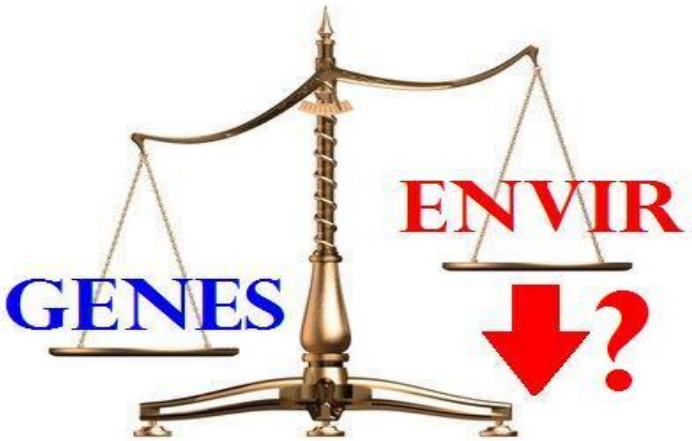

GENOTOXICOLOGICAL EFFECT

OF IONIZING RADIATION

ON MEDICAL OCCUPATIONALLY EXPOSED WORKERS

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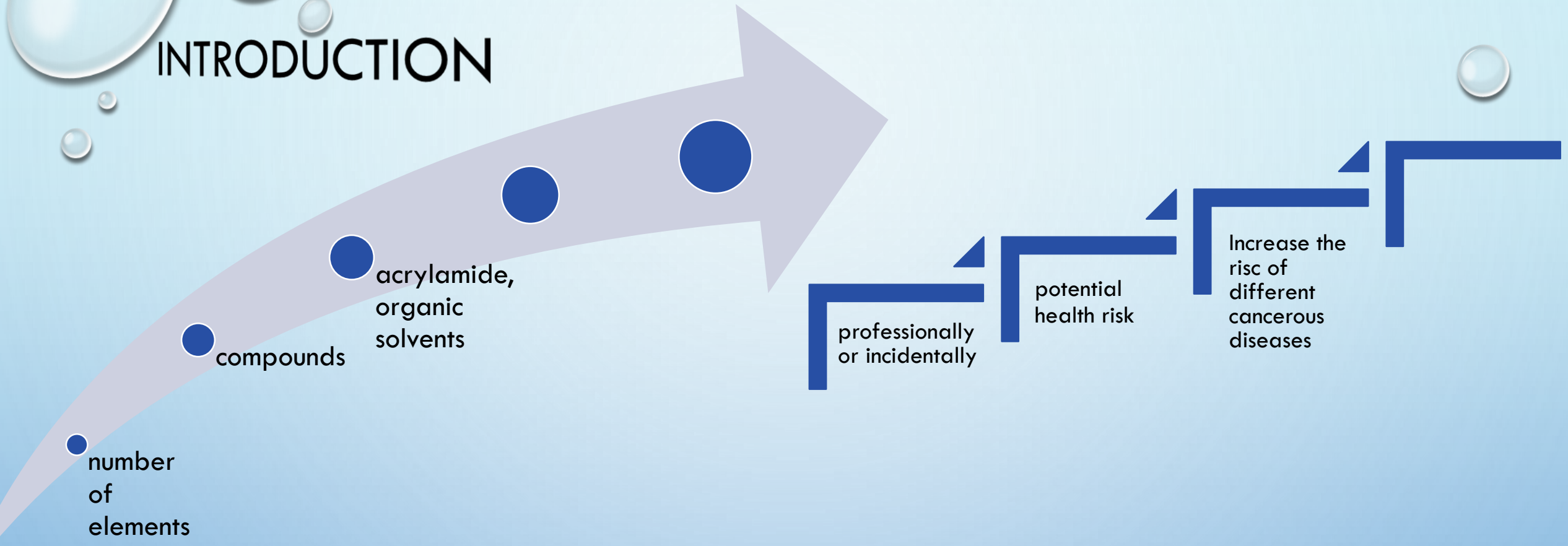
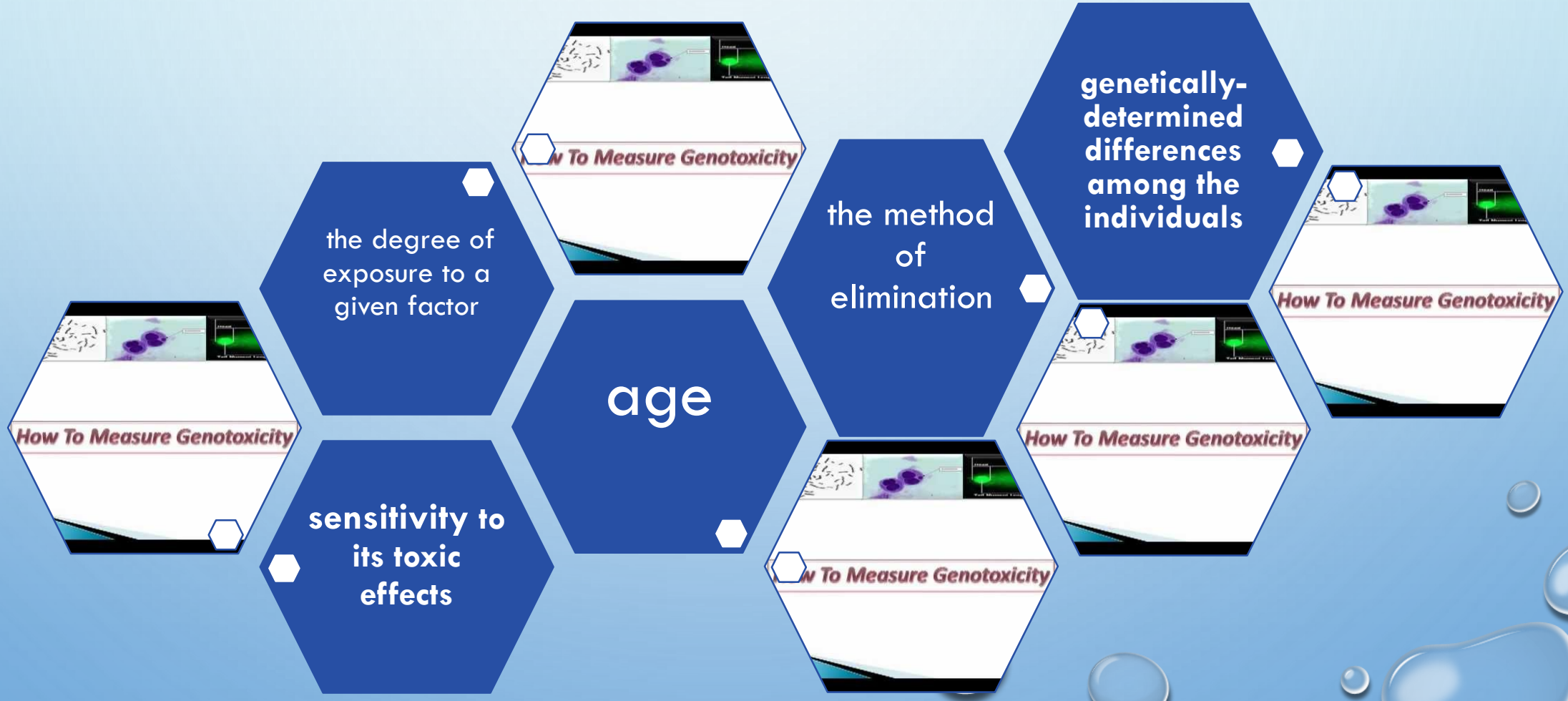
INTRODUCTION



