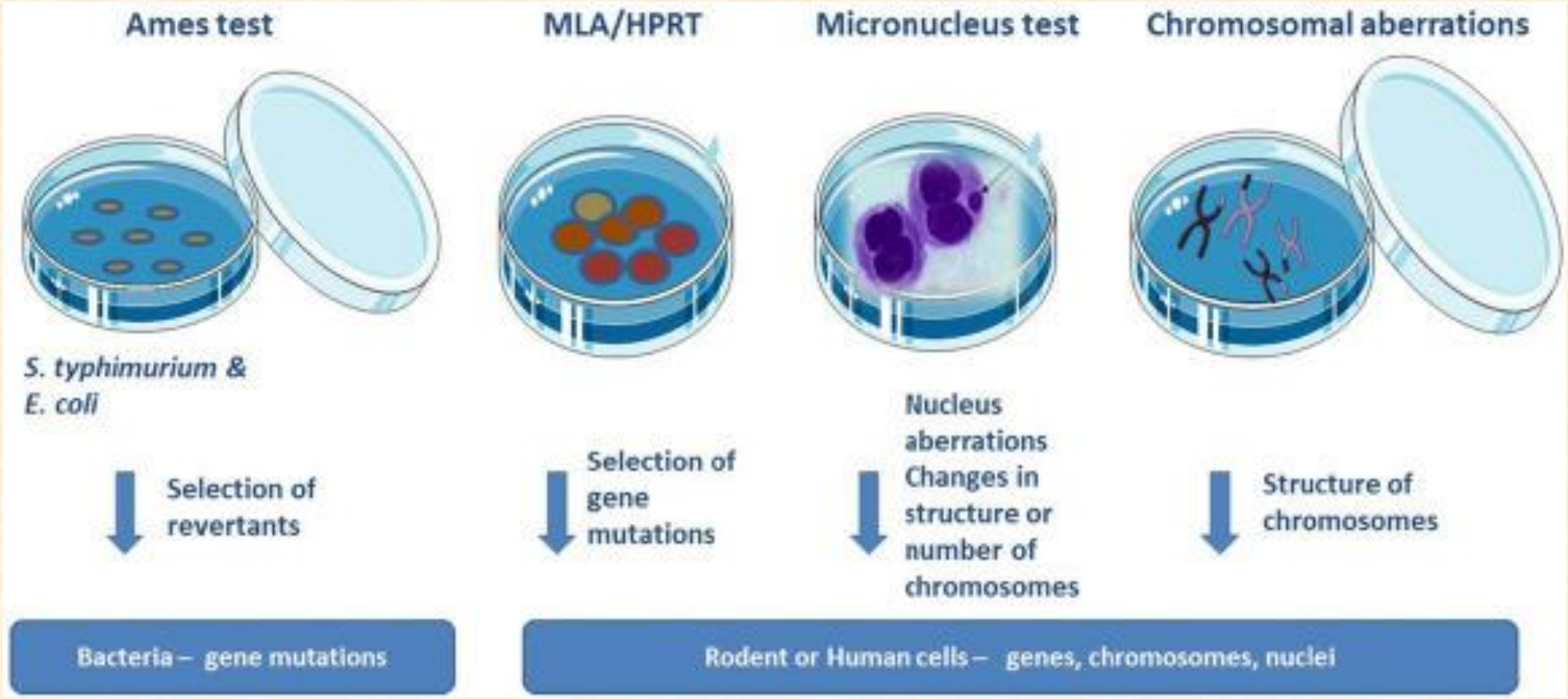
Bioindicators Used in Genetic Biomonitoring	**Cell/Tissue Samples**
Chromosomal Abberation (CA)	Lymphocytes
Sister Chromatid Exchange (SCE)	Lymphocytes
Micronucleus (MN)	Lymphocytes
Point Mutation (HPRT)	Lymphocytes and other tissues
DNA adducts	DNA isolated from cells or tissues
Protein adducts	Hemoglobin, Albumin
DNA strand breaks (COMET)	DNA isolated from cells or tissues
Oncogen activation	DNA or isolated specific proteins
Mutations/oncoproteins	Various cells and tissues
DNA repair	Cells isolated from blood samples

