

**3. hrvatski kongres
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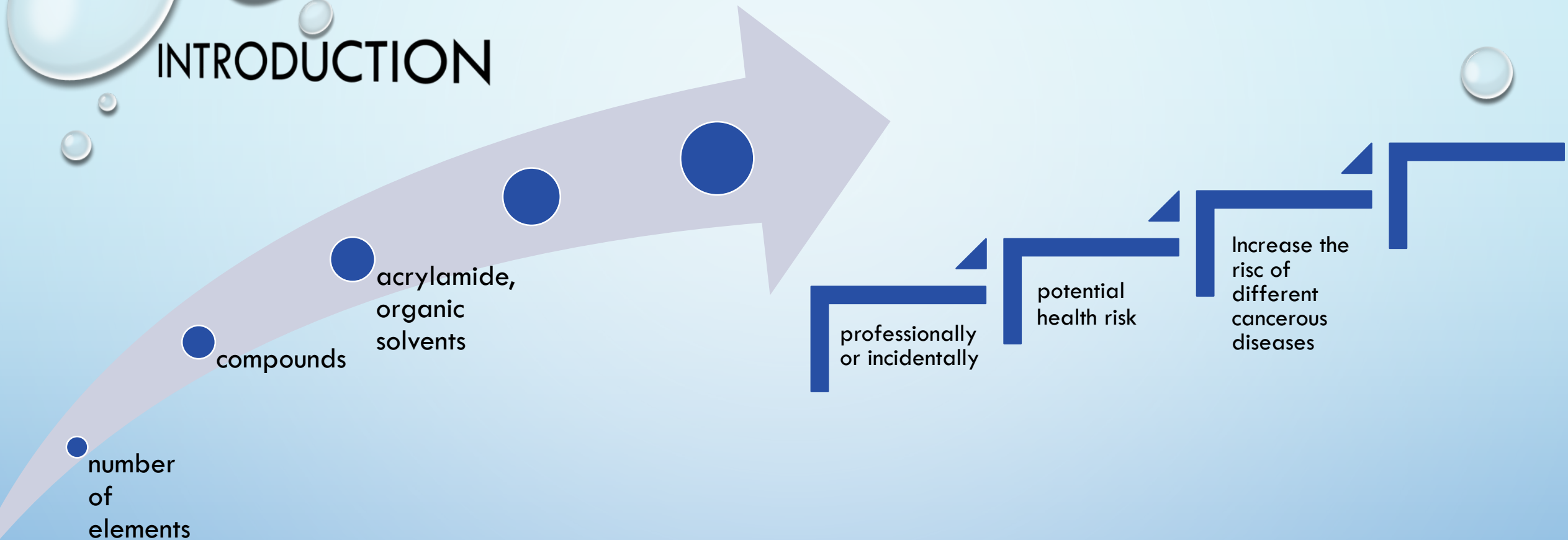
25. - 27.4.2017.
TERME TUHELJ
HOTEL WELL

**EVALUATION OF GENOTOXICOLOGICAL EFFECT ON IONIZING RADIATION
TO MEDICAL OCCUPATIONALLY EXPOSED WORKERS**

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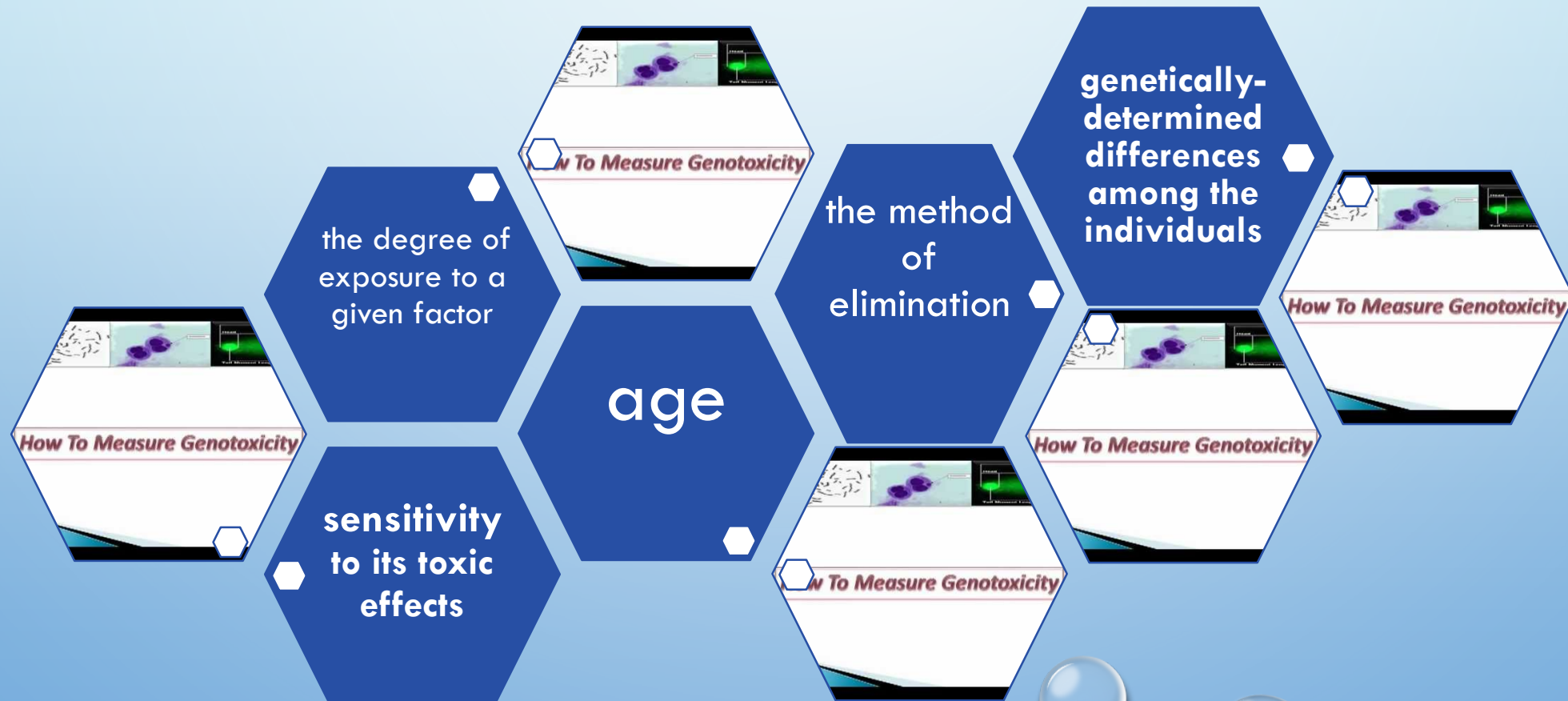
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INTRODUCTION



