PJ-04

"CYTOGENETIC ANALYSIS IN METHYL ISOCYANATE (MIC) EXPOSED POPULATION AND THEIR PROGENY"

Annual Progress Report of Intramural Project

October 2013- October2016

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Submitted to

National Institute of Research in Environmental Health

(Indian Council of Medical research)

Kamala Nehru Hospital Building

GMC Campus, Bhopal (MP)

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2385/264/2017

7th June, 2017

To

The Director
National Institute for Research in Environmental Health
Kamla Nehru Hospital Building
Gandhi Medical College Campus
Bhopal - 462 001 (M.P.)

Sub: Submission of final report of the Project Cytogenetic Analysis in the MIC exposed population and their proceny

Respected Sir,

ICMR has sanctioned the above project with the collaboration of NIREH Bhopal.

We hereby submitting the final report of the above mentioned project for the duration October 2013 - 2016 in 3 hard copies.

Kindly acknowledge the receipt.

Thanking you,
Yours Sincerely,

(Dr. N. Ganesh) 7/6/17
Sr. Scientist - Research Deptt.

Encl : As above

Received on 7/6/2017

CONTENTS

1. **Title of the project:**
   "Cytogenetic Analysis in Methyl Isocyanate (MIC) Exposed Population and their Progeny" (IEC/JNCH/01/18-05-13)

2. **Unique ID of the Project (provided by ICMR):**

3. **Principle Investigator and Co-Investigator**

   Name & Designation of the Chief Investigator:

   Dr. N. Ganesh, Sr. Scientist & Head, Dept. of Research, Jawaharlal Nehru Cancer Hospital & Research Centre, Bhopal

   Name & Designation of the Co-Investigator:
   Dr. N. Banerjee, Head, National Institute for Research in Environmental Health, Bhopal

4. **Implementing Institution and other collaborating Institutions**

   Implementing Institution: Dept. of Research, Jawaharlal Nehru Cancer Hospital & Research Centre, Bhopal

   Other collaborating Institutions: National Institute for Research in Environmental Health, Bhopal

5. **Date of commencement:** 29-11-2013

6. **Duration: 3 years**

7. **Date of completion:** 30-10-2016

8. **Objectives as approved**

   To investigate the cytogenetic status in methyl isocyanate (MIC) exposed population and their progeny.

9. **Deviation made from original objectives, if any, while implementing the project and reasons thereof.**

   The experts of ICMR have suggested to reduce the number from 3600 to half. Therefore, we had confirmed our total number of subjects for three years 1800. The project is funded for two years only. Therefore, 1200 subjects only targeted.

10. Field/Experimental work giving full details of summary of methods adopted.

Please refer Annexure (Page No. 22-33)

Selection of subjects was done purely on the basis of their exposure to the gaseous cloud released from Union Carbide's pesticide plant on midnight of 2nd -3rd December 1984. The selection was made as per the selection criteria so as to exclude the false positive exposures. All the subjects were selected from hospital registration and their accompanied relatives.

The investigations were done to study the various cytogenetic status in different groups as follows: (for 03 years total 1800 subjects)

- Exposed individual's age between 29-59 years (150 male and 150 female). (Healthy Individuals)
  Non-exposed individual's age between 18-59 years (150 male and 150 female) [75 from Bhopal and 75 from out stationed of each sex] (Healthy Individuals)
- Progeny born after exposure 1st generation (150 male and 150 female).
- Progeny born after exposure 2nd generation (150 male and 150 female).
- 150 male and 150 female of different ailments diagnosed after the exposure of the gas (non-inherited).
- 150 male and 150 female with congenital malformation/ malformation.

Project sanctioned for 02 years (Oct 2013 – Oct 2015, extension given for one year Oct 2016) – 1200 subjects

- Exposed individual's age between 29-59 years (100 male and 100 female). (Healthy Individuals)
  Non-exposed individual's age between 18-59 years (100 male and 100 female) [50 from Bhopal and 50 from out stationed of each sex] (Healthy Individuals)
- Progeny born after exposure 1st generation (100 male and 100 female).
- Progeny born after exposure 2nd generation (100 male and 100 female).
- 100 male and 100 female of different ailments diagnosed after the exposure of the gas (non-inherited).
- 100 male and 100 female with congenital malformation/ malformation.

The criteria followed for identifying the exposed subjects are as under:

a. A person must hold a gas victim official card or any other evidence which depicts the physical presence of his / her during the time of exposure.
b. Any other evidence which shows hospitalization on or within 48 hours of the episode.

c. It was ascertained that none of the subjects (exposed or control) was more than 55 years of age and does not have smoking or tobacco chewing habit. Persons having exposure to chemicals or radiations were excluded.
Please refer Annexure for full details

Pedigree analysis

A detailed demography of the individual of the entire group was recorded along with their family history. On this basis, all the subjects were selected for the investigation.

Chromosomal aberrations assay

One of the few direct methods which do exist to measure mutations or other forms of induced damage in human exposed to potential mutagen or carcinogen has employed.

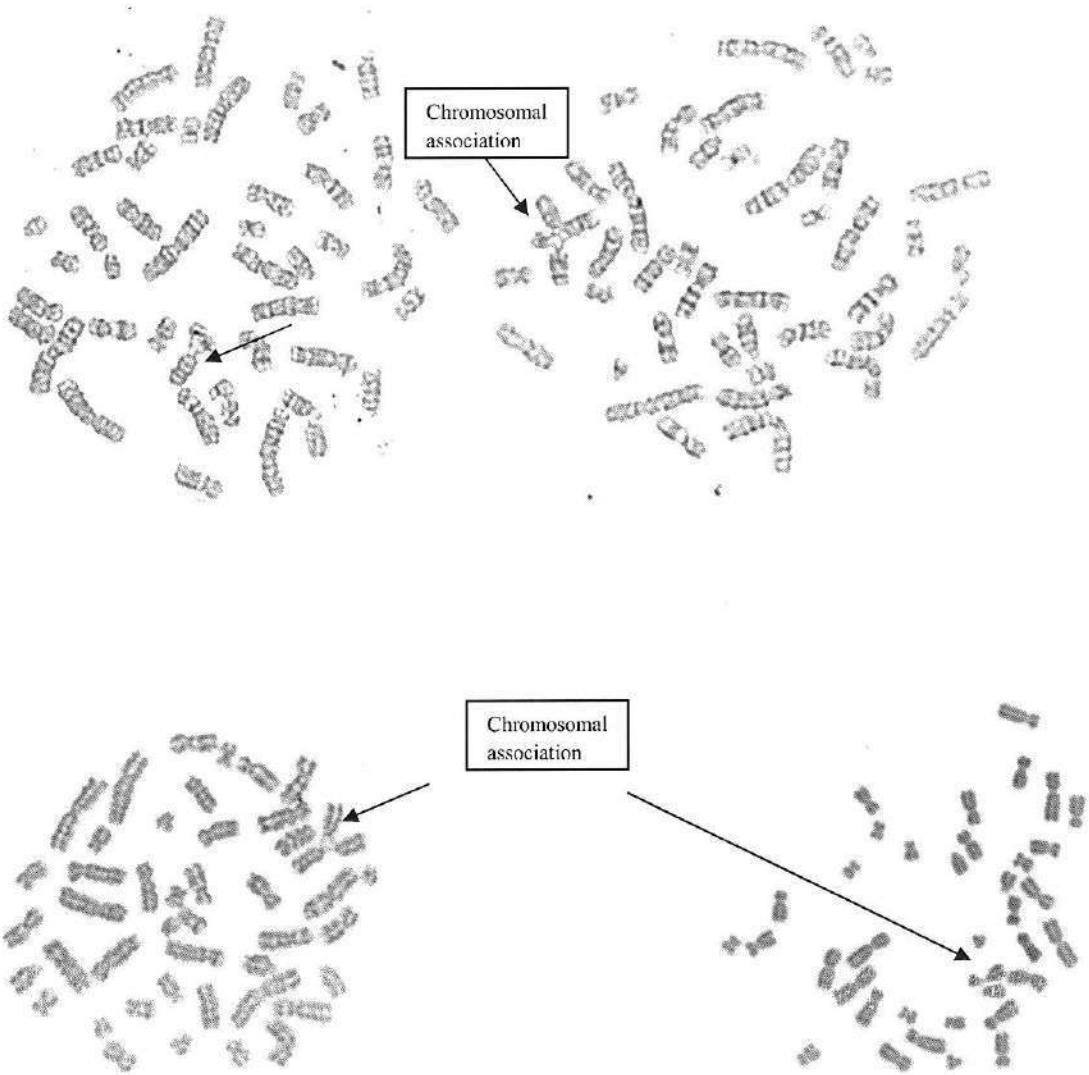
Chromosome preparations were made from peripheral blood lymphocyte cultures stimulated with PHA following modification of **Moorhead *et al.*, (1960).**

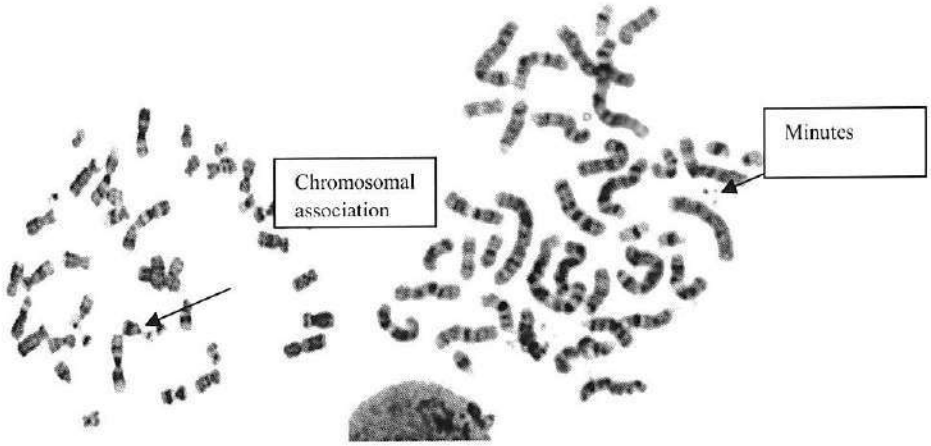
The gross alterations would include structural aberrations such as breakage, translocation (if any), chromosomal associations, minutes etc. The introduction of chromosome banding technique has made it possible to utilize chromosomal aberrations assay for studying genotoxic effects in greater details. In the present investigation, the G & C banding techniques was employed to assess the chromosomal aberrations [5, 6].