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

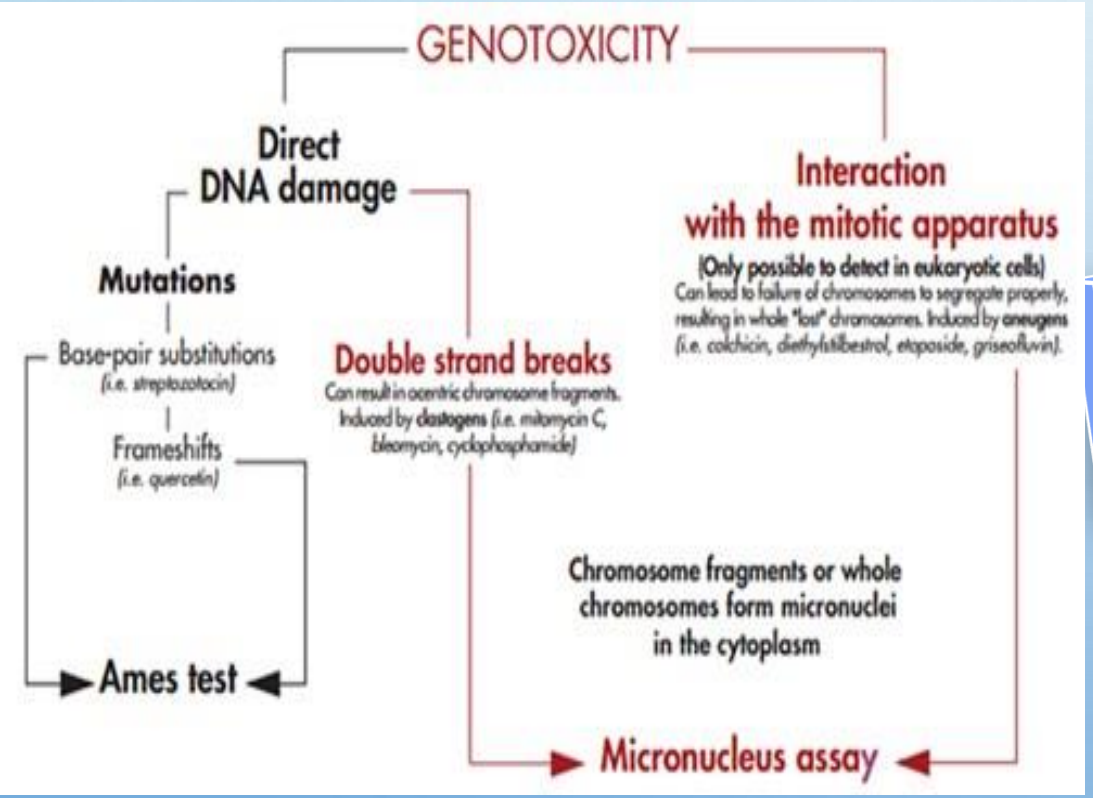
genotoxic agents induced mutations

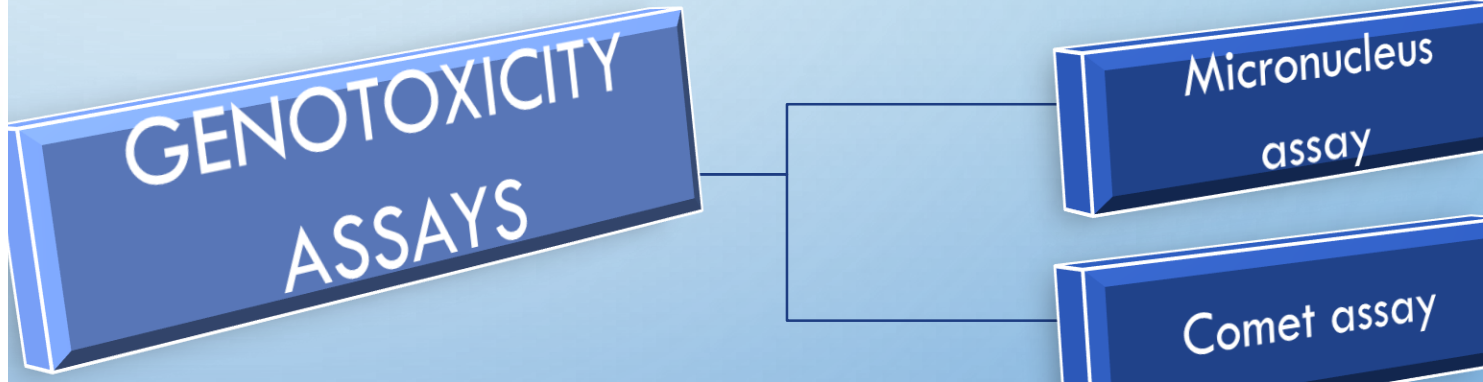
chromosome mutagen effect:

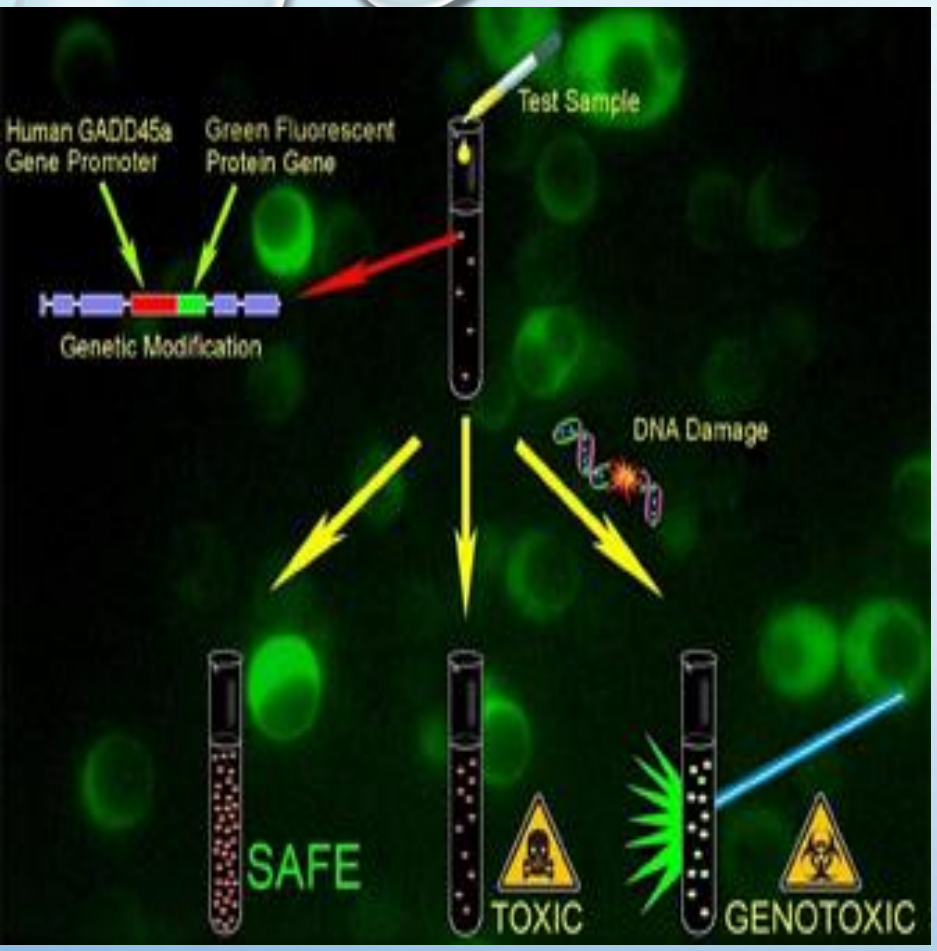
chromosome breakage and rearrangement

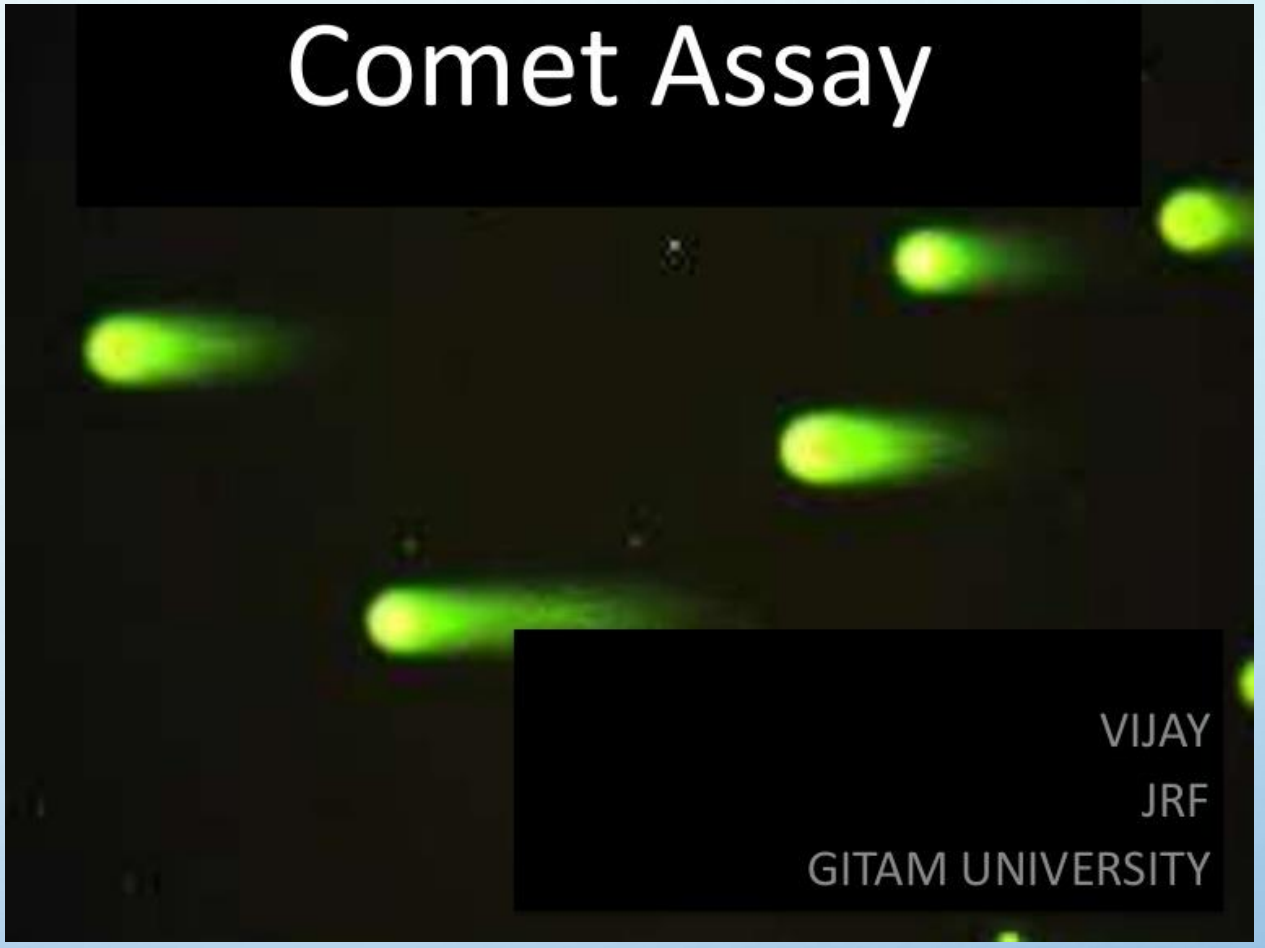
chronic exposure of the organism to small doses of chemical or physical mutagens leads to potential genotoxicity