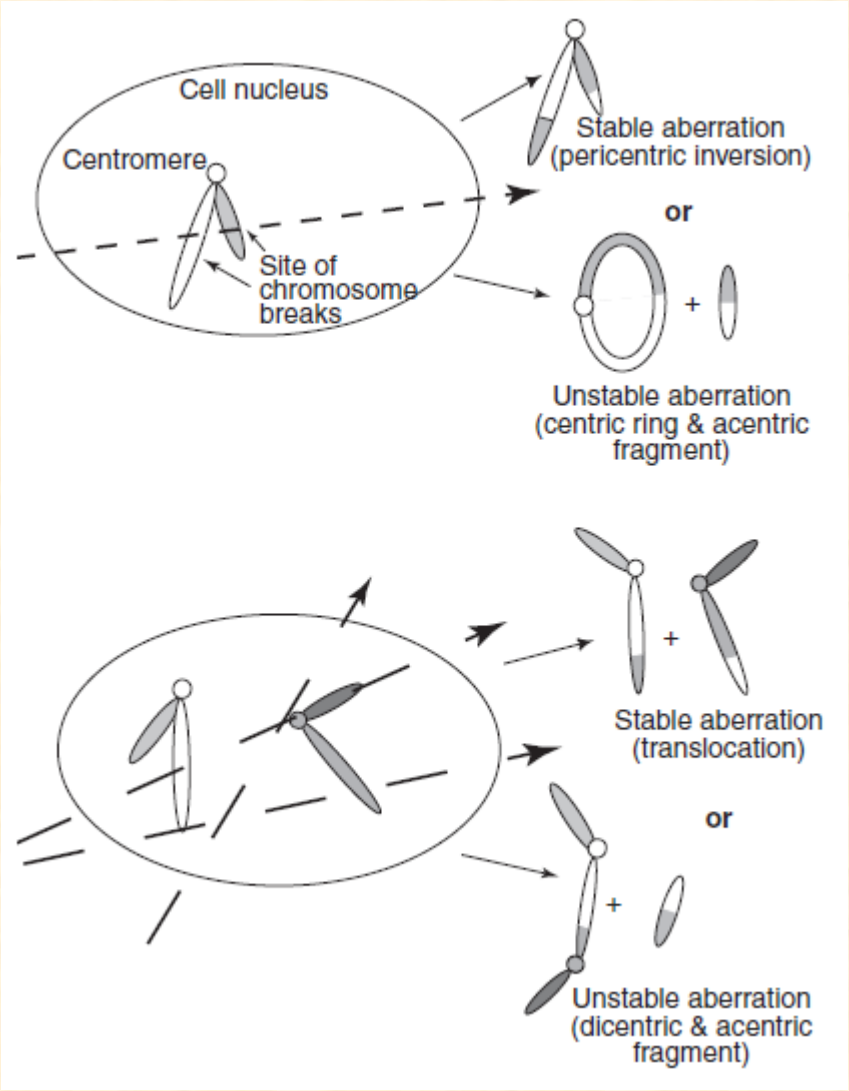
EU Regulatory Overview

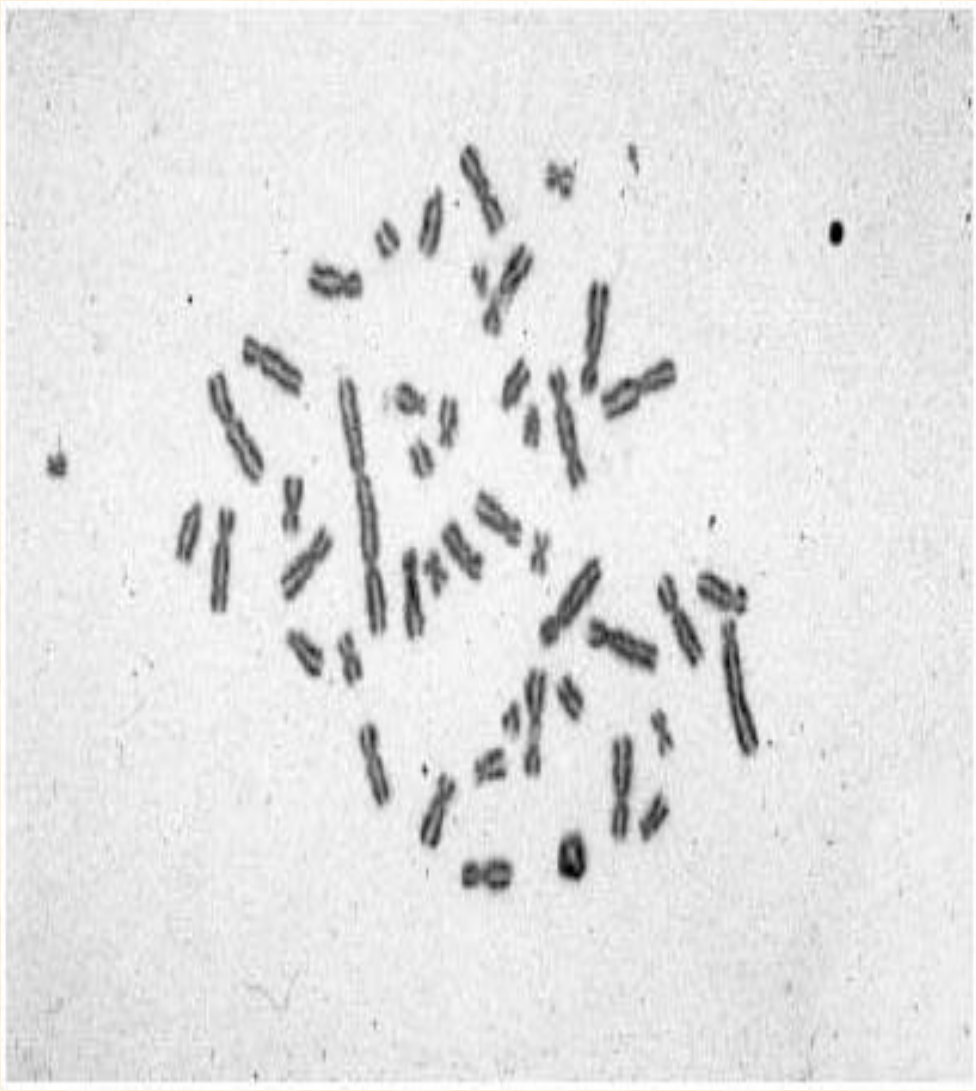


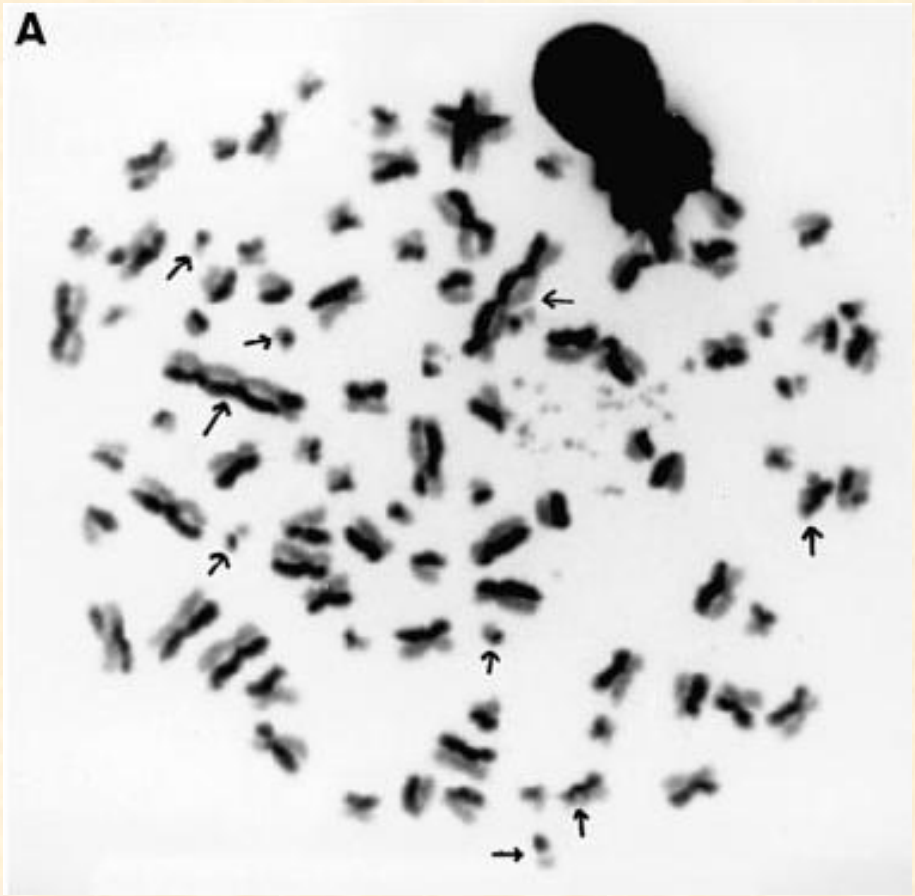
IN VIVO GENOTOXICITY TESTS

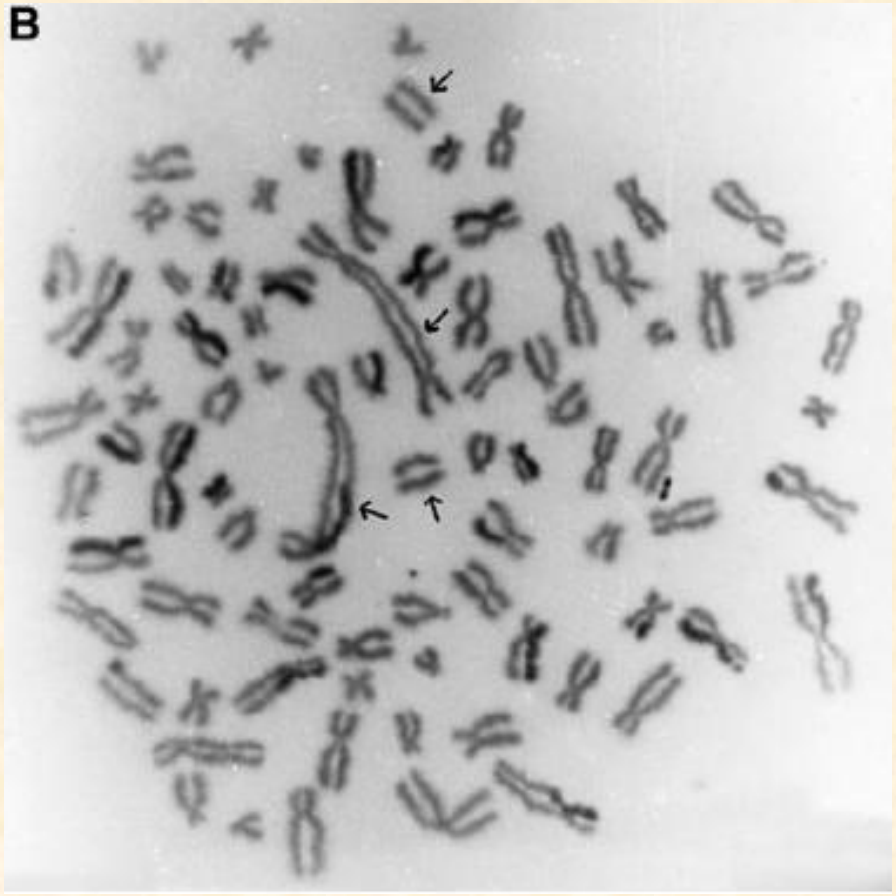


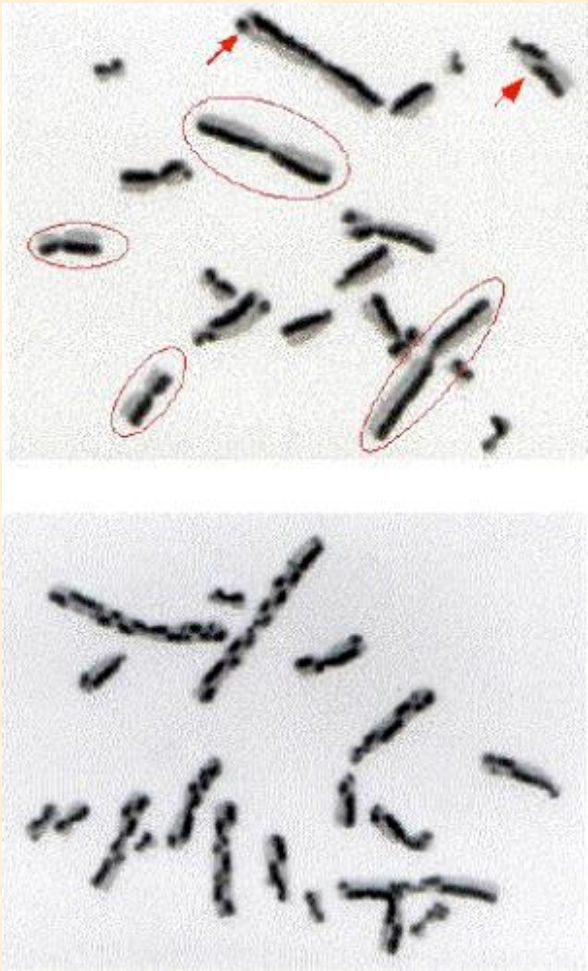

Ames test
MLA/HPRT
Micronucleus test
Chromosomal aberrations
S. typhimurium &
E. coli
Selection of
revertants
Selection of
gene
mutations
Nucleus
aberrations
Changes in