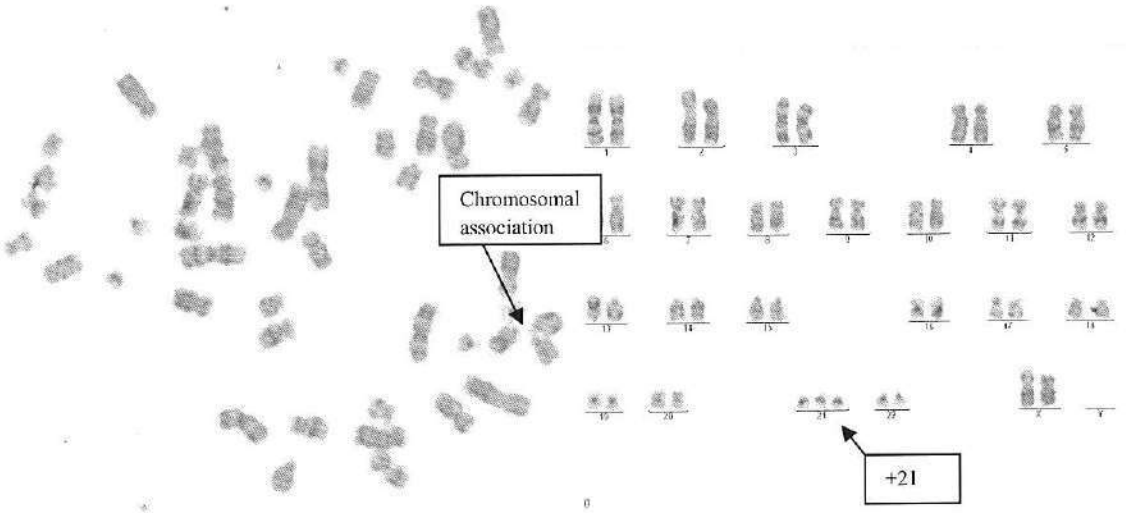
11. Supported by necessary tables, charts, diagrams and photographs

Please refer Annexure (Page No. 34-68)

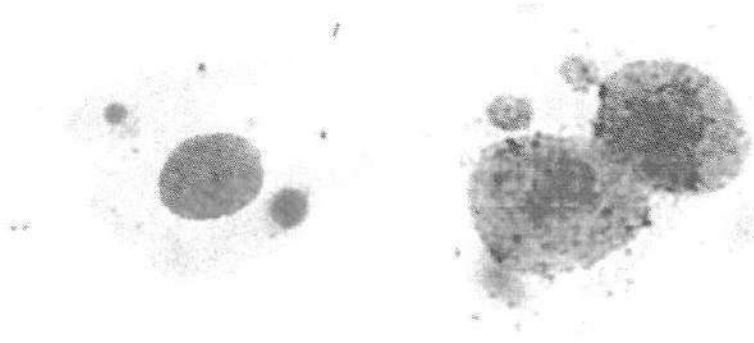
Sample Microphotograph







Micronucleus



C banded Metaphase spread

Table No. 1: Total No. of Identified Individuals (01-01-2014 to 30-10-2016) and enrolled individuals after Randomization					
Group	**Sub-groups**	**Male**	**Female**	**Total Individuals Identified**	**Total Individuals Enrolled after Randomization**
Normal Healthy MIC Exposed		**224**	**197**	**421**	**200**
Normal Healthy Non Exposed		**252**	**242**	**494**	**200**
1st Progeny of MIC exposed individual		**362**	**297**	**659**	**200**
2nd Progeny of MIC exposed individual*		**27**	**19**	**46**	**46**
Congenital Malformations	**A: With MIC Exposure***	**50**	**37**	**87**	**87**
	B: Without MIC Exposure	**64**	**60**	**124**	**100**
Other Ailments	**A: With MIC Exposure**	**125**	**117**	**242**	**100**
	B: Without MIC Exposure*	**46**	**50**	**96**	**96**
Total	**-**	**1150**	**1019**	**2169**	**1029**

NB*: Through pedigree analysis only these number of individuals identified, hence these much number only could manage to enroll in the final total number of subjects.

Table No. 2: Detail of Samples Processed				
Groups	**Sub-groups**	**No. of Males**	**No. of Females**	**Total No. of Subjects**
Normal Healthy MIC Exposed n=200		**100/100**	**93 /100**	**193/200**
Normal Healthy Non Exposed n=200		**100/100**	**72/100**	**172/200**
1st Progeny of MIC exposed individual n=200		**72/100**	**59/100**	**131/200**
2nd Progeny of MIC exposed individual n=200		**27/100**	**19/100**	**46/200**
Congenital Malformations n=200	**A: With MIC Exposure**	**50/50**	**37/50**	**87/100**
	B: Without MIC Exposure	**50/50**	**50/50**	**100/100**
Other Ailments n=200	**A: With MIC Exposure**	**38/50**	**19/50**	**57/100**
	B: Without MIC Exposure	**46/50**	**50/50**	**96/100**
No. of samples processed	-	**483/600**	**399/600**	**882/1200**

NB: Only 882 individuals sample got processed because of their availability during the blood sample collection time or because of the preoccupied duties they failed to visit the centre, or because of their religious functions like Nawratere, Mohoram, Ramzan, etc.

Table No. 3: Types of aberrations in number of Male individuals of all the groups (n= 483)									
Group	**MN**	**MIN**	**DL**	**NUM ABR**	**FRG**	**CHR ASSC**	**PCD**	**PP**	**PUL**
MIC Exp n= 100	26 **(26%)**	11 **(11%)**	07 **(07%)**	3 **(3%)**	04 **(4%)**	42 **(42%)**	09 **(9%)**	2 **(2%)**	2 **(2%)**
Non Exp n = 100	11 **(11%)**	02 **(2%)**	0	0	03 **(2%)**	08 **(2%)**	02 **(2%)**	0	0
1st Progeny n = 72	12 **(16.6%)**	7 **(9.7%)**	0	0	1 **(1.4%)**	11 **(15.2%)**	1 **(1.4%)**	0	0
2nd Progeny n = 27	4 **(14.8%)**	2 **(7.4%)**	0	1 **(3.7%)**	0	3 **(3.7%)**	0	0	0
Other Ailments/ W Exp n = 38	**12 (31.5%)**	9 **(23.6%)**	0	0	0	07 **(18.4%)**	0	0	0
Other Ailments/ WO Exp n = 46	19 **(41.3%)**	13 **(28.26%)**	0	3 **(6.5%)**	2 **(4.3%)**	6 **(13%)**	03 **(6.5%)**	1 **(2.1%)**	1 **(2.17%)**
CM W Exp n = 50	08 **(16%)**	2 **(4%)**	0	50 **(100%)**	2 **(4%)**	11 **(22%)**	0	0	0
CM/ WO Exp n = 50	06 **(12%)**	0	0	50 **(100%)**	6 **(12%)**	10 **(20%)**	1 **(2%)**	0	0

Legend:

CM W Exp- Congenital Malformed with exposure, CM/WO Exp- Congenital Malformed without exposure, MN- Micronucleus, MIN- Minute, DL- Deletion, NUM ABR- Numerical Aberration FRG- Fragment, CHR ASSC- Chromosomal Association,PCD- Premature Centromeric Division PP- Polyploidy, PUL- Pulverized Chromosomes

Table No. 4: Types of aberrations in number of female individuals of all the groups (n=399)									
Group	**MN**	**MIN**	**DL**	**NUM ABR**	**FRG**	**CHR ASSC**	**PCD**	**PP**	**PUL**
MIC Exp (n = 93)	11 **(11.8%)**	1 **(1%)**	03 **(3.2%)**	0	02 **(2.1%)**	23 **(24.7%)**	0	0	0
Non Exp (n = 72)	04 **(5.5%)**	1 **(1.3%)**	0	0	1 **(1.3%)**	6 **(8.3%)**	1 **(1.3 %)**	0	0
1st Progeny (n = 59)	3 **(5%)**	1 **(1.6%)**	0	0	1 **(1.6%)**	4 **(6.7%)**	0	0	0
2nd Progeny (n =19)	1 **(5.2%)**	1 **(5.2%)**	0	0	0	0	0	0	0
Other Ailments/ W Exp (n = 19)	3 **(15.7%)**	3 **(15.7%)**	0	0	0	6 **(31.5%)**	0	0	0
Other Ailments/ WO Exp (n = 50)	18 **(36%)**	7 **(14%)**	0	2 **(4%)**	1 **(2%)**	14 **(28%)**	0	0	0
CM W Exp (n = 37)	1 **(2.7%)**	0	0	37 **(100%)**	0	7 **(18.9%)**	0	0	0
CM/ WO Exp (n = 50)	4 **(8%)**	0	0	50 **(100%)**	1 **(2%)**	16 **(32%)**	0	0	0

Legend:
CM W Exp- Congenital Malformed with exposure, CM/WO Exp- Congenital Malformed without exposure, MN- Micronucleus, MIN- Minute, DL- Deletion, NUM ABR- Numerical Aberration FRG- Fragment, CHR ASSC- Chromosomal Association, PCD- Premature Centromeric Division PP- Polyploidy, PUL- Pulverized Chromosomes

12. **Detailed analysis of results**

Please refer Annexure (Page No. 34-68)

13. **Contributions made towards increasing the state of knowledge in the subject.**

14. **Conclusions summarizing the achievements and indication of scope for future work.**

Please refer Annexure (Page No.75-80)

15. **Science and Technology benefits accrued: (NIL)**

I. List of research publications with complete details:
Authors, Title of the paper, Name of Journal, Vol. Page, year

II. Manpower trained in the project:

a. Research Scientists or Research Fellows

1. **Ms. Poonam Sharma – SRF (Resigned)**
2. **Ms. Nidhi Puranik – JRF (Resigned)**
3. **Mr.Arun R Nair Lab Astt. (Resigned)**
4. **Mr. Immamul Haque - JRF**
5. **Mr. Ravindra Singh – Lab Astt. (Resigned)**

b. No. of PhDs produced. (NIL)

c. Other Technical Personnel trained.