HUMAN GENOTOXICANTS COME AS POLLUTANTS FROM TECHNOLOGICAL PROCESSES AND ARE ALSO A RESULT OF THE UNCONTROLLED MANUFACTURING OF CERTAIN CHEMICAL SUBSTANCES AND THEIR PRODUCTS

THE EFFECT ON EACH EXPOSED INDIVIDUAL DEPENDS OF



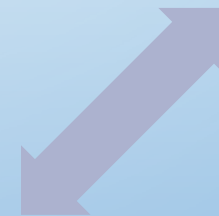
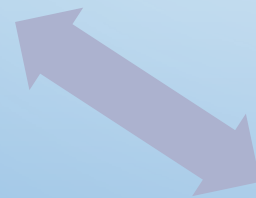
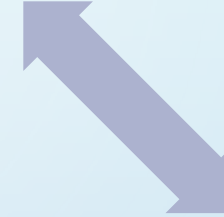
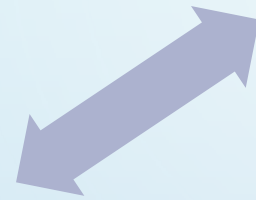
Cytogenetic monitoring

T-lymphocytes are commonly used cell line

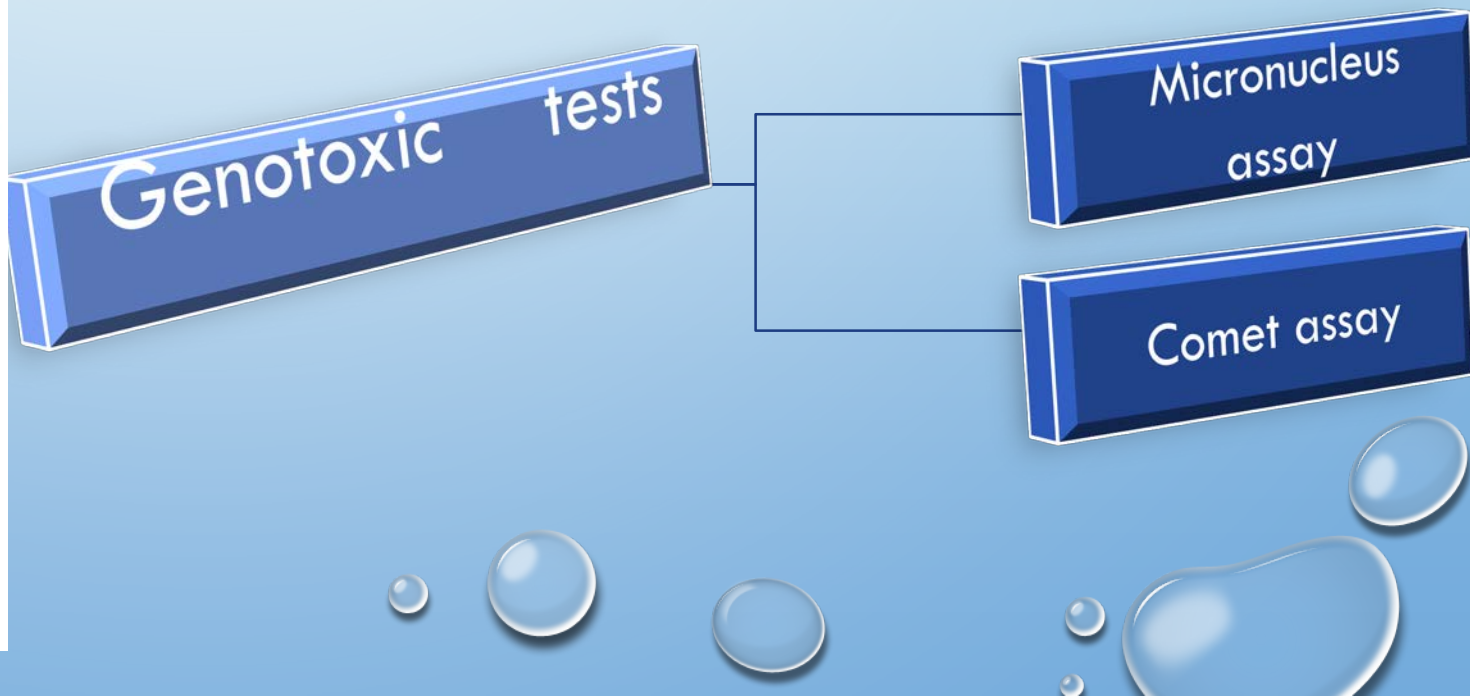
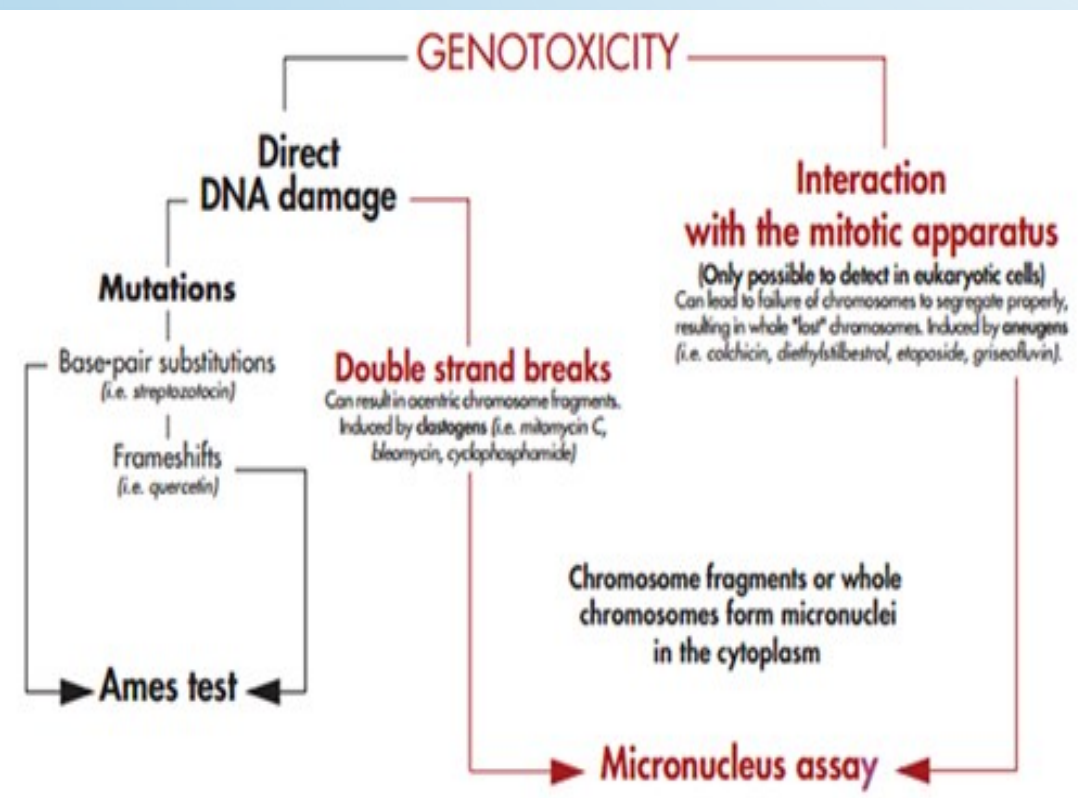
chronic exposure of the organism to small doses of chemical or physical mutagens leads to potential genotoxicity