structure or
number of
chromosomes
Structure of
chromosomes
Bacteria – gene mutations
Rodent or Human cells – genes, chromosomes, nuclei

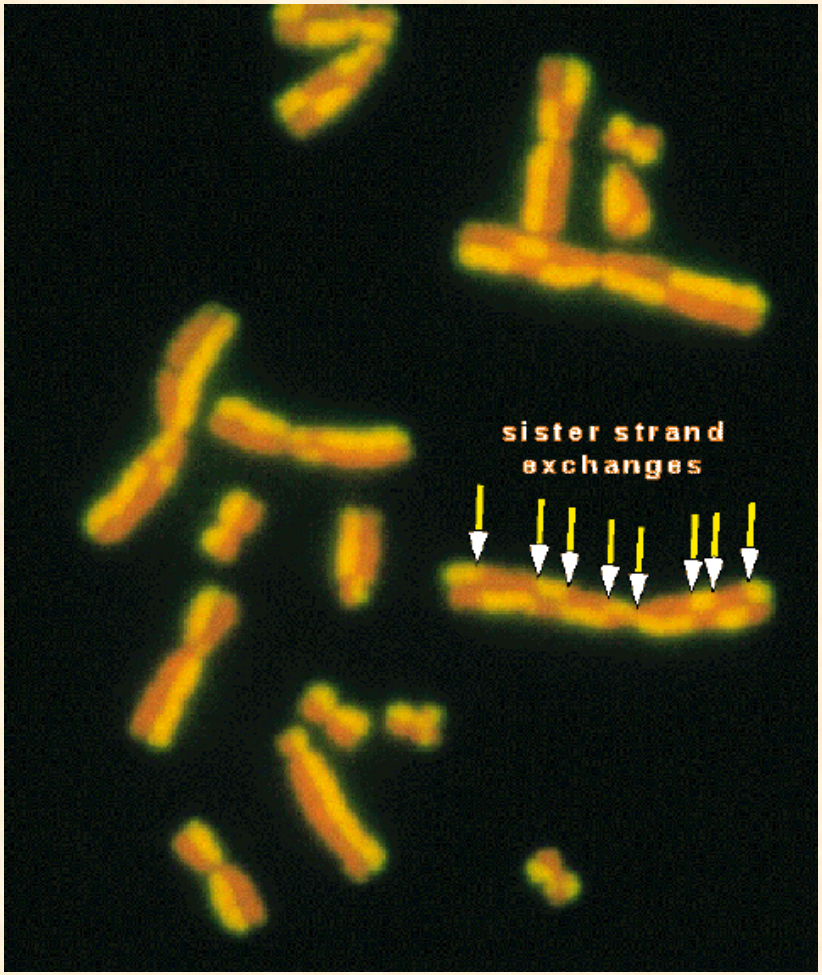

Cell nucleus
Centromere
Site of chromosome breaks
Stable aberration (pericentric inversion)
or
+
Unstable aberration (centric ring & acentric fragment)
+
Stable aberration (translocation)
or
+
Unstable aberration (dicentric & acentric fragment)