III. Patents taken, if any: NA

IV. Products developed, if any. NA

16. Abstract (300 words for possible publication in ICMR Bulletin).

Bhopal gas tragedy of 1984 was the world's worst industrial disaster that leads to deaths of thousands of residents as a consequence of exposure to the toxic gases released at the time of the incident. Many studies reported genotoxicity and mutagenicity in the exposed population soon after the exposure but, no study was conducted to address the long-term genotoxic effects among the survivors. The frequency and pattern of chromosome instability was studied through conventional chromosome aberration assay in the peripheral blood of exposed individuals to unveil the long-term genotoxicity of the exposure. In the present study six major groups has been taken. Among all the groups age, sex, and area were matched. The MIC exposed individuals and their progenies were selected from the nearby vicinity of the factory and the control was selected from the non-exposed area of Bhopal and out skirts of the Bhopal as well as the neighbouring district of Bhopal. Primarily, the cases were selected from the registered MIC subjects of Jawaharlal Nehru Cancer Hospital Idgah Hills, Bhopal. Total 1029 subjects were enrolled after randomization and 882 subject's samples were processed for cytogenetic study. Structural and numerical aberrations were recorded. The mean percentage of chromosomal aberrations like micronucleus, chromosomal associations, minutes and deletions in MIC exposed groups was significantly higher ($p<0.05$) as compared to non-exposed and rest of the aberrations in this group have revealed non-significant values. It was also noticed that chromosomal association in female subjects of MIC exposed congenital malformation (CM) female of non- MIC exposed group are significantly higher ($p<0.05$) as compared to the CM of non- MIC exposed groups and rest of the aberrations are non-significant. It was also noticed that chromosome type and chromatid types aberrations were statistically more in healthy male exposed groups. However, micronucleus, minute, deletion, chromosomal associations etc. are not shown significant in rest of the male groups. The study concludes that chromosome instability persist as a long term effect in the survivors of the MIC gas tragedy in Bhopal. The higher frequency of chromosomal aberrations may play an unambiguous role in the pathway of genetic diseases. However, definite conclusion could not be drawn because of the multiple associated aetiologies in the present scenario.

17. Procurement/usage of Equipment. (NA)

a. Table

S.No.	Name of Equipment	Make/Model	Cost Fe/ Rs.	Date of Installation	Utilisation rate %	Remarks regarding maintenance/Breakdown

b. Suggestions for disposal of equipment.

c. Name and signatures with date

1. Dr. N. Ganesh 7/6/17

(Principle Investigator)

2. Dr. N. Benerjee

(Co-Investigator)

18. References

Please refer Annexure

INTRODUCTION

Massive releases of chemical agents are rare, but they can be disruptive to the lives of individuals at many levels. Exposure to occupational or industrial contaminants is a major contributor to human health problems. Inhalation of gases, vapors, aerosols and mixtures of these can cause a wide range of adverse health effects. (Klaassen, 2001; Greenberg *et al.*, 2003; Chauhan and Jhonston, 2003; Winder and Stacey, 2004).

On the intervening night of 2-3 December 1984, the release of toxic gases occurred from an insecticide factory in Bhopal, owned by American multinational Union Carbide Corporation. Based on quantity of the chemical released and area of spread, the Central Water & Air Pollution Control Board of Bhopal estimated methyl isocyanate (MIC) concentration to be about 27 ppm, a figure which is about 1400 times that of the occupational safety and health (OSH) workplace standard of 0.02 ppm for 8 hours. (Singh and Ghosh, 1987).

Although MIC was the major toxic chemical escaping the reservoirs of union carbide's plant, a number of other toxic decomposition byproducts such as hydrogen cyanide, nitrogen oxides and carbon monoxide were also suspected to be present with MIC. The gases and particulate matter that escaped were most probably in the form of aerosol. (Dhara & Gassert, 2002).

Bhopal gas tragedy, 1984 was the world's worst industrial disaster that resulted in the death of thousands of residents within days due to their exposure to the toxic gases released. About 2, 00,000 persons were exposed to the gas tragedy and the initial death toll, within a week following the accident, was over 2500 with estimates ranging from 3000-20,000 (Stringer *et al.,* 2002). Department of Relief & Rehabilitation (DRR), Government of Madhya Pradesh, placed the toll at 3598 and by 1994 the toll was estimated to be over 6000. (DRR, Madhya Pradesh, 1989). Over the last 20 years at least 15,000 and more people have died from illness related to gas exposure (Sriramachari, 2004).

Chemicals such as MIC, which are used primarily as captive intermediate in closed production processes, often do not receive attention for toxicity evaluations as do chemicals with a greater potential for human exposure. The Bhopal gas tragedy lead researchers to examine various toxicological effects of the isocyanates on environment and indirectly on humans.

The Indian Council for Medical Research (ICMR), New Delhi was one of the foremost organizations to initiate clinical research studies on the exposed population. It presented its technical report of the research findings on the 'Health effects of the toxic gas leak from the methyl Isocyanate plant in Bhopal'. According to ICMR, 2004 a large fraction of the exposed population still remains chronically ill with diseases of the respiratory, gastro-intestinal, reproductive, neurological and other systems. A study of humoral and cell mediated immunity in exposed subjects, two months after exposure, revealed that cell mediated immunity was suppressed and that MIC specific antibodies persisted for several months (Anderson, 1988).

In the survivors of Bhopal, 71% of the exposed population showed evidence of chromosomal damage 12 months after the exposure, compared to the 21% incidence in a control population residing 20-50 km from the plant (Goswami *et al.*, 1986). Immunologic, mutagenic and genotoxic effects observed in the exposed population show that MIC reacts with enzymes involved in DNA replication and repair. This may be the cause of some of the problems with the pregnancies. It was found that 43.8% of pregnancies in women residing near the Union Carbide pesticide plant did not result in the birth of a live child. (Varma *et al.*,, 1987). There have been many studies regarding the genotoxicity and mutagenicity of MIC in the exposed population. The studies revealed increased chromosomal aberrations as compared to the controls (Saxena *et al.*, 1988; Ghosh *et al.*,, 1990), higher frequency of sister chromatid exchange and chromosomal anomalies in the exposed group (Goswami, 1984; 1986 and Goswami *et al.*, 1990) and a gradual yearly increase of different types of cancers in the Bhopal gas survivors as compared to the unexposed population (Ganesh *et al.*,, 2005).

Besides genotoxicity, studies have also been carried out regarding the reproductive disorders, ocular problems, respiratory problems, immunotoxicity, psychological and neurological health effects in the exposed population. It was found that 43.80% of pregnancies in exposed women resulted in still births and the unsuccessful pregnancies also involved spontaneous abortions (Varma, 1987). It has also been reported that the fetal loss among gas affected women was strikingly higher when compared to the controls. In an epidemiological study, the perinatal and the neonatal mortalities were found to be significantly higher in the affected area (Bhandari *et al.,* 1990). However, there has been no study regarding the long term genotoxicity of the Bhopal gas tragedy.

The aim of the present study was to determine the long term genotoxic effects of the Bhopal gas tragedy in the exposed human population. As many studies have been carried out stating the genotoxicity of the gas, there have been no follow up studies to rule out the genotoxicity in the surviving exposed population. Keeping in view the toxicity of the gas, it is very necessary to determine whether it has produced any long term genetic damage in the exposed population.

Cytogenetic biomarkers, being the most frequently used end point in human biomonitoring studies, are used extensively to assess the impact of environmental, occupational and medical factors on genomic stability. The present study was one such attempt to determine the current cytogenetic status of the Bhopal gas tragedy survivors with special references to long term genotoxicity produced by the exposure to the toxic gases released at the time of the incident.

Objectives as approved

To investigate the cytogenetic status in methyl isocyanate (MIC) exposed population and their progeny.

Study Design: The present research study is an observational, analytical, case-control research.

REVIEW OF LITERATURE

Historical background

The plant

The Union Carbide India Ltd (UCIL) plant in Bhopal was established in 1969 to produce pesticides to be used in agriculture, while the promotion of Green Revolution in developing countries was in full swing. Union Carbide Corporation (UCC), USA owned 50.9% of UCIL (Wood, 2001). The sharing of UCIL was prompted by India's efforts to gain control of its economic policies to encourage foreign companies to invest in local industry. UCC was asked to build a plant for manufacture of Sevin, a pesticide commonly used throughout Asia. As part of the deal, Indian government insisted that a significant percentage of the investment should come from local shareholders. The government itself had a 22% stake in the company's subsidiary i.e. Union Carbide India Limited. The company built the plant in Bhopal because of its central location and access to transport infrastructure. The specific site within the city was zoned for light industrial and commercial use, not for hazardous industry (Fortun, 2001).

The plant was initially approved only for formulation of pesticides from component chemicals, such as methyl isocyanate (MIC) imported from the parent company, in relatively small quantities. However, pressure from competition in the chemical industry led UCIL to implement "backward integration" – the manufacture of raw materials and intermediate products for formulation of the final product within one facility. This was inherently a more sophisticated and hazardous process (Shrivastava, 1987a).

UCIL used to manufacture three different kinds of pesticides viz carbaryl (trade name Sevin), aldicarb (trade name Temik) and a formulation of carbaryl and gamma-hexachlorocyclohexane (γ-HCH) sold under the trade name Sevidol. Carbaryl and aldicarb fall under carbamate group of pesticides; both are moderately persistent, highly toxic, highly water soluble and mobile in soils (Johnson *et al.*, 2009).