genotoxic agents induced mutations

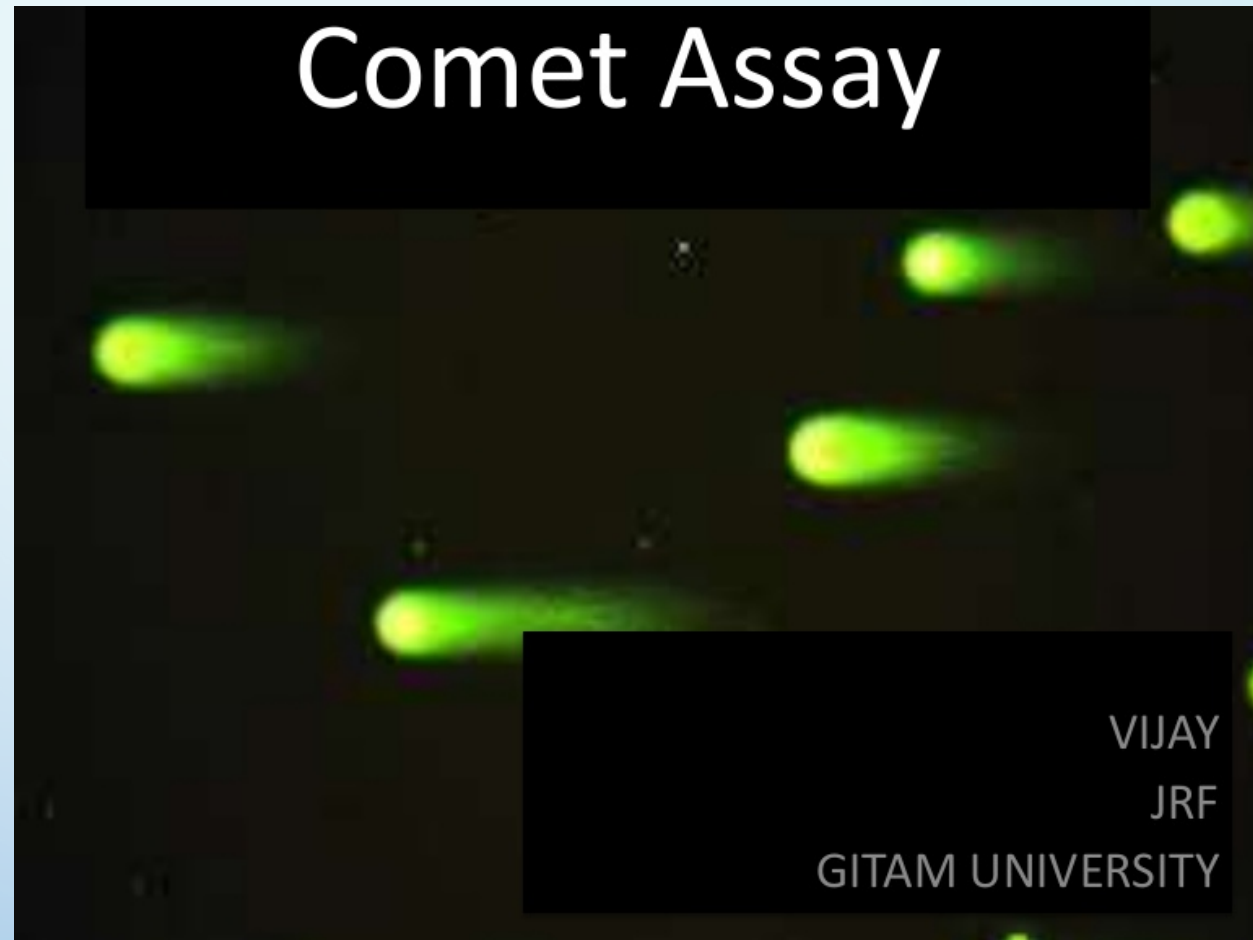
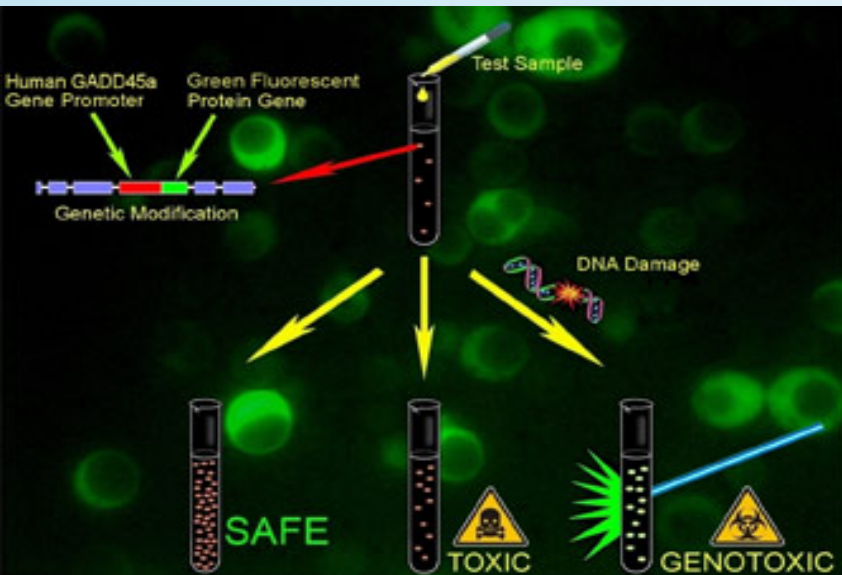
chromosome mutagen effect:
chromosome breakage and rearrangement



How To Measure Genotoxicity



Comet Assay

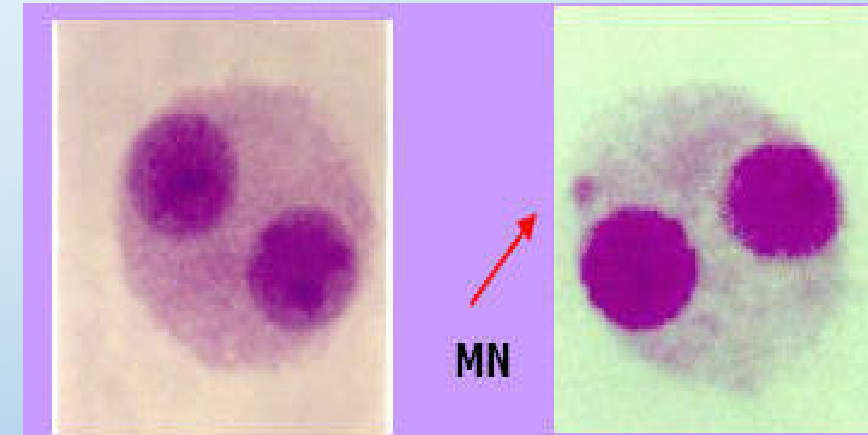


Comet assay is performed on individual cells in agarose gel used for rapid detection of any damage or repair in the DNA molecule. The short fragments travel faster through the gel due to the different molecular weight. These different fragments are stained with fluorescent colors and on fluorescent microscope are visible as comets