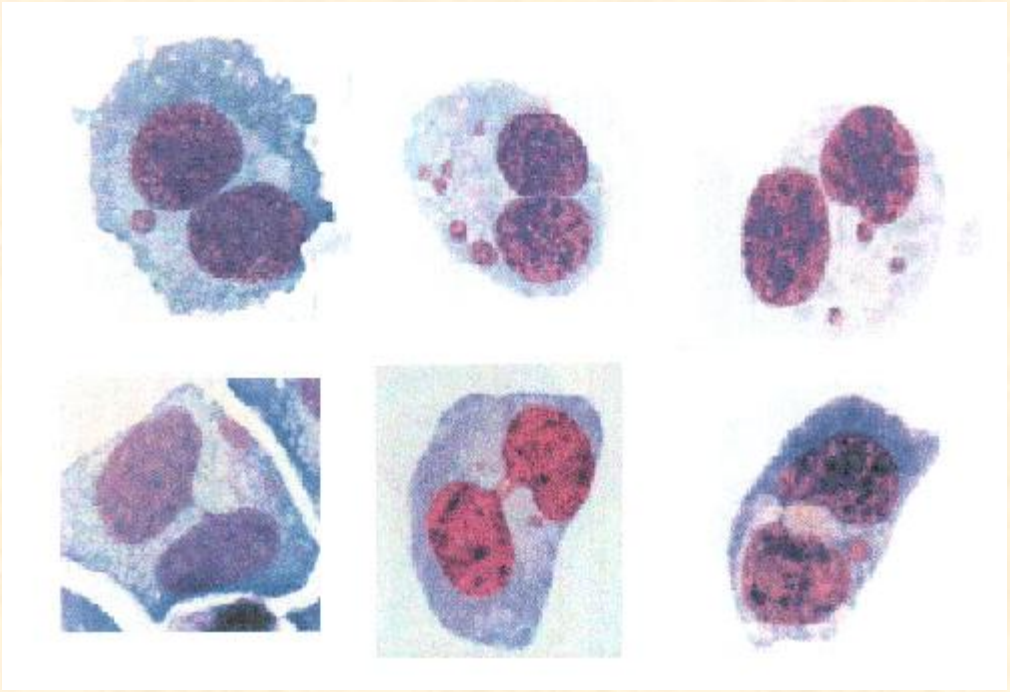



A

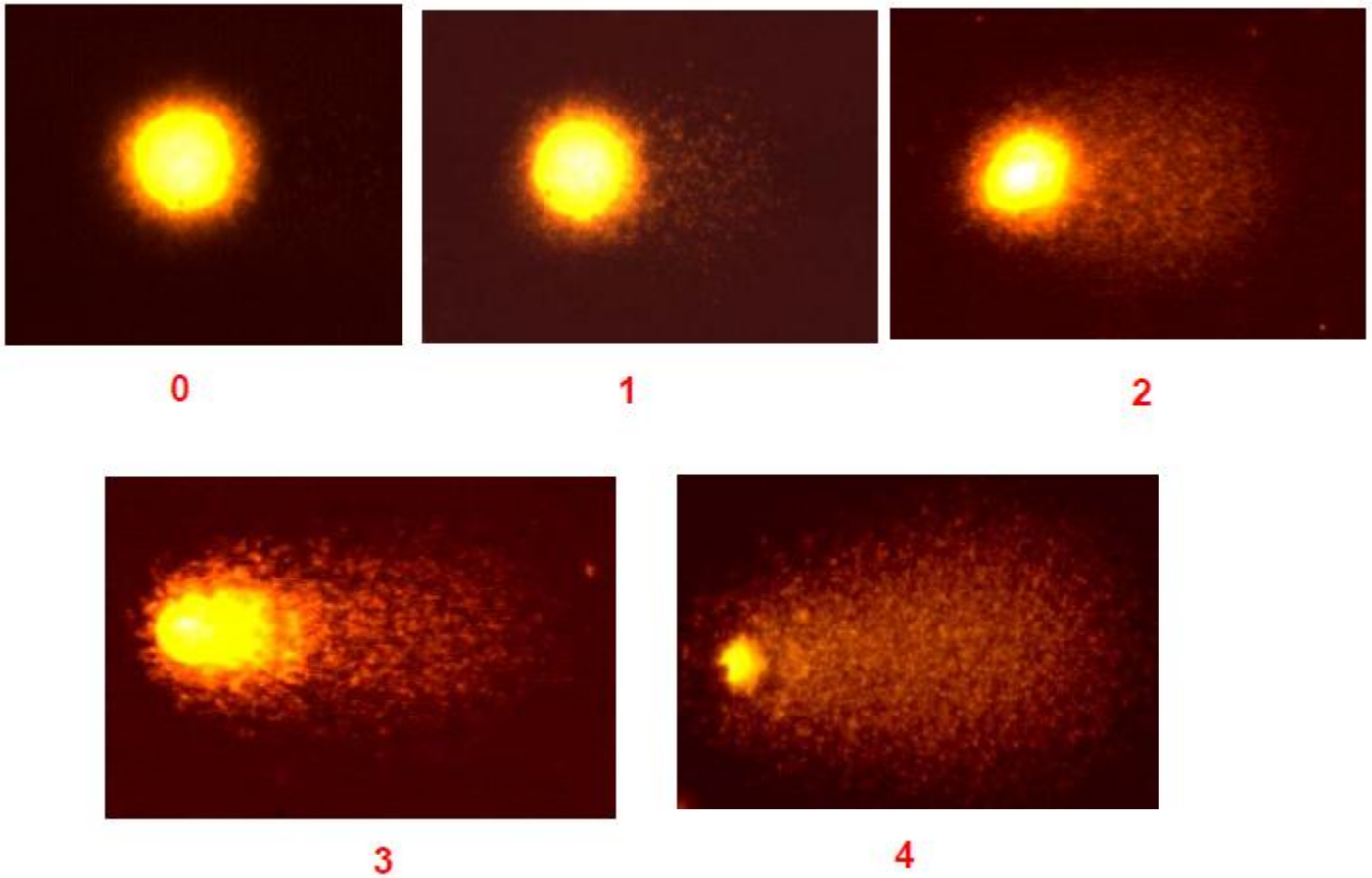

B




sister strand
exchanges

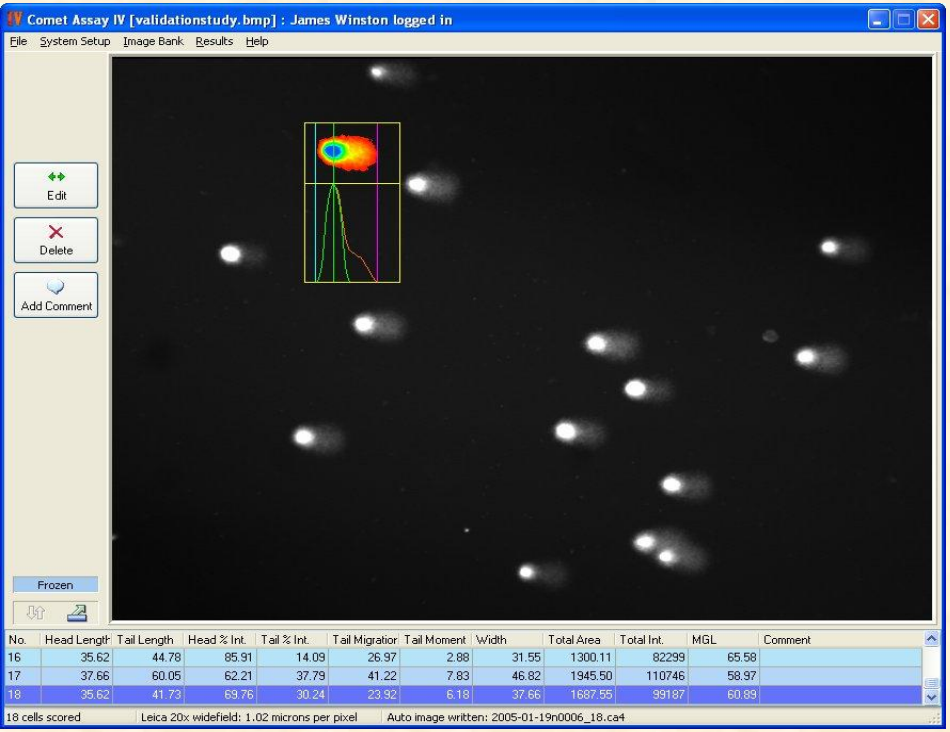


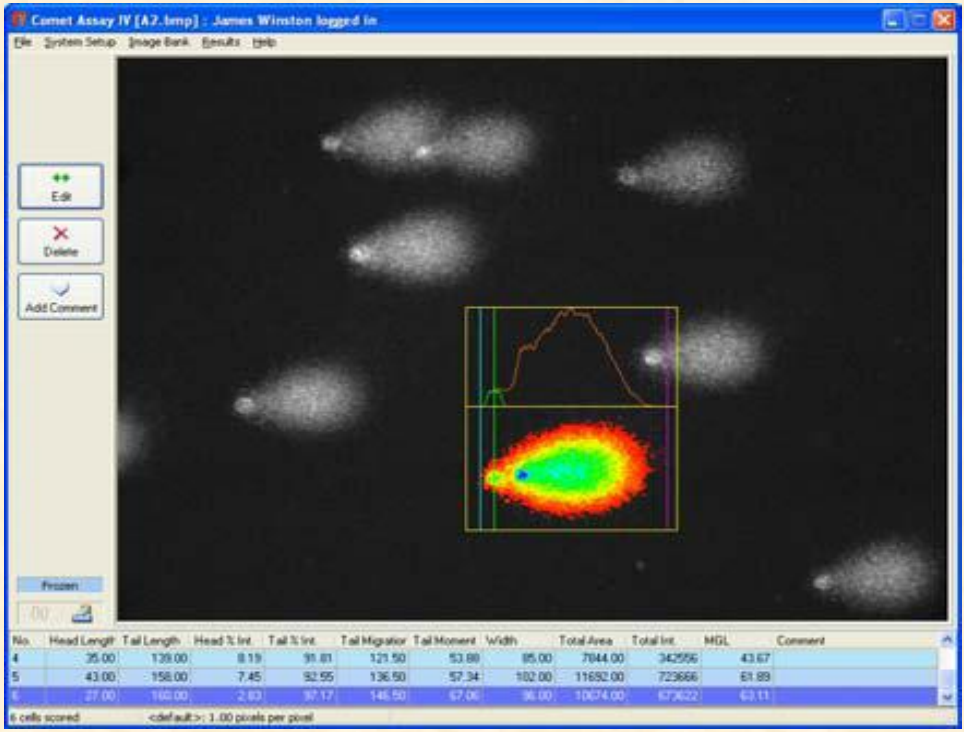