For manufacturing Sevidol, γ-HCH was extracted from the technical grade HCH which is a mix of several chemical forms (isomers) of HCH (mainly α, β, γ and δ−HCH). UCIL

used to buy technical grade HCH, extract γ-HCH and throw the remaining isomers as wastes (Johnson *et al.*, 2009).

HCH and its isomers are highly persistent and toxic organochlorine pesticides and presence of different isomers of HCH is likely because of the processing of HCH in the plant, use of γ-HCH for Sevidol formulation and dumping of other isomers within the factory and outside in the waste dump site (also called by UCIL as solar evaporation pond). Hexachlorobenzene (HCB) is an impurity in the technical grade HCH and was also produced as a by product of various chemical processes in the UCIL factory. Chlorinated benzene compounds are highly persistent and were either used by UCIL as solvents or are degradation products of HCH or HCB. For instance, 1,2 dichlorobenzene or orthodichlorobenzene was used as solvent for producing alpha-naphthol – a chemical used in the production of Sevin, the main product of UCIL. Chlorinated benzene compounds are also used as insecticides and fungicides. Heavy metal like mercury was used as a sealant in the Sevin plant and chromium was used as a coolant in the cooling plant of the UCIL factory (Johnson *et al.*, 2009).

In May 1982, a UCC team of safety experts conducted a detailed examination of the Bhopal plant. The report identified several deficiencies and warned that a leak could occur due to equipment failure or operating problems reflecting a lack of safety consciousness. A similar safety report was prepared at the West Virginia plant of UCC in 1984. The report included warnings of a possible run away reaction in the MIC storage tank of the plant. These reports were not made available outside management circles and were labeled as 'business confidential' (Chouhan, 2004).

In October 1982, a mixture of MIC, chloroform and hydrochloric acid escaped from the Bhopal plant, injuring 16 workers and endangering the neighboring community. This incident made very clear the potential public risk, yet there was no preparation of an evacuation plan. When the plant was installed, Carbide officials suggested an evacuation plan that was ignored because the public relations advisors suggested that pursuing such a plan with local authorities would highlight the hazardous nature of the plant (Chouhan, 2004).

In 1984, the plant was manufacturing Sevin at one quarter of its production capacity due to decreased demand for pesticides. Widespread crop failures and famine on the subcontinent in the 1980s led to increased indebtedness and decreased capital for farmers to invest in pesticides. Local managers were directed to close the plant and prepare it for sale in July 1984 due to decreased profitability. When no ready buyer was found, UCIL made plans to dismantle key production units of the facility for shipment to another developing country. In the meantime, the facility continued to operate with safety equipment and procedures far below the standards found in its sister plant in West Virginia, USA. The local government was aware of safety problems but was silent to place heavy industrial safety and pollution control burdens on the struggling industry because it feared the economic effects of the loss of such a large employer (Shrivastava, 1987b).

Gas tragedy December 2/3, 1984

On the intervening night of December 2-3, 1984 a faulty valve allowed one ton of water for cleaning internal pipes to mix with forty tons of MIC inside tank 610 (Fortun, 2001). A 30 ton refrigeration unit that normally served as a safety component to cool the MIC storage tank had been drained of its coolant for use in another part of the plant (Shrivastava, 1987).

The water flowing into tank also carried iron rust fillings from corroding pipe walls, residue of the salt compounds that had blocked the lines being washed and other contaminants. The entry of water with contaminants set off an exothermic reaction which caused catalytic trimerization. Pressure and heat from the vigorous exothermic reaction in the tank continued to build. The gas flare safety system was out of action for three months (Chouhan, 2004).

Mixing water with MIC alone would have caused a similar reaction but over a much more extended time span. At ambient temperature (20°C), a violent reaction would have taken 23 hours to occur. Iron rust and other contaminants, such as chloride and sodium compounds, were carried with the water. This resulted in a catalytic trimerization (a run

away reaction), causing a massive rise in pressure and temperature over a short time span. The result was the release of the tank's contents through various outlets into the atmosphere (Chouhan, 2004).

About 40 tons of toxic gases were released from the Carbide's Bhopal plant resulting into the death of thousands of people and animals. Local hospitals were overcrowded with the injured, a crisis further compounded by a lack of knowledge of exactly what gas was involved and what its effects were (Fortun, 2001). It became one of the worst chemical disasters in history and the name Bhopal became synonymous with industrial catastrophe (MacKenzie, 2002). The gas cloud is thought to have contained not only MIC but also numerous other toxic chemicals including hydrogen cyanide, carbon monoxide, carbon dioxide, nitrogen oxides, monomethylamine, monomethylamine hydrochloride, dimethylamine hydrochloride, trimethylamine hydrochloride, 1,3-dimethyl urea, trimethyl urea, 1,3,5-trimethyl biuret, tetramethyl biuret, 1,3-dimethyl isocyanurate and 1,3,5-trimethyl isocyanurate (Anonymous, 1985 and Subramanian 1985). MIC, hydrogen cyanide and monomethylamine (MMA) are believed to have been responsible for many of the immediate deaths and toxic effects (Subramanian 1985).

Methyl isocyanate is a clear colorless liquid. It is sparingly soluble in water and hydrolyzes in an exothermic reaction, the rate of which is highly temperature dependent. MIC has the simplest chemical configuration of all industrially used chemicals of this group. It is highly reactive on the bases of double bonds which constitute the basis of its being intermediate in the synthesis of several industrial products. Methyl isocyanate (CH3-N=C=O) is an intermediate product in the manufacture of carbaryl (Sevin), a carbamate pesticide. The process begins with a mixture of carbon monoxide and chlorine to form phosgene. Phosgene is then combined with monomethylamine to form methyl isocyanate. Methyl isocyanate is further mixed with naphthol to produce the end-product, carbaryl. (Worthy, 1985 and Anonymous, 2005).

About 2, 00,000 persons were exposed to the gas tragedy and the initial death toll, within a week following the accident, was over 2500 with estimates ranging from 3000-20,000 (Stringer *et al.,* 2002). Department of Relief & Rehabilitation (DRR), Government of

Madhya Pradesh, placed the toll at 3598 and by 1994, the toll was estimated to be over 6000. (DRR, Madhya Pradesh, 1989).

Minutes of the meeting of the Group of Ministers (GoM), Government of India, to examine all the issues relating to Bhopal gas leak disaster held from 18th to 21st June 2010 reveal that there are 5259 death cases, 3199 cases of permanent disability, about 2000 cases of cancer, 1000 total renal failure cases and 33,672 cases of temporary disability. A total of 5,74,375 cases were awarded original compensation including the 5,295 death cases. The GoM suggested that research and rehabilitation work is necessary in the areas of respiratory diseases, eye-related diseases, cancer, total renal failures, genetic disorders, women-related medical issues and second-generation children related issues.

After the exposure

The Bhopal plant never operated after the accident, though Union Carbide India Limited continued to exist under the new name of Eveready Industries Limited. In 2001, Union Carbide became a wholly owned subsidiary of the Dow Chemical Company. Today the abandoned factory is guarded by a commercial security company, though local people frequently enter it, whether to graze their animals or, in the case of the children, to play. It retains the toxic legacy from its industrial days. Dumping and release to atmosphere of over 1500 tons of chemicals in and around the site between the years of 1969 and 1984 is recorded by a former plant operator (Stringer *et al.*,, 2002).

Pollution Monitoring Laboratories, New Delhi, in 2009, studied the chemistry of the processes used for producing various pesticides in UCIL and based on it, selected four groups of chemicals for testing soil and water samples. In chlorinated benzene compounds it tested 1,2 dichlorobenzene (1,2 DCB), 1,3 dichlorobenzene (1,3 DCB), 1,4 dichlorobenzene (1,4 DCB) and 1,2,3 trichlorobenzene (1,2,3 TCB). In organochlorine pesticides it tested hexachlorobenzene (HCB) and α, β, γ and δ isomers of HCH. In carbamates, it tested carbaryl and aldicarb – the two main products of UCIL. In heavy metals it tested five heavy metals – lead, cadmium, chromium, mercury and arsenic (Johnson *et al.*, 2009).

The National Environmental Engineering Research Institute (NEERI), Nagpur, conducted a number of environmental surveys in and around the UCIL premises. In 1997, Eveready Industries India Ltd, which bought Union Carbide's share in Union Carbide India Ltd., commissioned NEERI to find out the extent of contamination at the Bhopal plant site. NEERI found high levels of toxins and identified hot spots. Presence of carbaryl, lindane, alpha-napthol etc. was reported in their findings. A study by NEERI (2003) collected the ground water samples around UCIL premises. 1,2,3, TCB was detected in some of the groundwater samples. Pesticides like lindane, α-endosulfan, heptachlor, aldrin, dieldrin, BHC, endrin and 4,4 DDT were also detected in some of the samples (Johnson *et al.*, 2009).

Heavy metals, chlorinated hydrocarbons and pesticides were detected in all samples of soil, ground water, vegetables and breast milk around residential areas adjoining UCIL factory premises. In all the samples four heavy metals viz. Cr, Ni, Pb and Hg were found in very high concentrations. The total HCH pesticide concentration was very prominent in all four samples particularly it was up to 9 mg/kg in soil sample (Johnson *et al.*, 2009).

In 1999, Greenpeace International carried out surveys in order to gain an insight in to the nature and severity of chemical contamination. Greenpeace analyzed samples of solid wastes, soils and groundwater within UCIL and its surrounding areas. Greenpeace found samples to be contaminated with volatile organic compounds and heavy metals. Later in 2002 Greenpeace Laboratory collected twelve "stockpile" samples from six locations inside the site and four soil samples were collected from the Solar Evaporation Ponds. Eleven of the twelve stockpile samples were found to contain carbaryl at concentrations ranging from 0.013µg/kg to 1.84µg/kg. Ten contained hexachlorocyclohexanes, with total concentrations varying between 100µg to 84,000,000µg/kg dry weight of the sample. HCB was detected in many samples and the concentration ranged between 100µg to 100,000µg/kg. According to the report, the local populations are vulnerable to exposure to all chemicals found in the study through routes such as direct contact with contaminated soil or inhalation of contaminated dust. The HCH and other organochlorines may moreover be passed on in the milk of cattle that the locals graze on the site. (Stringer *et al.*, 2002).