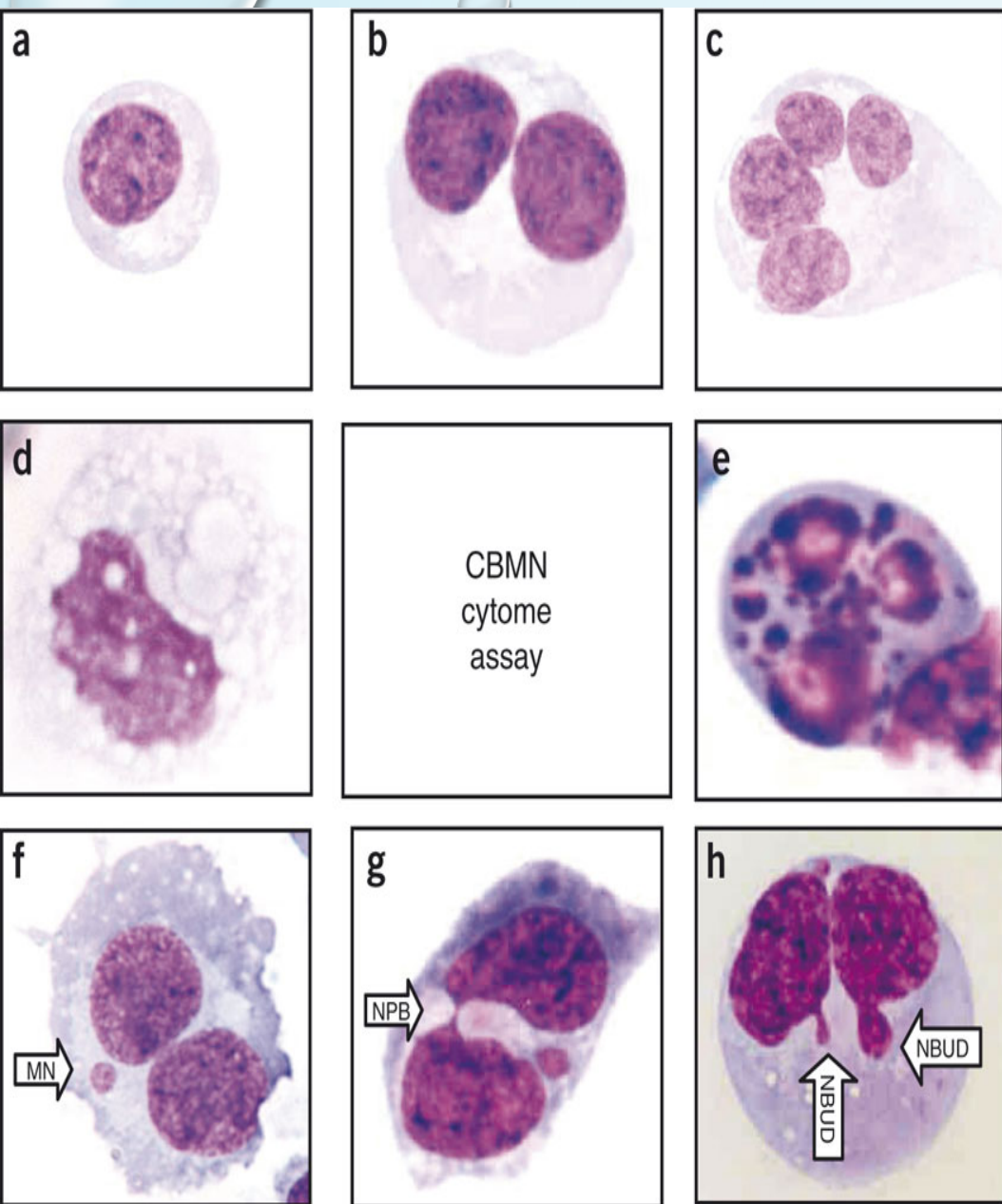
MN-ASSEY

DIAGNOSTIC TOOL AND PROCEDURE FOR MEASURING MICRONUCLEUS FREQUENCY IN PERIPHERAL BLOOD LYMPHOCYTES

- MICRONUCLEI ARE GENERATED AS A RESULT OF EXPOSURE OF THE BODY TO CLASTOGENIC AGENTS, ESPECIALLY THE IONIZING RADIATION
- MICRONUCLEI ARE INDEPENDENT CHROMATIN STRUCTURES THAT ARE COMPLETELY SEPARATED FROM THE CORE
- THEY ARE CREATED AS A RESULT OF CONDENSATION OF ACENTRIC CHROMOSOME FRAGMENTS OR WHOLE CHROMOSOMES THAT ARE LATE IN ANAPHASE
- AVERAGE SIZE OF MICRONUCLEI MAY VARY FROM 1/3 OR 1/16 OF THE CELL SIZE
- IMPORTANT QUANTITATIVE BIOMARKER WHICH PROVES THE EXISTENCE OF STRUCTURAL CHROMOSOMAL ABERRATIONS WHICH ARE THE RESULT OF DIFFERENT GENOTOXIC AGENTS *IN VITRO* OR *IN VIVO* CONDITIONS