Comet categories for visual scoring - classify 100 comets as 0-4

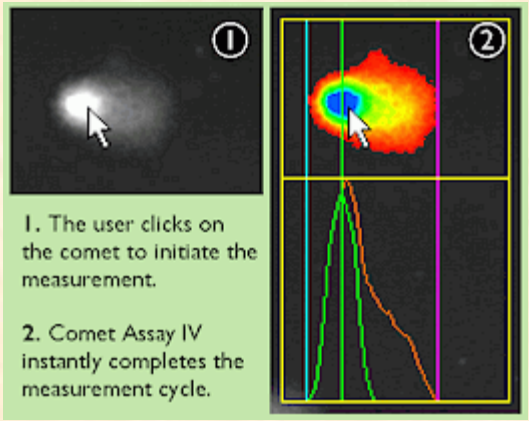


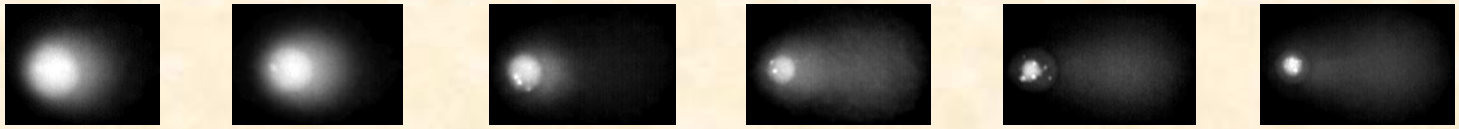
CHO cells treated with Ethyl methanesulfonate; Magnification 400X