Associated health problems

Symptom prevalence surveys conducted by the Indian Council of Medical Research (ICMR), New Delhi indicate that morbidity was higher in the exposed areas (26%) as compared to the unexposed area (18%) when assessed during the period November 1988 to March 1990. About 11% of people experienced two or more spells of illness in one year period. Respiratory, ocular and gastro-intestinal symptoms accounted for most of this morbidity. This trend appeared to be persistent in the survey conducted in the later part of 1990 (ICMR, 1991).

Results from the 1994 International Medical Commission on Bhopal (IMCB) survey showed that a large number of subjects reported general health problems and episodes of fever. Respiratory, neurological, psychiatric and ophthalmic symptoms also showed a higher gradient by exposed category.

Ocular problems

It is reported that the intensely irritating effect of MIC on the cornea resulted in severe ocular burning, watering, pain and photophobia. Examination of the eye showed involvement of the corneal and conjunctival epithelium with redness of the eye, corneal ulceration and lid swelling. (Andersson *et al.*, 1988)

Andersson *et al* (1986) performed a follow-up study on the eyes of survivors nine months after the accident and reported that no case of blindness could be found that could be attributed to gas exposure among the nearly 20,000 persons attending Bhopal Eye Hospital. However, they did find persistent eye watering and other chronic irritant symptoms like burning, itching and redness.

Raizada and Dwiwedi (1987) studied eye pathology among 1,140 exposed persons and found that the main chronic lesions were chronic conjunctivitis, deficiency of tear secretion and persistent corneal opacities.

Khurrrum and Ahmad (1987) conducted a large follow-up study of ocular lesions during the period from November 1986 to December 1987. About 2280 patients who had an

active lesion on exposure were selected from exposed area and 2000 subjects of similar social status were selected from unexposed areas. The results showed the persistence of chronic lesions in the exposed area: these included conjunctivitis (14%), tear secretion deficiency (6.7%), and corneal opacity (9%). However, no information is available on the prevalence of these conditions in the unexposed area.

Animal experiments conducted by Salmon *et al.,* (1985) on male lister hooded rats indicate that the most severe effects on the eye occur at exposure levels around 65 ppm.

Andersson *et al.,* (1990) performed a follow-up of 93% of exposed and unexposed Bhopal residents three years after exposure. The ocular effects surveyed included photophobia, burning and a watering sensation, signs of red eye, superficial interpalpebral erosion, bitot spots, corneal opacity, pterygium, discharge and fundal changes. Their findings indicated an increased risk of eye infections, hyperresponsive phenomena, (watering, irritation), excess cataracts and resolution of the corneal erosions in exposed persons.

Respiratory problems

Mishra and Nag (1988) concluded from their study on Bhopal gas tragedy that the acute symptoms of the respiratory tract were mainly due to the irritant action of the released gas on tissues. Predominant symptoms were cough accompanied by frothy expectoration, a feeling of suffocation, chest pain and breathlessness.

Spirometry was carried out on 783 exposed subjects by Rastogi *et al.,* (1988) to determine respiratory impairment. The results showed that 39% of the sample was found to have some form of respiratory impairment. The combined pattern of impairment (obstructive and restrictive disease) had the highest prevalence in the sample (22%). Smoking had no effect on the prevalence of this impairment. Females suffered more mild and moderate impairment whereas severe impairment was present in both the sexes.

Irani and Mahashur (1986) conducted a survey of Bhopal children 105 days after the accident and reported symptoms of persistent cough and breathlessness in 83% and 47%

of the exposed and the unexposed samples respectively. Besides, abnormal radiological findings were noted in 66% and 8% of children in the exposed group and in unexposed respectively. The investigators attributed the above findings to chronic effects of gas exposure.

Kamat *et al.,* (1987 and 1992) studied a cohort of 113 exposed patients who were administered a symptom questionnaire and had physical examination, chest radiography and spirometry performed sequentially at three and six months and two years after exposure. Initial respiratory symptoms included cough, sputum, chest pain and dyspnea. Over the first year there was an improvement of patients with worsening over the subsequent year. Similarly pulmonary function improved over the first year and declined over the next year with a predominant restrictive pattern.

Vijayan *et al.,* (1989) analyzed broncho-alveolar lavage fluid in 36 mild, moderate and severely exposed persons and 12 unexposed normal controls 1-2.5 years after exposure. The results indicated that severely exposed smokers and non-smokers showed a significant increase in alveolar macrophages. In a subsequent study, Vijayan and Sankaran (1989) studied 60 gas exposed patients presenting with dyspnea and cough and classified them as mild, moderate and severely exposed on the basis of respiratory and ophthalmic symptoms on the day of exposure. Their results showed that pulmonary function abnormalities may be due to accumulation of lung inflammatory cells and were most severe in those with higher exposures.

Karol *et al.,* (1987) concluded from his study on Bhopal gas tragedy victims that though isocyanates are known to be allergic in the lung, the respiratory toxicity of MIC appears to be primarily due to its irritant nature. Follow-up studies with lung biopsies done six months after exposure showed evidence of interstitial fibrosis and bronchiolitis obliterans. These findings were similar to those found in several animal studies by Boorman *et al.,* 1987 and Fowler and Dodd (1987) separately, thus revealing the close association between animal data and clinical findings in Bhopal victims.

Immunotoxicity

Immune function was studied in exposed subjects at the Indian Toxicology Research Centre, Lucknow, two and a half months after exposure to ascertain whether any change had occurred in the immune status. Humoral immunity and cell-mediated immunity (CMI) was assessed that revealed no difference in mean immunoglobulin level in the exposed individuals when compared to unexposed. The T-cell population (28%) was found to be less than half of that found normally in the Indian population (65%). Significant depression of phagocytic activity of lymphocytes was also found in the exposed individuals (Saxena *et al.,* 1988).

Concurrent with the human studies, immuno-toxicological evaluation of rats exposed to MIC showed a number of harmful results. Alveolar and peritoneal macrophage function was depressed and exposed rats were susceptible to *E. coli* endotoxin. Delayed type hypersensitivity was assessed by injecting sheep RBC's into the foot pads and was found to be impaired. Based on these results, it was concluded that the gas had a suppressive effect on cell-mediated immunity. (Dwiwedi *et al.,* 1988).

Karol *et al.,* (1987) found MIC-specific antibodies in guinea-pigs injected with MIC as well as in 12 of 144 human survivors. This showed that MIC was capable of eliciting an immunogenic response. The antibody titers in the human studies were low and transient suggesting a weak response.

Psychological and neurological health effect

The survivors of the Bhopal gas tragedy were reported with significant neurological, neurobehavioral and psychological effects. The ICMR, New Delhi report illustrated that the appearance of psychological implications in the exposed population led to anxiety and depression. In a randomised study of out patients at ten government run clinics three to five months after the disaster, 22.6% were found to be suffering from psychological disorders. A similar number suffered from neurotic depression, anxiety and social adjustment problems (ICMR, 2004). According to the ICMR (1991), neuromuscular defects such as tingling numbness, sensation of pins and needles in the extremities, and muscle aches, have continued among the victims after exposure. Such effects of MIC

exposure could be attributed to the prevention of formation of muscle fibres in the culture at low doses and causing death of fibroblasts and myoblasts at higher doses (Andersson *et al,* 1988). It was observed that out of 208 persons suffering from psychological problems, 45% suffered from neuroses, 35% from anxiety states and 9% from exacerbation of preexisting adjustment reactions.

Cytogenetic studies

The ability of inhaled methyl isocyanate (MIC) to induce genotoxic and cytotoxic damage *in-vivo* was evaluated by assessing the induction of chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs) in bone marrow metaphase cells, the induction of micronuclei in polychromatic erythrocytes (MN-PCEs) and the inhibition of bone marrow cellular proliferation and erythropoiesis by Tice *et al.*, 1987. He analyzed the various cytogenetic endpoints in bone marrow and peripheral blood samples taken from bromodeoxyuridine tablet-implanted animals. A significant delay in cellular proliferation was reported but did not induce a significant increase in chromosomal abnormalities, sister chromatid exchanges or in bone marrow MN-PCEs. Also, it did not inhibit the rate of erythropoiesis. Exposure to MIC for an increased time duration resulted in a significant increase in CAs and SCEs in male and in female mice. It also resulted in a depressed rate of erythropoiesis, with male mice appearing to exhibit greater depression than female mice. The results demonstrated that exposure to MIC by inhalation results in bone marrow damage, indicating the systemic genotoxic activity of MIC and its reactive metabolites.

The genotoxic effects of methyl isocyanate were investigated by Mason *et al.,* (1987) using four short-term tests: the *Salmonella* reversion assay (Ames test), the *Drosophila* sex-linked recessive lethal assay and the sister chromatid exchange (SCE) and chromosomal aberration assays in cultured Chinese hamster ovary (CHO) cells. No evidence was found for the induction of mutations in either *Salmonella* or *Drosophila.* MIC did, however, induce SCEs and chromosomal aberrations in CHO cells both in the presence and absence of Aroclor-induced rat liver.

Meshram and Rao (1988) evaluated the ability of methyl isocyanate to induce mutagenic and cytotoxic effects *in vivo* in mice. The induction of micronuclei and depression of polychromatic erythrocytes in bone marrow and peripheral blood smears was recorded. Animals were exposed to MIC through intraperitoneal injection for two and five days in separate experiments, and bone marrow and peripheral blood were sampled six and 48 hours after the last injection, respectively. MIC did not significantly increase the frequencies of micronucleated polychromatic erythrocytes (MN-PCE) and micronucleated normochromatic erythrocytes (MN-NCE) in bone marrow and peripheral blood samples respectively in either twice or multiply treated mice. However, a dose-dependent depression in percentage PCE observed was significant. This indicated that MIC exposure led to the cytotoxic effect by inhibition of bone marrow cell proliferation.