BINUCLEATED CELLS BLOCKED AT CYTOKINETIC STAGE WITH AND WITHOUT MICRONUCLEUS



abnormal nuclear
shapes (MNI),
nucleoplasmic
bridges (NPBs)
and nuclear buds
(NBUDs)

to evaluate
the
genotoxicity
of ionizing
radiation

to
determine
the human
health risk

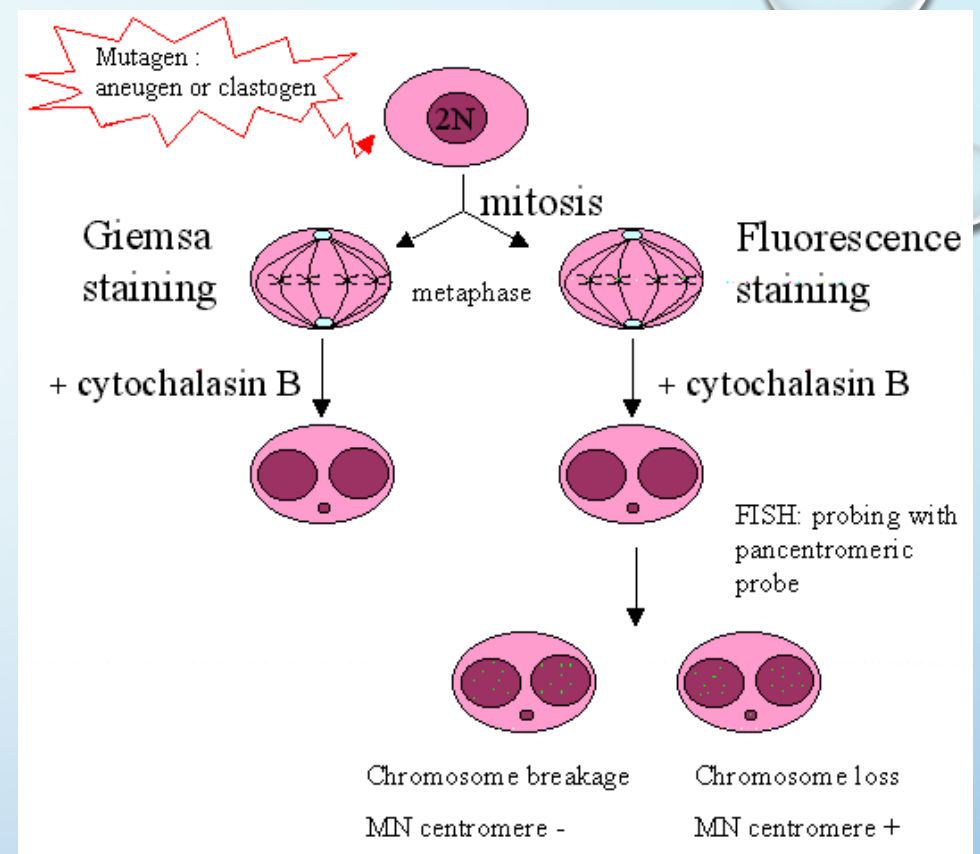
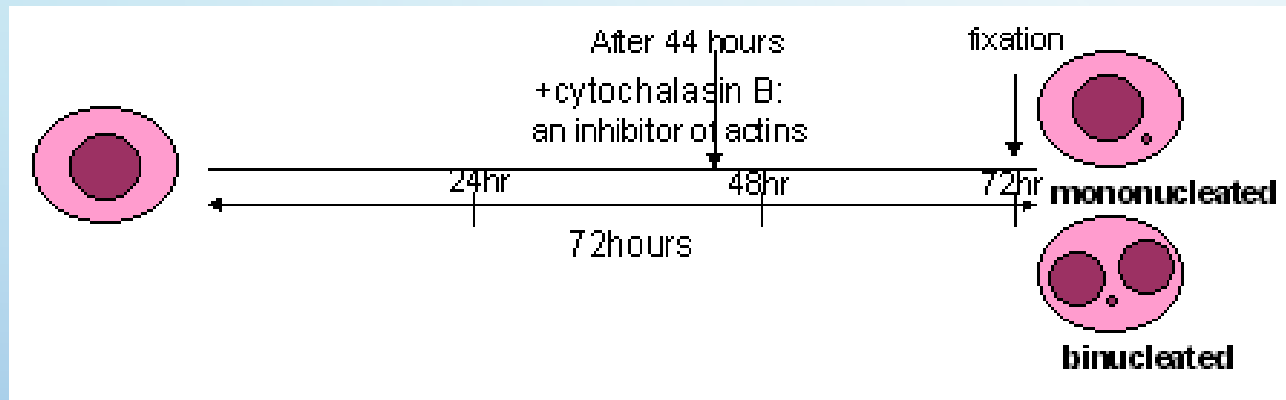
**AIMS
OF
THE
STUDY**

MATERIALS AND METHODS

- THE STUDY POPULATION INCLUDED:
- 20 INDIVIDUALS IN THE EXPOSED GROUP, MEDICAL PERSONNEL EXPOSED TO IONIZING RADIATION (RADIOLOGIST, TECHNICIANS AND NURSES)
- 20 INDIVIDUALS IN THE CONTROL GROUP, HEALTHY PEOPLE, (WHO HAVE NEVER BEEN EXPOSED TO IONIZING RAYS AND OTHER CHEMICAL OR PHYSICAL AGENTS).