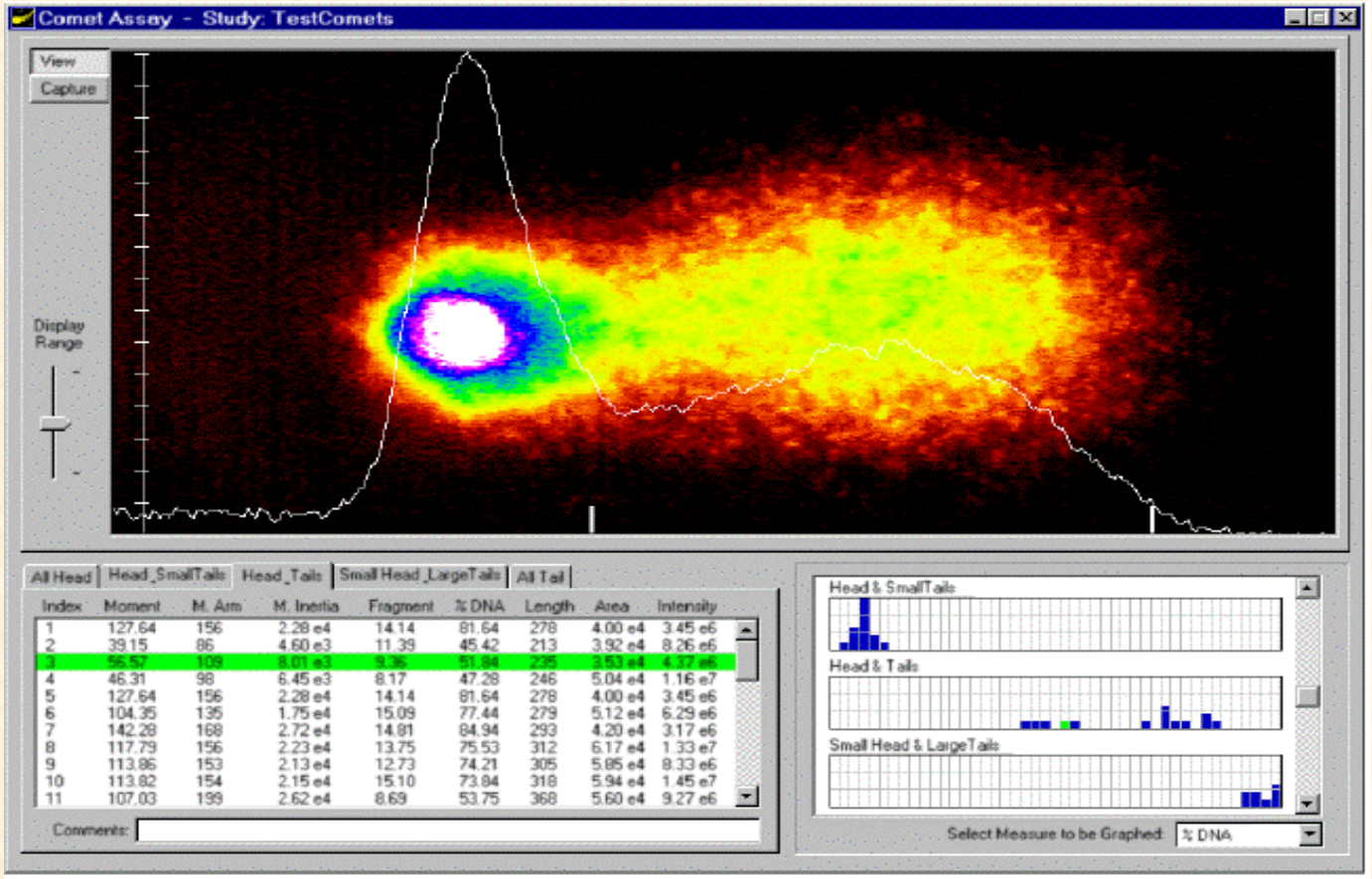
Ankara University
Faculty of Pharmacy










Comet Assay - Study: TestComets
View
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All Head
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Small Head_LargeTails
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Index Moment M. Arm M. Inertia Fragment % DNA Length Area Intensity
1 127.64 156 2.28 e4 14.14 81.64 278 4.00 e4 3.45 e6
2 39.15 86 4.60 e3 11.39 45.42 213 3.92 e4 8.26 e6
3 56.57 109 8.01 e3 9.36 51.84 235 3.53 e4 4.37 e6
4 46.31 98 6.45 e3 8.17 47.28 246 5.04 e4 1.16 e7
5 127.64 156 2.28 e4 14.14 81.64 278 4.00 e4 3.45 e6
6 104.35 135 1.75 e4 15.09 77.44 279 5.12 e4 6.29 e6
7 142.28 168 2.72 e4 14.81 84.94 293 4.20 e4 3.17 e6
8 117.79 156 2.23 e4 13.75 75.53 312 6.17 e4 1.33 e7
9 113.86 153 2.13 e4 12.73 74.21 305 5.85 e4 8.33 e6
10 113.82 154 2.15 e4 15.10 73.84 318 5.94 e4 1.45 e7
11 107.03 199 2.62 e4 8.69 53.75 368 5.60 e4 9.27 e6
Comments:
Head & SmallTails
Head & Tails
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% DNA

The relationship between Genetic biomonitoring and cancer risk evaluation;

Although the number of chemicals that induce cytogenetic changes in humans is limited, many known carcinogens have been found to cause damage in lymphocyte chromosomes.

The amount of genetic damage occurring as in alkylating agents used in the treatment of vinyl chloride, benzene, ethylene oxide and cancer is an indication of exposure, that is, the damage increases as the exposure increases. Therefore, for example; positive results from tests performed after exposure to certain chemicals occupationally; it shows the obligations to perform various applications to improve workplace working conditions.

The relationship between Genetic biomonitoring and cancer risk evaluation;

Much of the experience with cytogenetic biomonitoring applications results from high concentrations of occupational exposures. Many occupational exposures have been explored by different research groups. When these studies were compared with each other, it was seen that the studies on the detection of chromosomal damage and MN formation were more compatible with each other.

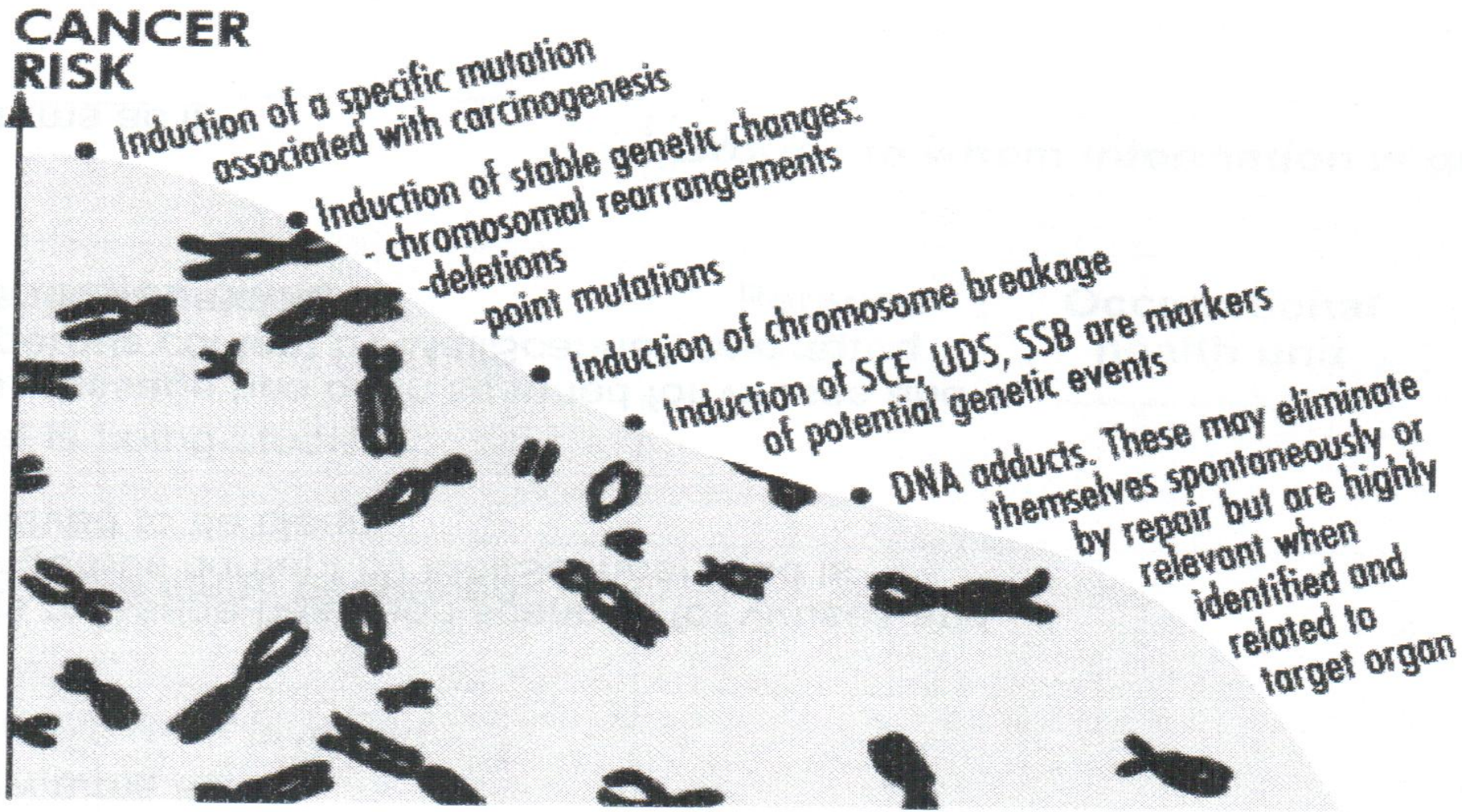
The relationship between Genetic biomonitoring and cancer risk evaluation;

There are many occupationally exposed and genotoxic chemicals in IARC monographs groups 1, 2A and 2B. It is understood from the chromosomal damage and MN tests performed in the first group that many chemicals which are human carcinogens are also clastogenic. This relationship is perceived to be carcinogenic chemical substances at the same time being clastogenic. While this applies to most chemicals, not all chemicals. It is a known fact that not all carcinogens, even if their numbers, cause cytogenetic damage.

	Cytogenetic data					
	Humans			Animals		
	CA	**SCE**	**MN**	**CA**	**SCE**	**MN**
Group 1, Carcinogens to humans						
Arsenic and Arsenic compounds	+	+		+		+
Asbestos		?		-		-
Benzene	+			+	+	+
Cyclophosphamide	+	+		+	+	+
Chrome compounds (+6)	+	+		+	+	+
Cigarette smoke	+	+	+		+	
Vinyl chloride	+	?		+	+	+
Radon	+			-		
Nickel compounds	+	-		?		