How To Measure Genotoxicity


GENOTOXICITY
Direct DNA damage
Interaction with the mitotic apparatus
(Only possible to detect in eukaryotic cells)
Can lead to failure of chromosomes to segregate properly, resulting in whole "lost" chromosomes. Induced by aneugens (i.e. colchicin, diethylstilbestrol, etoposide, griseofluvin).
Mutations
Base-pair substitutions
(i.e. streptozotocin)
Frameshifts
(i.e. quercetin)
Double strand breaks
Can result in acentric chromosome fragments. Induced by clastogens (i.e. mitomycin C, bleomycin, cyclophosphamide)
Chromosome fragments or whole chromosomes form micronuclei in the cytoplasm
Ames test
Micronucleus assay


GENOTOXICITY ASSAYS
Micronucleus assay
Comet assay



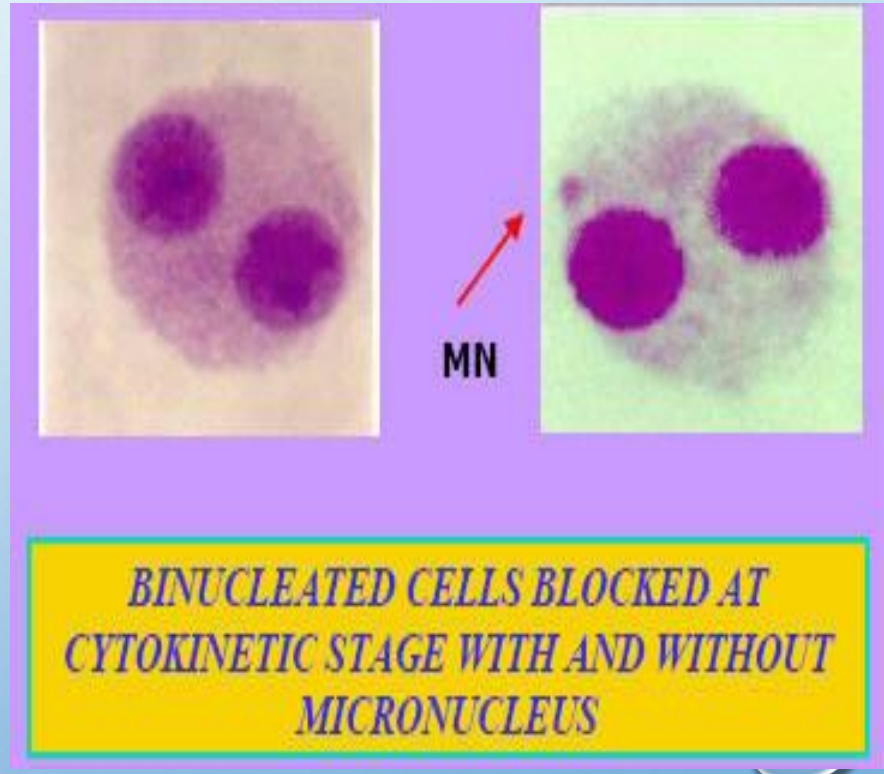


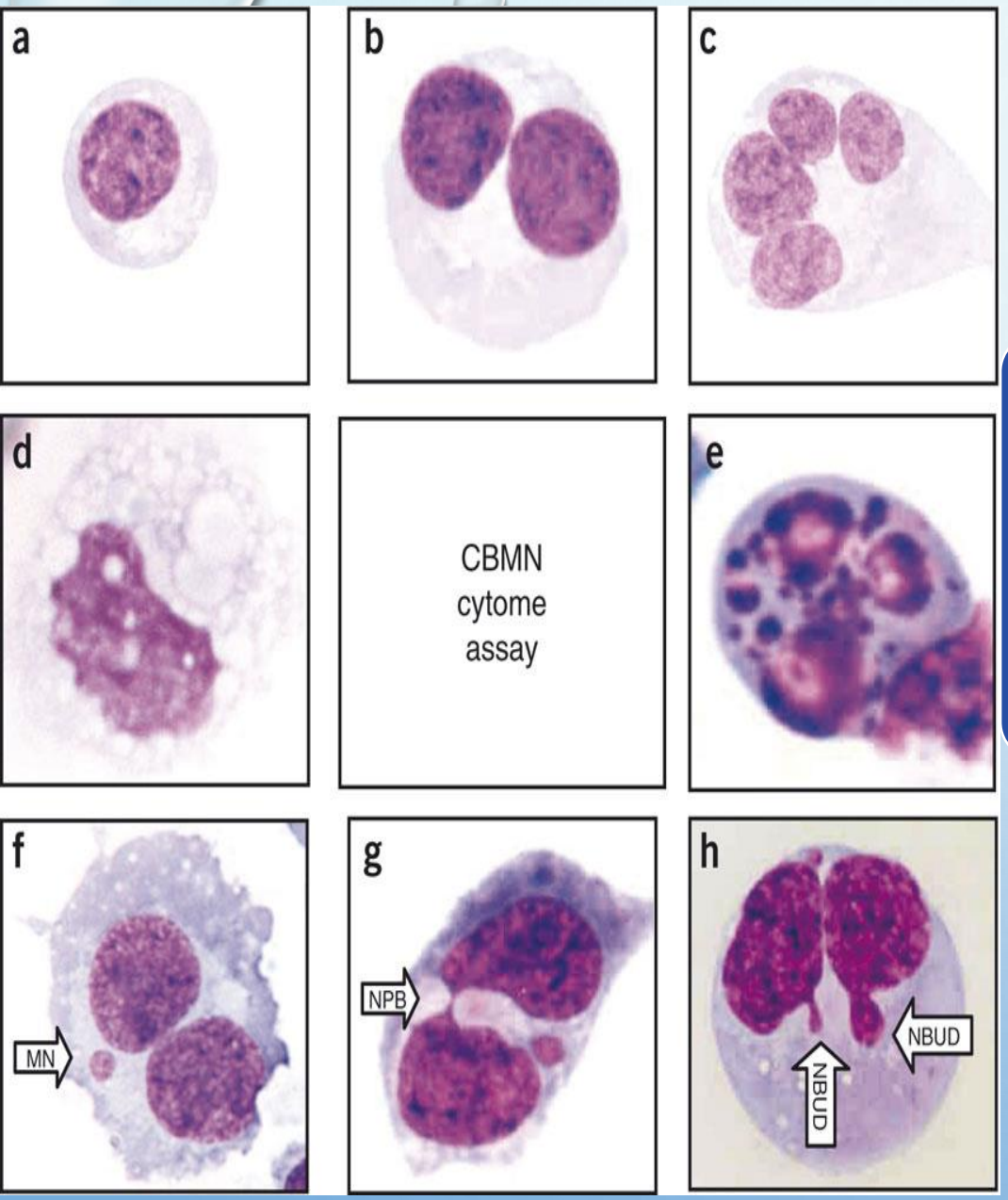
Comet assay is performed on individual cells in agarose gel used for rapid detection of any damage or repair in the DNA molecule. The short fragments travel faster through the gel due to the different molecular weight. These different fragments are stained with fluorescent colors and on fluorescent microscope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