Chromosomal studies were done two and a half months after the gas leak to evaluate genetic damage in the peripheral blood lymphocytes of gas-exposed survivors by Saxena *et al.,* (1988). The results showed a significantly increased ($P<0.001$) number of breaks and gaps in the exposed subjects.

Cytogenetic studies by Ghosh *et al.,* (1990) done after three years of the exposure on a sample of 40 male and 43 female exposed persons showed statistically higher frequencies of chromosomal aberrations in the exposed group as compared to 46 age and sex-matched unexposed controls. The aberrations were in the form of breaks, gaps, dicentrics, rings, tri and quadri-radial configurations (Robertsonian translocations) and were more pronounced in female subjects. However, sister chromatid exchanges were not significantly different.

In an initial study by Goswami (1986), it was observed that sister-chromatid exchanges (SCE) frequencies in lymphocytes were found to be increased more than three times in MIC-exposed persons. Chromosomal breaks were also observed in 10 out of 14 affected people (71.4%) studied, while only 6 out of 28 (21.4%) unexposed showed chromosomal breaks. Even chromatin bodies were observed in addition to the normal 46 chromosomes among some of the survivors.

In another study, Goswami *et al.*, (1990) formulated a chromosomal profile for 154 persons studied during 1986–1988. The exposed subjects developed at least two categories of chromosomal aberration, out of which Robertsonian translocations were repeatedly observed, mostly in acrocentric chromosomes 13 and 21. It is known that at least 50% of the subjects possessing such serious chromosomal abnormalities may have pathological implications such as tumors, recurrent miscarriage or transmission of defects to their offspring.

Shelby *et al.,* (1987) performed genetic toxicity testing on B6C3F1 mice exposed to various concentrations of MIC by inhalation using single (two hr, male mice) and multiple (six hr/day for four days, male & female mice) exposures. 50 Multiple exposure experiments in mice showed increased frequencies of SCE and chromosomal aberrations which were absent in two hour exposures.

Mishra *et al.,* (2009a) concluded from his studies on inflammatory response to isocyanates and the onset of genomic instability in cultured human lung fibroblasts, that isocyanates induce inflammation, DNA damage, cell cycle arrest, apoptosis, and more broadly genomic instability in the IMR-90 human lung fibroblasts.

The genotoxic potential of MIC in cultured mammalian cells after *in-vitro* exposure has been assessed by Mishra *et al.,* (2009c). The studies were performed to investigate cellular DNA damage response through qualitative phosphorylation states of ATM, γH2AX proteins and quantitative state of p53 phosphorylation; DNA cell cycle analysis and measure of cellular apoptotic index. It has been demonstrated that methyl isocyanate, by negatively regulating the DNA damage response pathway, might promote cell cycle arrest, and apoptosis in cultured mammalian cells suggestive of causing genetic alterations. Induction of genomic instability in cultured human colonocytes following exposure to methyl isocyanate was also investigated. Many treated cells were arrested at the G2/M phase of the cell cycle and had an increased apoptotic index and elevated inflammatory cytokine levels. Cytogenetic analyses revealed varied chromosomal anomalies, with abnormal expression of pericentrin protein.

Isocyanates may act as electrophilic agents and react with DNA to produce genetic damage (Shelby *et al.*, 1987). As a carbomoylating agent, MIC might be expected to react with nucleophilic sites on cellular macromolecules, including proteins, RNA and DNA. There are three possible ways in which carbamoylation may modify the action of other factors. Firstly modification of primary DNA damage produced by other agents and transformation of DNA into a non-repairable state, for example, the simultaneous formation of methylated and carbamoylated base pairs. Secondly carbamoylation may inhibit repair enzymes resulting in decreased repair of the primary damage produced by other agents. The third way is related to the possible reaction of the isocyanates with histones leading to a loosening and dissociation of DNA-protein bonds. It is known that 1,3 bis-(2-chloroethyl)-1-nitrosourea (BCNU), which releses 2-chloroethyl-isocyanate upon decomposition, induces DNA damage through DNA aminoethylation (Stahl *et al.*, 1992).

Methyl isocyanate and phenyl isocyanate are known to react with the exocyclic amino group of deoxycytidine, deoxyadenosine and deoxyguanosine to produce carbomoylated products (Tamura *et al.*, 1992).

A statistically significant increase in the risk of cancer for subjects with a high level of chromosomal aberrations compared to those with a low level in the Nordic cohorts was reported by Bonassi *et al.*, (2000) while studying whether chromosomal aberrations predict cancer. These estimates were not affected by the inclusion of occupational exposure level and the smoking habit supporting the fact that chromosomal damage itself is involved in the pathway of cancer.

Hagmar *et al.*, (2004) reported cancer risk for cytogenetically tested, healthy subjects with respect to frequency of chromosomal aberrations (CAs), chromosome-type aberrations (CSAs) and chromatid-type aberrations (CTAs) in peripheral blood lymphocytes, using Nordic (1981 subjects with CA data, 1871 subjects with CSA/CTA data) and Italian (1573 subjects with CA data, 877 subjects with CTA/CSA data) cohorts, with a median follow-up of 17 years. High levels of CAs at test were clearly associated with increased total cancer incidence in the Nordic cohorts and increased total cancer

mortality in the Italian cohort. In the Nordic cohorts, significantly elevated cancer risks were reported for subjects with both high CSAs and high CTAs at test, and these variables showed equally strong cancer predictivity. The results of the Italian cohort did not indicate any clear-cut difference in cancer predictivity between the CSA and CTA biomarkers. There was no significant effect modification by age at test, gender, country or time since test. The study suggests that both DNA double-strand breaks and other initial DNA lesions responsible for CSAs and CTAs are associated with cancer risk.

Rossner *et al.,* (2005) gathered cytogenetic records of 11,834 subjects, from 15 laboratories, who were free of cancer at the time of blood drawing and who went cytogenetic analysis for preventive purposes in the Czech Republic during 1975-2000. The results demonstrated a significant association between the overall cancer incidence and the presence of chromosome-type aberrations. However, the association was not found to be strong with that of chromatid-type aberrations. This study contributes to the validation of chromosomal aberrations as a predictive marker of cancer risk, in particular, of stomach cancer. These findings were strongly supported by the observations from a pooled study of 22358 subjects in 11 different countries by Bonassi *et al.,* (2008).

In earlier investigations on the somatic chromosomes of atomic bomb survivors of Hiroshima and Nagasaki, it was shown that cells with radiation-induced chromosomal aberrations persisted among the circulating lymphocytes for at least three decades after radiation exposure and that the frequency of aberrant cells was in general proportional to dose. The majority of such aberrant cells were identified as having symmetrical exchanges (reciprocal translocations and inversions) while the frequency of unstable aberrations (dicentrics and rings) was less (Awa, 1997).

Significantly increased chromosomal aberrations due to atomic bomb radiations are recognized in peripheral blood T-cells and bone marrow cells in proximally exposed atomic bomb survivors. Chromosome study was performed using colony forming cells induced by hemopoietic stem cells of peripheral blood of proximally exposed survivors whose chromosome aberrations were confirmed in peripheral blood T-cells. The same

chromosome aberrations in colony forming cells and peripheral T-cells were observed in several survivors (Ichimaru, *et al.,* 1991).

Findings emerging from the cytogenetic studies in atomic bomb survivors reveal differences in the frequencies and types of observed chromosomal aberrations and their age. Among 94 younger survivors exposed to more than 100 rad, the frequency of cells with dicentrics, rings, and fragments, was 0.50 per cent, almost precisely the same as it was among 77 older exposed persons. And yet, the proportion of cells with balanced translocations or pericentric inversions was only 0.10 per cent in the younger subjects, aged 20 to 49, and 1.10 per cent among those between 50 and 88 years of age. Furthermore, a recent review of 248 additional exposed persons shows that there is a steady increase in the frequency of cells with dicentrics, rings, and acentric fragments with an increase in dose in subjects estimated to have been exposed to doses ranging from 100 to over 700 rad (Bloom *et al.,* 1966;1967).

Statistically significant differences were found in the frequency of aberrant cells, chromosome breaks, dicentric chromosomes and total frequency of chromosomal aberrations (CA) per 100 cells in Chernobyl clean-up workers and control individuals. Even three to eight years after the Chernobyl accident, radiation-induced chromosomal damage was found to be present in lymphocytes of Chernobyl clean-up workers. As expected, the tendency of the increased frequency of dicentric and ring chromosomes was statistically significant. An increased frequency of acentric fragments has been reported in individuals occupationally exposed to low levels of ionizing radiation. The same was noted in individuals exposed to radiation from Chernobyl fallout and in another study of Chernobyl clean-up workers (Lazutka and Rimdeika, 2006).

Reproductive loss

An epidemiological survey by Varma (1987) showed pregnancy loss and infant mortality to be high in gas-exposed women. In a sample of 865 women who lived within one kilometer radius of the plant and who were pregnant at the time of the gas leak, 43% of the pregnancies did not result in live births. Of the 486 live births, 14% of babies died in

the first 30 days as compared to a death rate of 2.6% to 3% for previous deliveries in the two years preceding the accident in the same group of women.

Prakash (1986) reported a pregnancy outcome survey nine months after the accident in three gas-exposed areas of Bhopal. A total population of 8165 in 1632 households was surveyed by random sampling. The results showed a four-fold increase in overall spontaneous abortion rate for the period after the gas leak.

The increase in spontaneous abortion rates was confirmed by a larger study on pregnancy outcome carried out in 18,978 households in the severely affected areas around the Union Carbide plant by Bhandari *et al.,* (1990). Though the stillbirth rate was similar in the exposed and the unexposed areas, the perinatal and neonatal mortality rates showed significant differences. Congenital malformation rates were not significantly different between the two areas.