	Cytogenetic data					
	Humans			Animals		
	CA	**SCE**	**MN**	**CA**	**SCE**	**MN**
Group 2A, Probably carcinogenic to humans						
Adriamicine	+	+		+	+	+
Cisplatin		+		+	+	
Epichlorohydrine	+			?	+	-
Group 2B, Possibly carcinogenic to humans						
DDT	?			+		-
Stiren	+	?	+	?	+	+

IARC: http://monographs.iarc.fr/


CANCER RISK
• Induction of a specific mutation associated with carcinogenesis
• Induction of stable genetic changes:
- chromosomal rearrangements
-deletions
-point mutations
• Induction of chromosome breakage
• Induction of SCE, UDS, SSB are markers of potential genetic events
• DNA adducts. These may eliminate themselves spontaneously or by repair but are highly relevant when identified and related to target organ

SCIENTIFIC STUDY SAMPLES PERFORMED IN OUR DEPARTMENT

Arch. Environ. Contam. Toxicol. 41, 241–246 (2001)
DOI: 10.1007/s002440010244

ARCHIVES OF Environmental Contamination and Toxicology

Correlation Between Lead Exposure Indicators and Sister Chromatid Exchange (SCE) Frequencies in Lymphocytes from Inorganic Lead Exposed Workers

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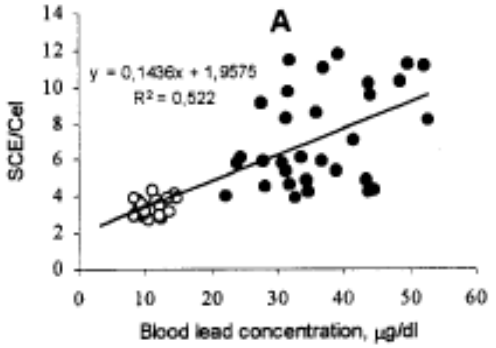
Received: 7 September 2000/Accepted: 25 February 2001

Abstract. Inorganic lead exposure was studied in 31 volunteers employed in storage battery plant. The genotoxicity of lead was measured in terms of sister chromatid exchange (SCE). Erythrocyte δ-aminolevulinic acid dehydrogenase (ALAD) activity. rect mechanism of lead-induced DNA damage (Hiraku and Kawanishi 1996). However lead ions have no ability to generate highly reactive hydroxyl radical (˙OH) from superoxide radical ($O_2^{\cdot-}$) or H_2O_2 (Hiraku and Kawanishi 1996)

Table 2. PbBs and SCE frequencies in groups A, B, and C

Parameters	Control Group	Group A (n = 21, < 40 μg/dl)	Group B (n = 8, 40–50 μg/dl)	Group C (n = 2, > 50 μg/dl)
Blood Pb (μg/dl) mean ± SD (range)	11.1 ± 2.13 (8.11–14.71)	31.56 ± 4.71[a] (22.22–39.01)	44.79 ± 2.76[ab] (41.2–49.42)	52.26 ± 0.36[ac] (52.0–52.51)
SCE/Cell mean ± SD (range)	3.46 ± 0.47 (2.81–4.31)	6.81 ± 2.53[a] (3.92–11.77)	7.73 ± 2.93[ad] (4.20–11.20)	9.64 ± 2.07[ae] (8.17–11.11)

[a] Statistically higher from the control group ($p < 0.001$).
[b] Statistically higher from group A ($p < 0.001$).
[c] Statistically higher from group B ($p < 0.001$).
[d] The difference was not statistically significant when compared with group A ($p > 0.05$).
[e] The difference was not statistically significant when compared with group B ($p > 0.05$).



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Influence of δ-aminolevulinic acid dehydratase (ALAD) polymorphism on the frequency of sister chromatid exchange (SCE) and the number of high-frequency cells (HFCs) in lymphocytes from lead-exposed workers

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Received 6 December 2002; received in revised form 22 May 2003; accepted 7 July 2003

Table 3
Comparison of Pb-B levels, SCE and HFC values in ALAD 1-1 and ALAD 1-2 workers

	Exposed workers ($n = 50$)	Pb-B (μg/dl)		
		Group A (<40 μg/dl, $n = 34$)	Group B (40–50 μg/dl, $n = 8$)	Group C (>50 μg/dl, $n = 8$)
ALAD 1-1 workers				
Pb-B	34.42 ± 1.56[a] (13.41–71.82)	29.74 ± 1.24 (13.41–39.54)	44.73 ± 1.27 (40.03–49.42)	58.75 ± 2.77 (51.51–71.82)
SCE per cell	8.07 ± 0.44 (2–28)	6.96 ± 0.53 (2–20)	8.80 ± 1.23 (2–20)	12.33 ± 1.72[b] (4–28)
HFCs (total cells)	642 (2500)	267 (1700)	125 (400)	250 (400)
HFC (%)	25.68[a]	15.71	31.25[c,**]	62.5[b]
HFC outliers	22	9	6	7
HFC outliers (%)	44	26.47	75	87.5
	Exposed workers ($n = 21$)	Pb-B (μg/dl)		
		Group A (<40 μg/dl, $n = 12$)	Group B (40–50 μg/dl, $n = 6$)	Group C (>50 μg/dl, $n = 3$)
ALAD 1-2 workers				
Pb-B	34.94 ± 1.49 (19.22–69.61)	30.07 ± 1.42 (19.22–39.03)	44.07 ± 0.66 (41.24–45.61)	58.63 ± 5.78 (50.04–69.61)
SCE per cell	7.73 ± 0.59 (3–19)	7.06 ± 0.76 (3–16)	7.35 ± 1.17 (3–16)	13.15 ± 2.06[b] (6–19)
HFCs (total cells)	236 (1050)	84 (600)	47 (300)	104 (150)
HFC (%)	22.48	14	15.67	69.33[b]
HFC outliers	6	1	2	3
HFC outliers (%)	28.57	8.33	33.33	100

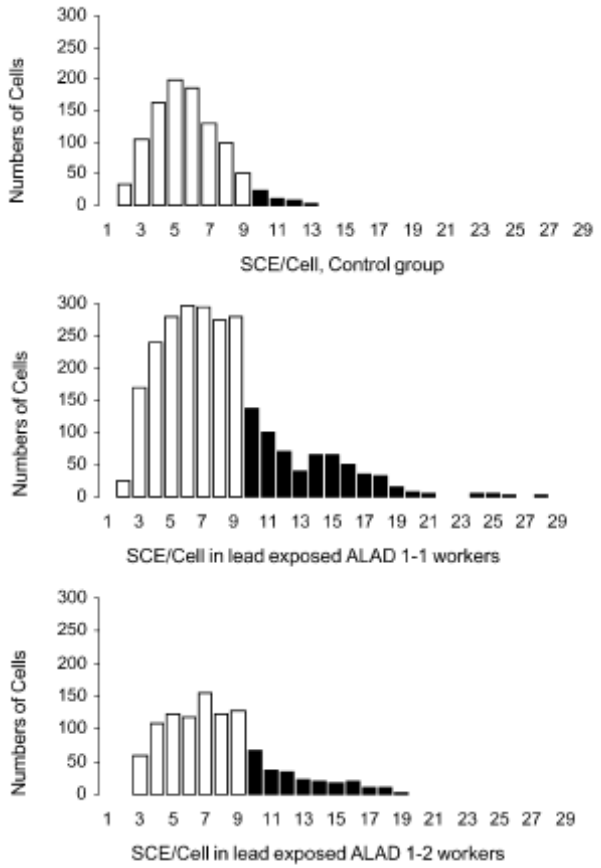

Fig. 1. Frequency histograms of SCE per cell values from control group, lead-exposed workers with ALAD 1-1 genotype, and ALAD 1-2 genotype. Black bars represent the HFCs.