AIMS OF THE STUDY

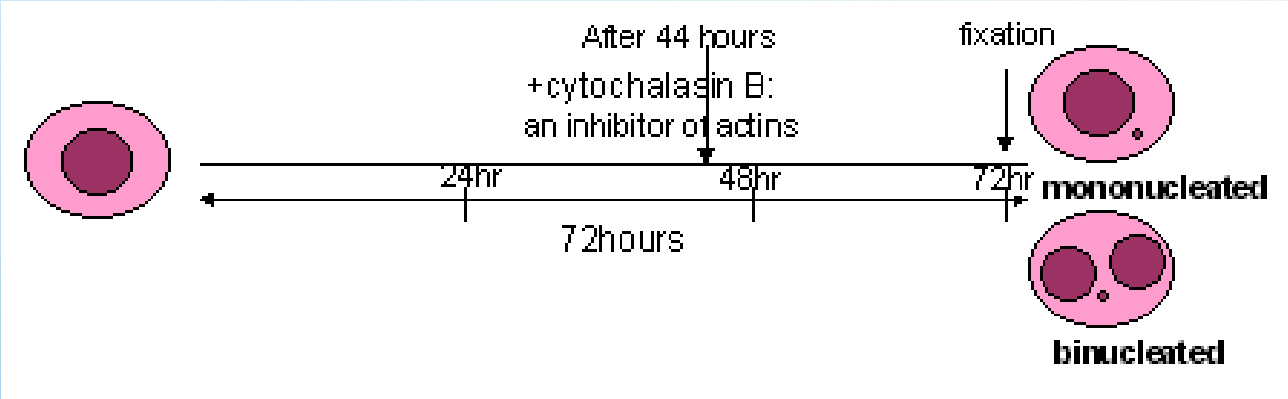
- to evaluate the genotoxicity of ionizing radiation
- abnormal nuclear shapes (MNi), nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs)
- to determine the human health risk

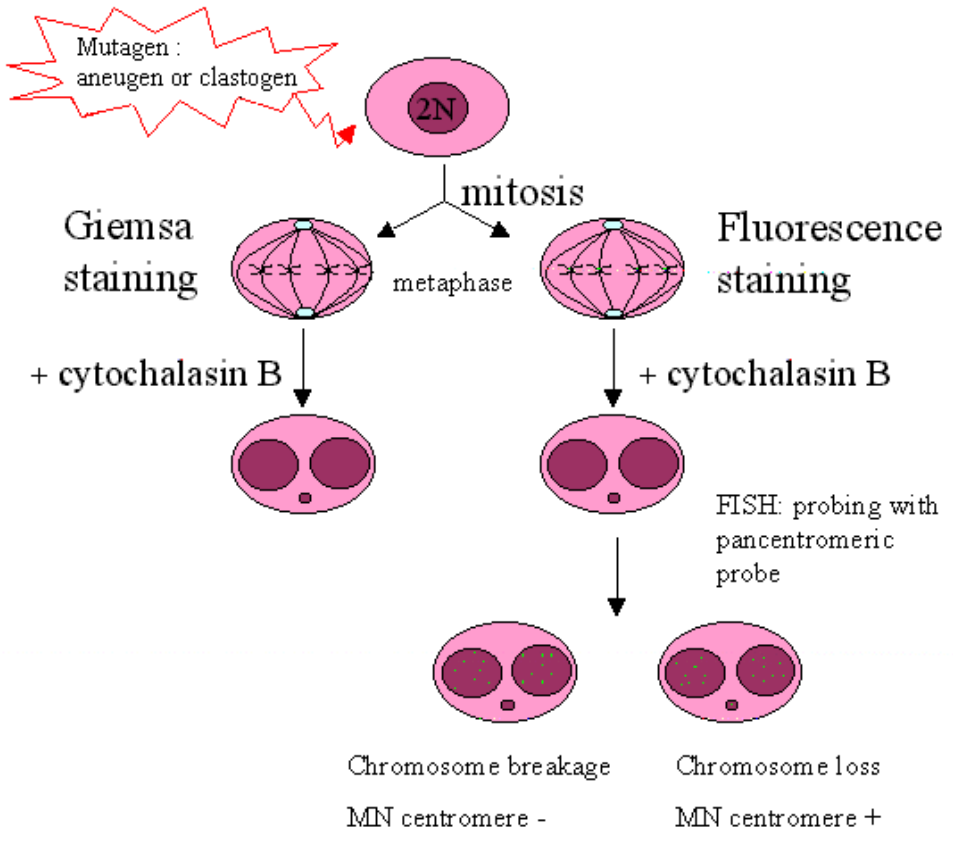
MATERIALS AND METHODS

- 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)