Animal experiments conducted by Schwetz *et al.,* (1987) exposing pregnant mice to MIC by inhalation showed that this exposure does indeed have a fetotoxic effect. This finding was replicated by Varma *et al.,* (1987), who observed a concentration-dependant increase in embryo loss, decrease in fetal and placental weights and a 20% reduction in mandible length and bones of the extremities.

Varma *et al.,* (1990) studied the contribution of maternal hormonal changes and pulmonary damage to fetal toxicity of MIC in rats and mice. His findings showed that fetal toxicity of MIC was partly independent of maternal pulmonary damage and that MIC could be directly fetotoxic.

Cancer risk assessment

No information is available on the carcinogenic effects of MIC in humans. Increases in relatively rare gallbladder adenocarcinomas in Bhopal survivors are being examined (Mishra *et al.*, 2009b).

A population-based cancer registry has been established in Bhopal in 1986 to record possible carcinogenic effects of the gas leak. Dikshit and Kanhere (1999) analyzed

incidence of cancer in males for the period 1987 to 1992. Relative risks of 1.4, 1.3 and 0.7 (all non-significant) were found for lung, oropharynx and oral cavity cancers, respectively for 1992 in comparison to the years 1987-1990 and gas unaffected regions combined. A marginally increased risk was found only for oropharyngeal cancer.

Studies carried out by Ganesh *et al.,* (2005) on the exposed population of Bhopal revealed a gradual increase in the number of cases of cancer recorded per year in MIC exposed population as compared to the unexposed population. The types of cancers found were of breast, lung, liver, bone, alimentary canal, cervix, blood and brain.

MATERIAL AND METHODS

MATERIAL

Glassware

Glassware used in the present study was obtained from "Borosil" Mumbai, India. Glass bottles (100, 250 and 500ml capacity), test tubes (15 ml capacity), culture bottles (20ml capacity) and filtration assembly (500ml capacity) etc. were used in the study.

Plastic wares

Plastic wares used in the present study were obtained from "Laxbro" Pune, India and "Tarsons" Kolkata, India. Sterile disposable syringes were obtained from "Hindustan Syringes and Medical Devices Ltd" India. Micro tips (0-50µl, 100-1000µl capacity) and micropipettes were obtained from "Fine Care Biosystems" Gujarat, India.

Chemicals

All the chemicals including basic medium (RPMI 1640), buffering agents, antibiotics, growth supplements, colchicine and trypsin etc. used in the present study were of analytical grade and were obtained from Gibco, USA, Qualigens, Mumbai, India and Bengal Chemicals, Kolkata, India.

Instruments

The main instruments used in the present investigation are listed in Table 1.

Table 1: Main instruments used in the present study.

S. No.	Name of the instrument	Make
1	CO_2 incubator	Hera Cell, Germany
2	Laminar air flow	Scientech, India
3	Centrifuge	Remi, Mumbai
4	Cyclomixer	Remi, Mumbai
5	Hot air oven	Scientech, Delhi
6	Weighing balance	Sartorius, Mumbai
7	Autoclave	Scientech, Delhi
8	Magnetic stirrer	Scientech, Delhi
9	Slide warming plate	Scientech, Delhi
10	Vacuum pump	Scientech, Delhi
11	Phase contrast trinocular microscope	Olympus, Singapore

The study subjects

Selection of subjects was done purely on the basis of their exposure to the gaseous cloud released from Union Carbide's pesticide plant on midnight of 2nd -3rd December 1984. The selection was made as per the selection criteria so as to exclude the false positive exposures. All the subjects were selected from hospital registration and their accompanied relatives.

The investigations were done to study the various cytogenetic status in different groups as follows: (for 03 years total 1800 subjects)

- Exposed individual's age between 29-59 years (150 male and 150 female). (Healthy Individuals)
  Non-exposed individual's age between 18-59 years (150 male and 150 female) [75 from Bhopal and 75 from out stationed of each sex] (Healthy Individuals)
- Progeny born after exposure 1st generation (150 male and 150 female).
- Progeny born after exposure 2nd generation (150 male and 150 female).
- 150 male and 150 female of different ailments diagnosed after the exposure of the gas (non-inherited).
- 150 male and 150 female with congenital malformation/ malformation.

Project sanctioned for 02 years (Oct 2013 – Oct 2015, extension given for one year Oct 2016) – 1200 subjects

- Exposed individual's age between 29-59 years (100 male and 100 female). (Healthy Individuals)
  Non-exposed individual's age between 18-59 years (100 male and 100 female) [50 from Bhopal and 50 from out stationed of each sex] (Healthy Individuals)
- Progeny born after exposure 1st generation (100 male and 100 female).
- Progeny born after exposure 2nd generation (100 male and 100 female).
- 100 male and 100 female of different ailments diagnosed after the exposure of the gas (non-inherited).
- 100 male and 100 female with congenital malformation/ malformation.

The criteria followed for identifying the exposed subjects are as under:

a. A person must hold a gas victim official card or any other evidence which depicts the physical presence of his / her during the time of exposure.

b. Any other evidence which shows hospitalization on or within 48 hours of the episode.

c. It was ascertained that none of the subjects (exposed or control) was more than 55 years of age and does not have smoking or tobacco chewing habit. Persons having exposure to chemicals or radiations were excluded.

Table No. 2: Total No. of Identified Individuals (01-01-2014 to 30-10-2016) and enrolled individuals after Randomization

Group	Sub-groups	Male	Female	Total Individuals Identified	Total Individuals Enrolled after Randomization
1. Normal Healthy MIC Exposed		224	197	421	200
2. Normal Healthy Non Exposed		252	242	494	200
3. 1st Progeny of MIC exposed individual		362	297	659	200
4. 2nd Progeny of MIC exposed Individual*		27	19	46	46
5. Congenital Malformations*	5 a) With MIC Exposure	50	37	87	87
	5 b) Without MIC exposure	64	60	124	100
6. Other Ailments*	6 a)With MIC Exposure	125	117	242	100
	6 b)Without MIC Exposure	46	50	96	96
Total	-	1150	1019	2169	1029

NB*: From pedigree analysis above mentioned individuals were identified and after randomization they were enrolled. The enrollment number is less in group 4, 5a, and in 6b because these much of individuals have shown interest and given their consent.

METHODS

Maintenance of aseptic conditions

The culture room was regularly fumigated once in a month with 3% $KMnO_4$ in 1: 1 N - Butanol: Formic Acid (1:1) and was kept closed overnight. The sterility of culture room was monitored by exposing nutrient agar plates (composition given in Table 2) at regular intervals. All the cultures were performed in the laminar air flow chamber. The working area was cleaned both before and after use with 70% ethanol.

Table 3: Composition of nutrient agar medium (1L)

S. No.	Component	Quantity
1	Beef extract	3.0 gm
2	Peptone	10.0 gm
3	NaCl	5.0 gm
4	Agar	15.0 gm
5	Double Distilled Water	1 liter

Cleaning of glassware's

Fresh borosilicate glasswares including filtering flask, culture tubes, conical flasks, beakers, measuring cylinders and all other glasswares were dipped in 5% chromic acid and kept overnight to remove all the traces of impurities. These were then washed with running tap water followed by boiling in 2.5% extran solution (Merck, Mumbai) for 30 minutes. The glassware was again washed under running tap water followed by rinsing thoroughly in single and double distilled water and was dried in hot air oven.

Sterilization of glasswares

Filtering flasks, reagent storage bottles and conical flasks were plugged with cotton wrapped with aluminum foil and brown paper. Screw caps and rubber corks were packed in a separate beaker which was then covered by aluminum foil and paper. Filtration assembly was wrapped first with aluminum foil and then with brown paper. All the packed culture vessels were labeled and sterilized by autoclaving at 15 lbs/ $inch^2$ at 121°C for 30 minutes.

Preparation of RPMI 1640 medium

16.30 gm of the powdered medium from one packet of RPMI 1640 was dissolved in 900 ml of sterile double distilled water. Antibiotics, penicillin (5000 IU/ml and streptomycin (5000 µg/ml) in 0.85% saline were added followed by the addition of 0.29 gm of L-glutamin. 3.40gm of sodium bicarbonate was also added to maintain the pH of the prepared medium. The medium was filtered by a filtration assembly with a membrane filter of the pore size of 0.22µm. Phytohaemagglutinin (PHA-M) and 10% of fetal bovine serum (HiMedia, USA) was added to the medium prior to use. The complete medium composition is given in Table 3.

Table 4: Composition of RPMI 1640 medium (1L).

S. No.	Contents	Quantity
1	RPMI-1640 (with L-glutamine)	16.40 gm
2	$NaHCO_3$	3.40 gm
3	Pen-strep solution	20.00 ml
4	Double distilled water	900.00 ml
5	Fetal bovine serum	100.00ml
6	Gentamycin	8-10 drops
7	Amphotericin-B	500-1000µl

Sterility test

Sterility test of the medium was done by incubating small aliquots from the prepared medium for 24 hours at 37 °C. After incubation, the medium was checked for microbial contamination.

Preparation of reagents

Hypotonic solution

0.075 M KCl solution was used as a hypotonic solution for the lymphocytes. It was prepared by dissolving 0.57gm of KCl in a final volume of 100ml of double distilled water.

Fixative

Carnoy's fixative used for the fixation of the cultured lymphocytes was prepared by the mixture of one part of glacial acetic acid and three parts of methanol.

Buffer solution

Buffer solutions were prepared by dissolving 1.42gm of di-sodium hydrogen phosphate anhydrous (Na_2HPO_4) in 100ml of double distilled water and 1.56gm sodium dihydrogen phosphate monohydrate ($NaH_2PO_4.H_2O$) in 100ml of double distilled water separately.

Stains and related reagents

Giemsa stain (stock solution)

Giemsa stock solution was prepared by adding 1gm of Giemsa powder to 50ml of glycerol and 50ml of methanol was added followed by overnight incubation at 60 °C and the solution was stirred at room temperature over a magnetic stirrer. The stain was filtered and stored at 4°C in an amber colored glass bottle.