Environmental and Molecular Mutagenesis 47:185–191 (2006)

Increased Sensitivity to Mitomycin C-Induced Sister Chromatid Exchange in Lymphocytes From Patients Undergoing Hyperbaric Oxygen Therapy

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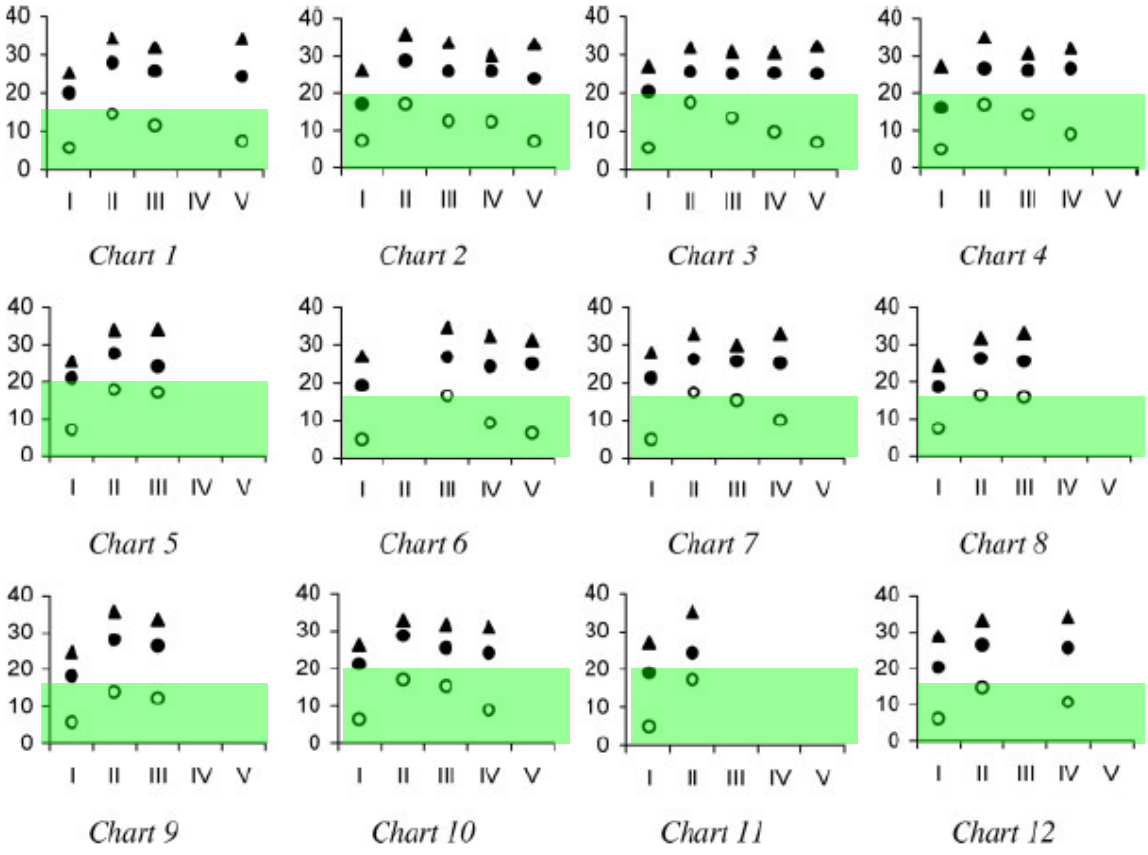

Fig. 1. Chart 1–12: mean SCE frequencies for individual volunteer patients, I: immediately before the first HBOT session; II: at the end of the 1st HBOT session; III: at the end of the 5th HBOT session; IV: at the end of the 10th HBOT session; V: 1 day after completion of HBOT (○: without MMC; ●: 20 ng/ml MMC; ▲: 40 ng/ml

Environmental and Molecular Mutagenesis 49:232–237 (2008)

Research Article

Cytogenetic Monitoring of Coal Workers and Patients with Coal Workers' Pneumoconiosis in Turkey

Ozge Cemiloglu Ulker, Aylin Ustundag, Yalcin Duydu, Berran Yucesoy, and Asuman Karakaya*
Department of Toxicology, Faculty of Pharmacy, Ankara University, Ankara, Turkey

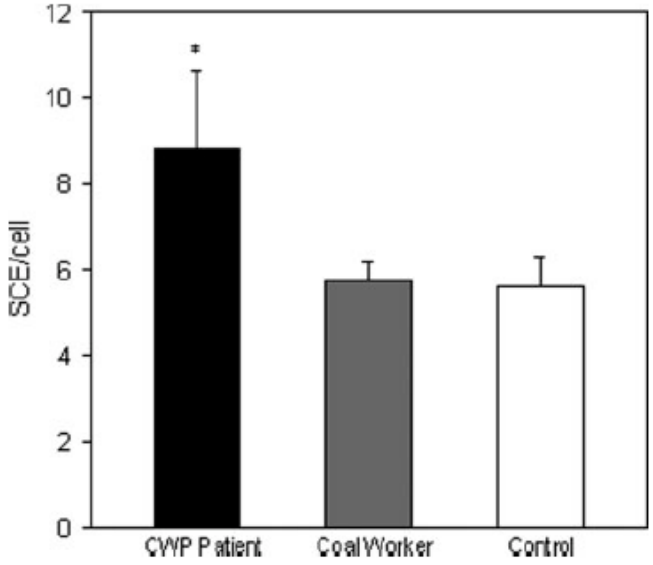

Fig. 1. SCE frequencies of CWP patient, coal worker, and control groups (mean ± S.E.). *$P < 0.01$ compared to coal worker and control groups (Student *t*-test).

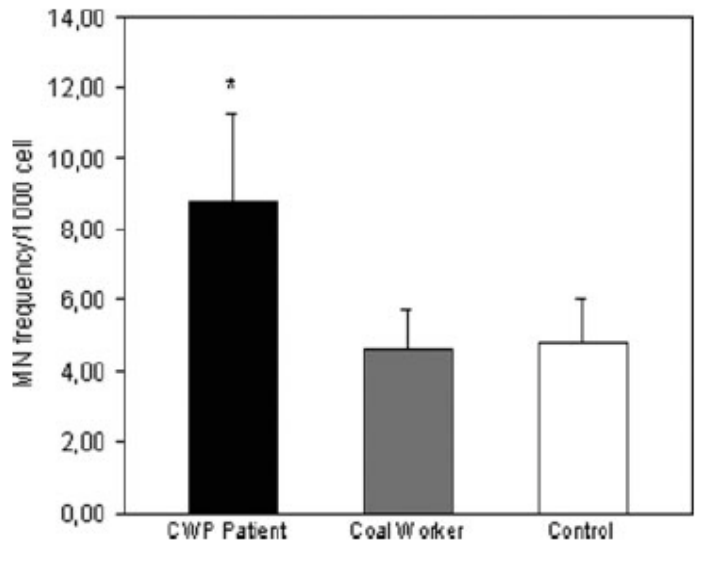

Fig. 2. MN frequencies of CWP patient, coal worker, and control groups (mean ± S.E.). *$P < 0.01$ compared to coal worker and control groups (χ^2 test).