	Exposed group	Control group
Number of men	13	10
Number of women	7	10
Age range	45±15	18±22
Range of years of professional exposure	15-35	/
Smokers	13	7

MATERIALS AND METHODS

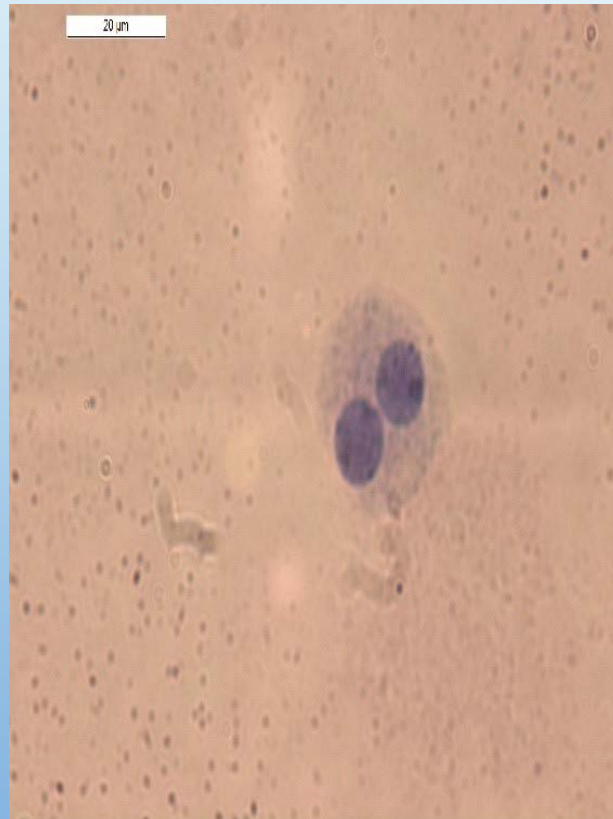
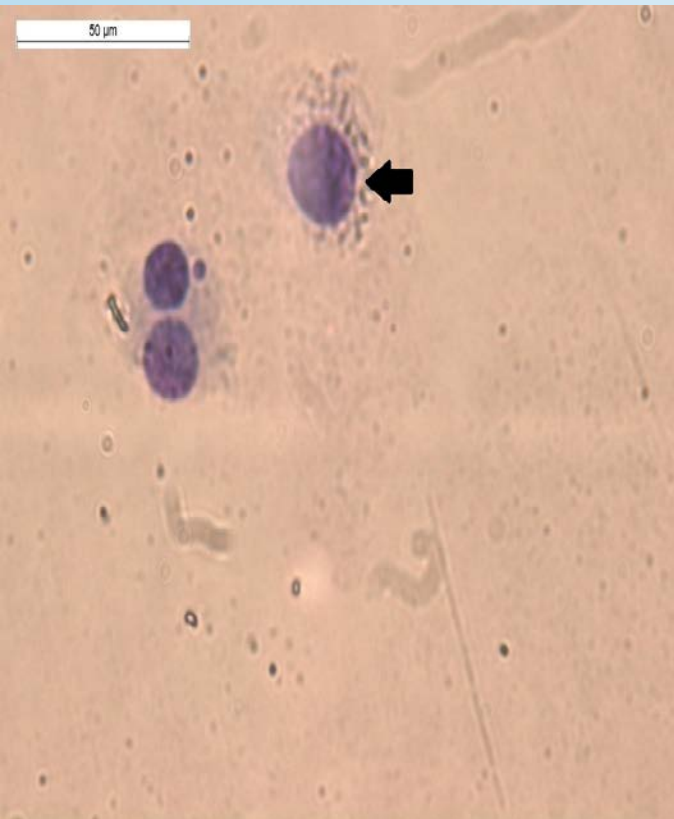


Venous blood sample (3 ml) was collected in heparinized tubes. Blood culture protocol was done according to Fenech (2007). 0.5-ml of blood sample was added to the culture tubes containing 4.5 ml of RPMI 1640 media (enriched with 20% fetal bovine serum, L-glutamine and 0.2 ml of phytohemagglutinin 1 % and each supplemented with 100 units/mL penicillin and 100 µg/mL streptomycin. The tubes was incubated 44h at 37 °C. Cytochalasin B was then added to each culture to block cell cytokinesis and cultures were reincubated at 37 °C for further 28 h.

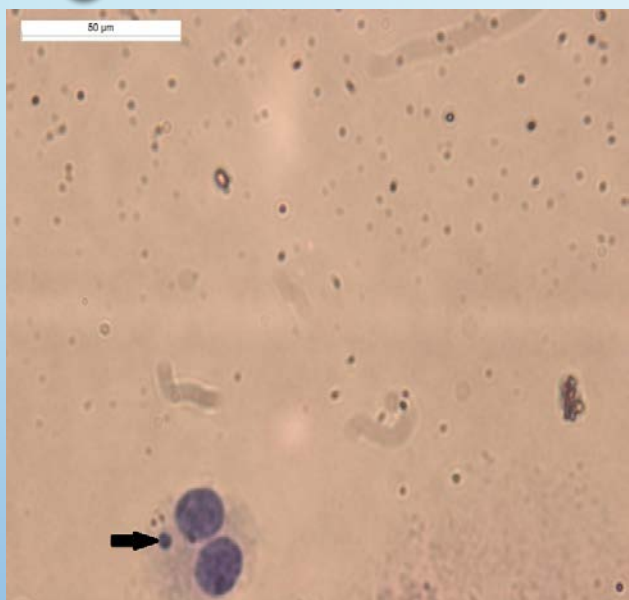
Fixation → Stained → examined by light microscope Leica DM4500 P (×40 and ×100)