	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



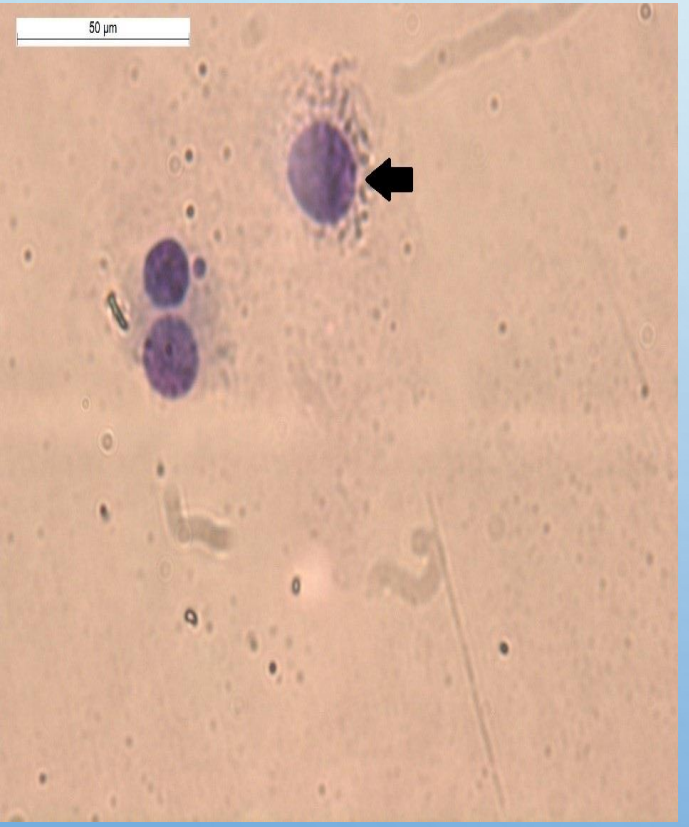


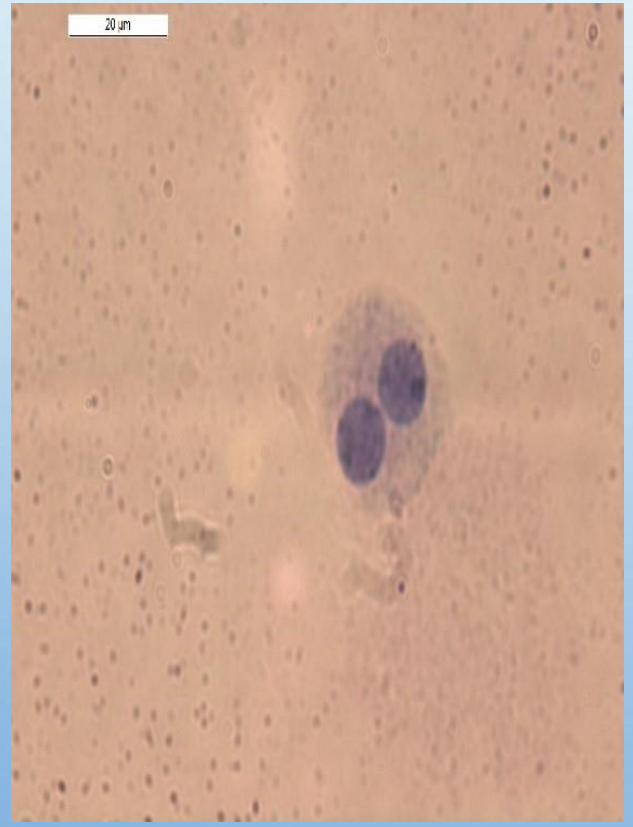
Venous blood sample (3 ml) was collected in heparinized tubes. Blood culture protocol was done according to Fenech (2007). 0.5-ml of blood sample was added to the culture tubes containing 4.5 ml of RPMI 1640 media (enriched with 20% fetal bovine serum, L-glutamine and 0.2 ml of phytohemagglutinin 1 % and eachsupplemented 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.

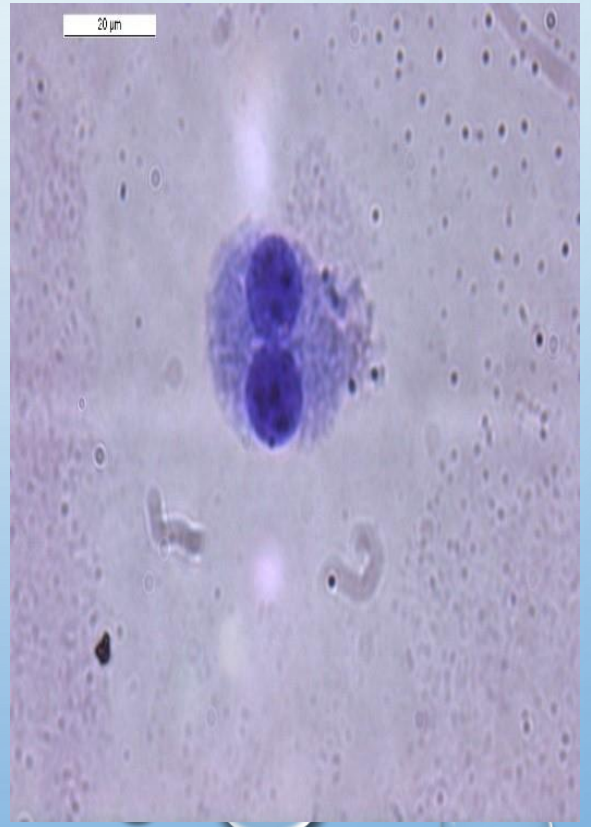
Fixation ➡ Stained ➡ examined by light microscope Leica DM4500 P (×40 and×100)