RESULTS AND DISCUSSION

Classified the cells as mononucleates, binucleates or multinucleate

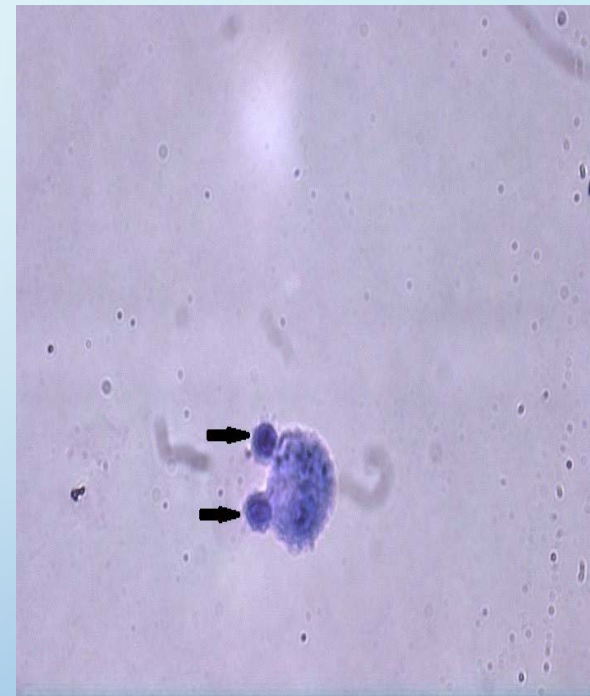


1,000 BN cells were evaluated



MNi are defined as small, round nuclei clearly separated from the main cell nucleus

SAMPLE	EXPOSED GROUP					CONTROL GROUP			
1.	M/F	AGE	PROFES.EXPOS	S/NS	MN	M/F	AGE	S/NS	MN
2.	M	60	32	S	16	M	21	NS	5
3.	M	51	24	S	12	M	19	NS	0
4.	M	50	15	NS	5	M	27	S	4
5.	F	59	35	S	18	M	18	NS	1
6.	F	45	15	S	21	M	33	S	8
7.	M	48	27	S	9	M	20	NS	2
8.	M	46	18	NS	14	M	25	NS	3
9.	M	45	17	NS	8	M	25	NS	2
10.	F	57	33	S	19	M	21	S	0
11.	M	45	16	NS	8	M	21	NS	2
12.	F	55	31	S	17	F	31	NS	2
13.	F	45	23	NS	21	F	28	NS	4
14.	M	45	26	S	8	F	28	NS	5
15.	F	60	35	S	18	F	28	S	3
16.	M	58	24	S	16	F	32	S	11
17.	M	46	16	S	19	F	18	NS	4
18.	M	52	24	NS	11	F	20	NS	6
19.	M	55	25	NS	6	F	32	S	9
20.	M	59	32	S	23	F	24	NS	6
21.	F	60	30	S	19	F	40	S	13
Σ					289				90
Average (x)					14.5				4.5
STDV (s)					5,51				3,52
V (%)					38.07				78.15



BN cells containing NPB

BN cells containing NBUDs

CONCLUSIONS

- **MN-ASSEY IS ONE OF THE BIOMARKERS IN GENOTOXICOLOGY, FOR ASSESSING THE CHROMOSOMAL INSTABILITY AND DAMAGE IS SCORING OF MN FREQUENCY'S IN LYMPHOCYTES**
- **THE MEAN OF MN FREQUENCIES IN THE EXPOSED GROUP IS GREATER IN COMPARISON WITH THE MEAN OF MN FREQUENCIES IN THE CONTROLLED GROUP**
- **CHROMOSOMAL INSTABILITY IS IN CORRELATION WITH MN FREQUENCIES IN MEDICAL WORKERS EXPOSED TO IONIZING RADIATION**
- **THE FORMATION OF SMALL AND LARGE MNI, NPBS, NBUDS ETC. INDICATES THAT MEDICAL WORKERS ARE EXPOSED ON CLASTOGENIC AND ANEUGENIC AGENTS, LIKE IONIZING RADIATION AND HAVE CHROMOSOMAL INSTABILITY AND HIGH RISK OF CANCER**
- **STUDENT'S T-TEST SHOWED SIGNIFICANT STATISTICAL DIFFERENCES BETWEEN THE TOTAL NUMBER OF BN CELLS WITH MN WITHIN THE TWO GROUPS (THE EXPOSED AND THE CONTROL) ($T=6,812$; $P<0,05$).**

CONCLUSIONS

THE NEED OF CYTOGENETIC MONITORING

**THE IMPORTANCE OF THE CHROMOSOMAL
MUTAGENIC EFFECT, WHICH WE CAN ANALYZE
CYTOLOGICALLY WITH GENOTOXIC TESTS**

MN-assey
Comet assey