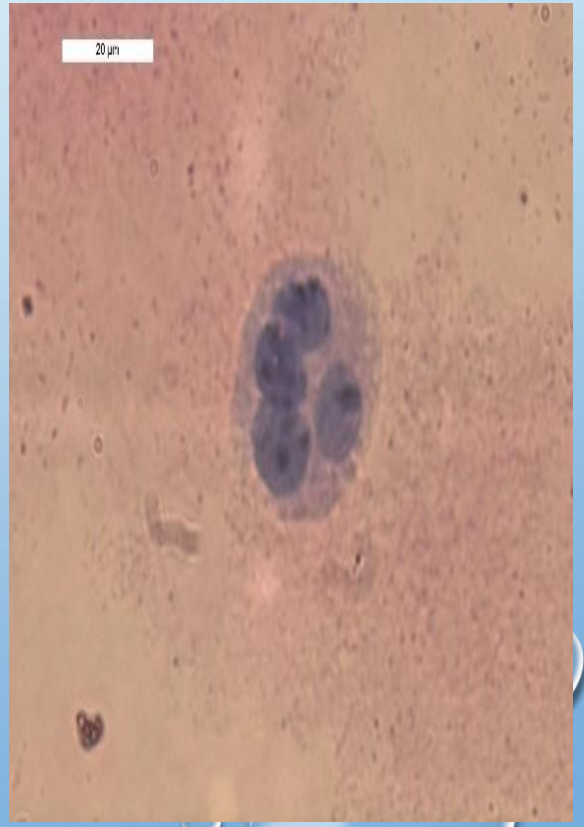
RESULTS AND DISCUSSION

Classified the cells as mononucleates, binucleates or multinucleate

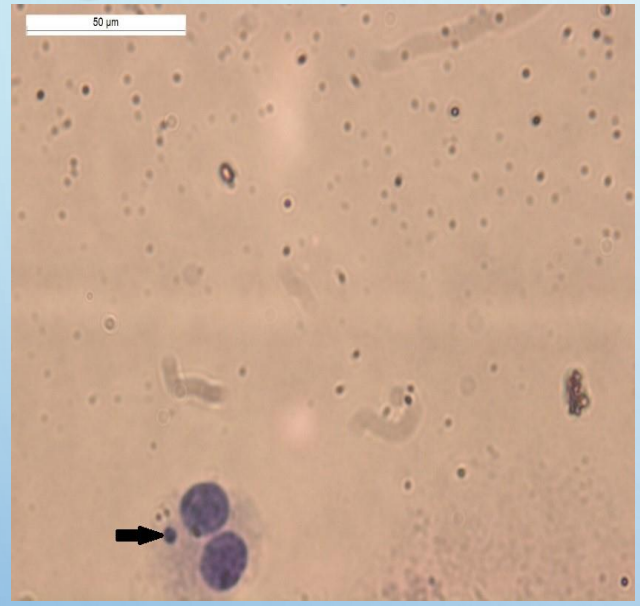


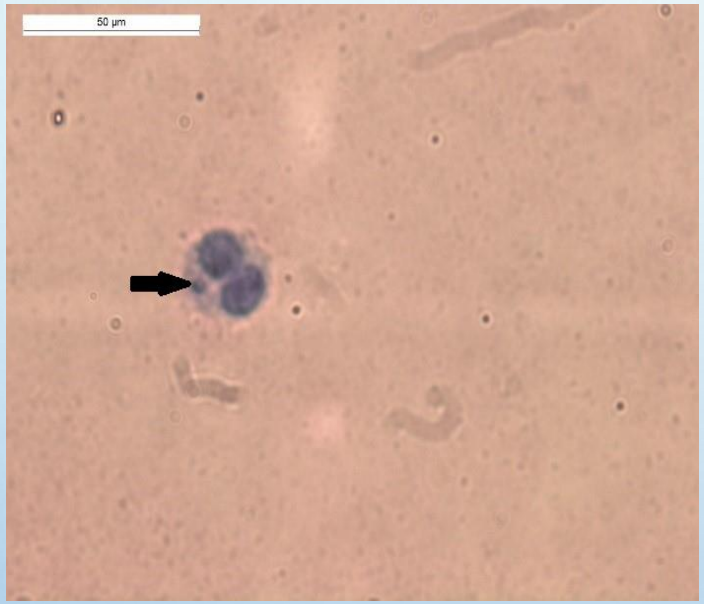


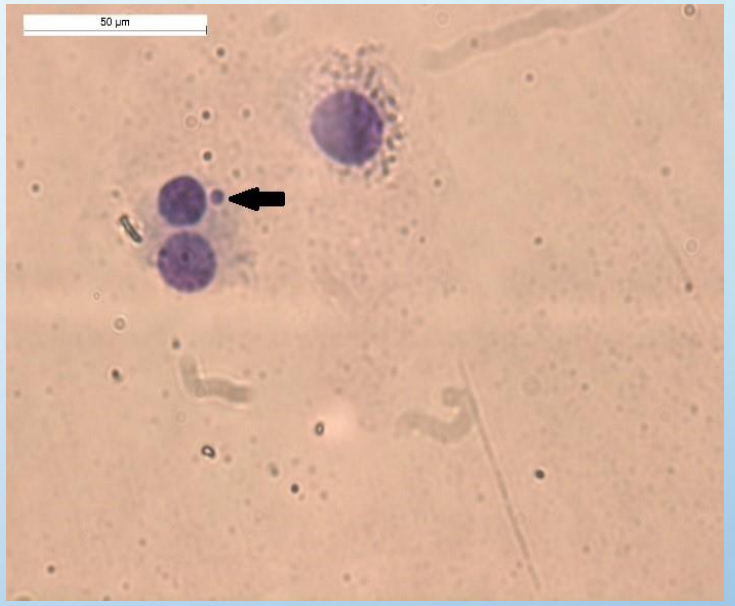




1,000 BN cells were evaluated

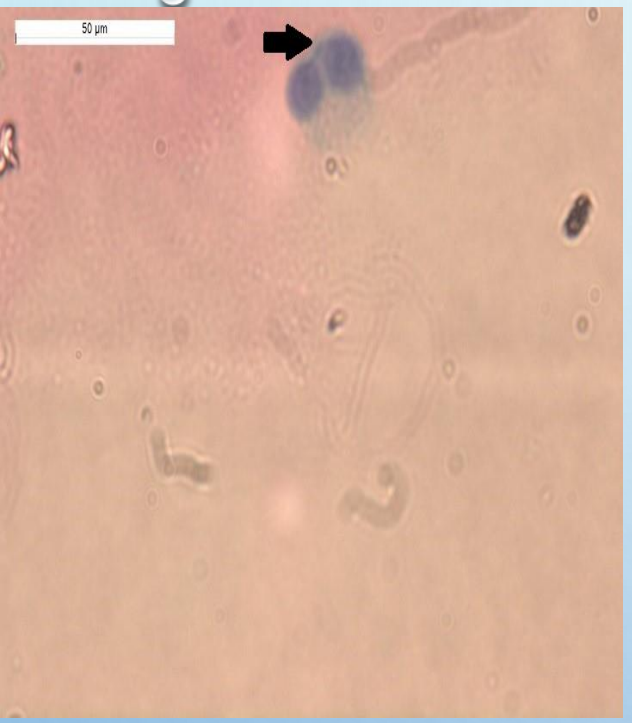


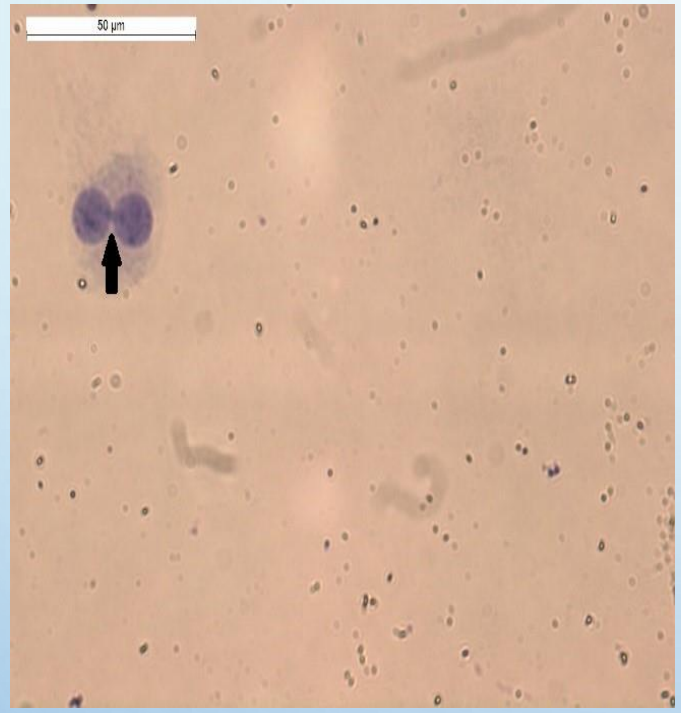


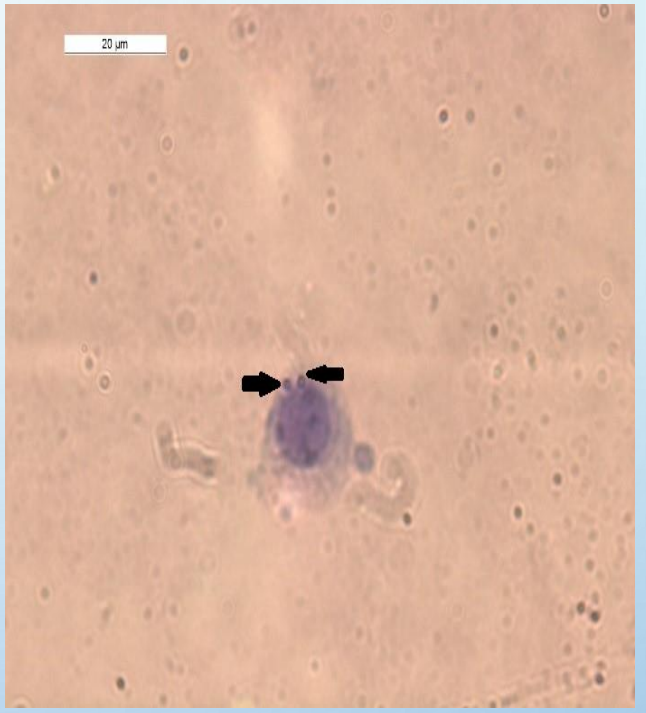


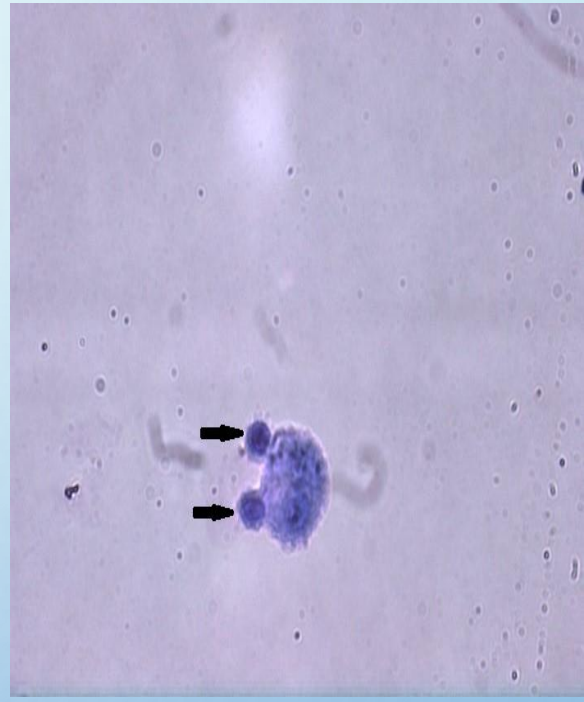
MNi are defined as small, round nuclei clearly separated from the main cell nucleus

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









BN cells containing NPB

BN cells containing NBUDs

CONCLUSIONS

- **MN-ASSEY IS ONE OF ASSAY 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 PERSONNEL 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

This study has a practical importance

- indicates the need of introducing a permanent genotoxicological monitoring

MN-assey

Comet assey

application of other molecular cytogenetic methods for observation of the chronic exposure of organism to a potentially genotoxic agents

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CYTOGENETIC ABNORMALITIES IN LYMPHOCYTES EVALUATED WITH MICRONUCLEUS ASSAY IN MEDICAL PERSONNEL OCCUPATIONALLY EXPOSED TO IONIZING RADIATION

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Velickova N., M. Milev, T. Ruskovska, B. Petrova, B. Nedeljkovik, P. Gorgieva (2015): *Cytogenetic abnormalities in lymphocytes evaluated with micronucleus assay in medical personnel occupationally exposed to ionizing radiation*.–Genetika, Vol 47, No. 3, 927-939.

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PRESENT KNOWLEDGE AND EXPERIENCE ON THE STRATEGIES EMPLOYED BY MYCOPLASMA CONTAMINATION OF THE HUMAN CELL CULTURES

Nevenka Velickova[1], Misko Milev[2], Gorgi Sumanov[3], Biljana Petrova[4]

THANK YOU