Giemsa stain (working solution)

6ml of Giemsa stock was added to 40ml of double distilled water followed by addition of 2ml each of Na_2HPO_4 and NaH_2PO_4 buffer solutions.

Normal saline solution

0.90gm of sodium chloride was dissolved in 100ml of double distilled water and was used as normal saline solution.

Trypsin solution

Trypsin solution was prepared by dissolving 20mg of trypsin powder in 50ml of normal saline solution.

Ethical approval

The present study was approved by the Institutional Human Ethical Committee, Jawaharlal Nehru Cancer Hospital & Research Centre, Idgah Hills, Bhopal (IEC/JNCH/01/18-05-13)

Pedigree construction

Pedigrees of the subjects included in the samples were constructed in a standard format.

Sample collection

Samples were collected from each subject after his/her informed consent as per the ethical norms. 2ml of heparinized blood sample was withdrawn from each subject with a disposable syringe.

Cytogenetic analysis

Setting up of lymphocyte cultures

The heparinized peripheral blood samples were aseptically transferred into a

sterile culture bottle with 5-8ml of RPMI-1640 medium each supplemented with L- glutamine, fetal bovine serum and phytohemagglutinin. The cultures were incubated in a CO_2 incubator for 72 hours following protocol given by Moorhead *et al.,* 1960 (Figure 1).

Harvesting of cultures

After 70 hours of incubation 50µl colchicine solution was added as the pretreatment agent. After 72 hours of incubation, the cell suspensions were transferred into labeled centrifuge tubes and centrifuged for 10 minutes at 1000 rpm. The supernatant was discarded and the pellet was treated with the hypotonic solution (0.075M KCl) by gentle flushing and mixed with the help of cyclomixer. The centrifuge tubes were again incubated at 37 °C for 45 minutes. The tubes were again centrifuged carefully at 1000 rpm for 15 minutes. The supernatant was removed and 5-8 ml of freshly prepared pre-chilled Cornoy's fixative was added to the pellet while mixing on cyclomixer. The tubes were allowed to stand overnight and washed with freshly prepared pre-chilled Cornoy's fixative repeatedly for 3-4 times.

Preparation of slides

Slides were prepared by air drop method. Cleaned frosted slides were dipped in methanol and chilled prior to the slide preparation and each slide was marked with a identification number. The slides were then dried at room temperature.

Staining of the slides

Chromosome preparations were stained with Giemsa working solution for 5-8 minutes and the extra stain was removed by single rinse of the slides with double distilled water following protocol as given by **Seabright, 1971.**

G- banding of metaphases (Moorhead *et al.*, 1960)

G – banding of metaphases was done by treating them with trypsin solution for 20-60 seconds and then immediately rinsing them with normal saline solution so as to remove the extra trypsin. The slides were then stained with Giemsa working solution as described above.

C- Banding (Gustashaw K.M. 1991).

C-banding is used to specifically stain the centromeric regions and other regions containing constitutive heterochromatin, i.e., the secondary constrictions of human chromosomes 1, 9, 16, and the distal segment of the Y chromosome long arm. C-banding can be used to detect increases, decreases, inversions or rearrangements of heterochromatic regions. The breakdown of euchromatic DNA to leave the darkly staining C-bands (heterochromatic regions) is believed to occur by a three-step process:
a.Depurination of the DNA by acid treatment (HCl),
b.Denaturation of the DNA by alkaline treatment (barium hydroxide),
c. Breakdown and removal of the denatured DNA chain at the depurinated sites by treatment with hot salts (2 x SSC).

Observation of the slides

All the slides were observed under 10X objective of Olympus BX60 (phase contrast) microscope to locate the metaphases in them and for the observation of minute chromosomal aberrations 100X (oil immersion) objective was used. 50 well spread metaphases were analyzed per sample and the chromosomal aberrations were recorded in a standard format and classified according to the International Nomenclature (ISCN, 1995). For the study of acrocentric associations, the following criteria used by **Hansson, 1970** were applied.

1. The satellite ends of the associating chromosomes had to be directed towards each other with their longitudinal axes meeting between their short arms.
2. The distance between the centromeres of associated chromosomes should not exceed the total length of one 'G' chromosome after excluding its satellite.

Statistical analysis

The statistical analysis was done with the help of GraphPad PRISM version 4 software on PC. Student's *t* test was performed to test the significance of difference between means of chromosomal aberrations recorded.

Fig.1: Conducting Counseling, Registration, Consent Form filling, Blood sample Collection, Setting of Culture, Slide Preparation, Staining and microscopy.

Table No.5 : Detail of Samples Processed				
Groups	**Sub-groups**	**No. of Males**	**No. of Females**	**Total No. of Subjects**
Normal Healthy MIC Exposed n=200		**100/100**	**93 /100**	**193/200**
Normal Healthy Non Exposed n=200		**100/100**	**72/100**	**172/200**
1st Progeny of MIC exposed individual n=200		**72/100**	**59/100**	**131/200**
2nd Progeny of MIC exposed individual n=200		**27/100**	**19/100**	**46/200**
Congenital Malformations n=200	**A: With MIC Exposure**	**50/50**	**37/50**	**87/100**
	B: Without MIC Exposure	**50/50**	**50/50**	**100/100**
Other Ailments n=200	**A: With MIC Exposure**	**38/50**	**19/50**	**57/100**
	B: Without MIC Exposure	**46/50**	**50/50**	**96/100**
No. of samples processed	-	**483/600**	**399/600**	**882/1200**

NB: Only 882 individuals sample got processed because of their availability during the blood sample collection time or because of the preoccupied duties they failed to visit the centre, or because of their religious functions like Navratri, Moharram, Ramzan, etc.

RESULTS

Structural and Numerical chromosomal aberrations

Structural and numerical aberrations were recorded at mitotic metaphases. These were observed slightly higher in the healthy exposed group as compared to the healthy control group (Table 6 & Table 7). Percentage of aberrant metaphases in male and female individuals of all the groups were observed and it was found that chromosomal association followed by Micro nucleus and single and double minutes was statistically higher than that of control. Chromosomal aberrations comprised of both chromosome-type and chromatid-type aberrations.

Table No. 6: Types of aberrations in number of Male individuals of all the groups (n= 483)

Group	MN	MIN	DL	NUM ABR	FRG	CHR ASSC	PCD	PP	PUL
MIC Exp n= 100	26 (26%)	11 (11%)	07 (07%)	3 (3%)	04 (4%)	42 (42%)	09 (9%)	2 (2%)	2 (2%)
Non Exp n = 100	11 (11%)	02 (2%)	0	0	03 (2%)	08 (2%)	02 (2%)	0	0
1st Progeny n = 72	12 (16.6%)	7 (9.7%)	0	0	1 (1.4%)	11 (15.2%)	1 (1.4%)	0	0
2nd Progeny n = 27	4 (14.8%)	2 (7.4%)	0	1 (3.7%)	0	3 (3.7%)	0	0	0
Other Ailments/ W Exp n = 38	12 (31.5%)	9 (23.6%)	0	0	0	07 (18.4%)	0	0	0
Other Ailments/ WO Exp n = 46	19 (41.3%)	13 (28.26%)	0	3 (6.5%)	2 (4.3%)	6 (13%)	03 (6.5%)	1 (2.1%)	1 (2.17 %)
CM W Exp n = 50	08 (16%)	2 (4%)	0	50 (100%)	2 (4%)	11 (22%)	0	0	0
CM/ WO Exp n = 50	06 (12%)	0	0	50 (100%)	6 (12%)	10 (20%)	1 (2%)	0	0

Legend: **CM W** Exp- Congenital Malformed with exposure, **CM/WO** Exp- Congenital Malformed without exposure, **MN**- Micronucleus, **MIN**- Minute, **DL**- Deletion, **NUM ABR**- Numerical Aberration, **FRG**- Fragment, **CHR ASSC**- Chromosomal Association, **PCD**- Premature Centromeric Division, **PP**- Polyploidy, **PUL**- Pulverized Chromosomes.

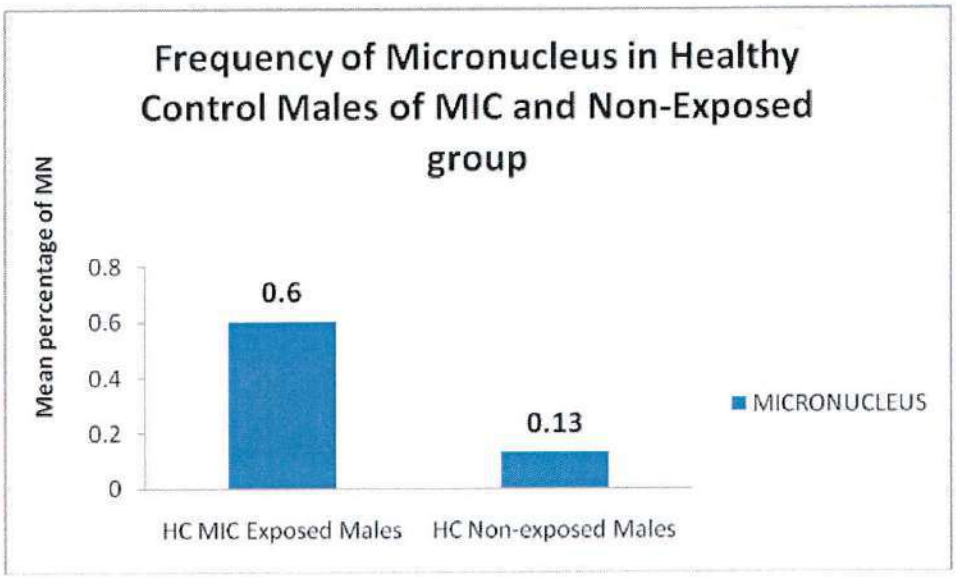
Table No. 7: Types of aberrations in number of female individuals of all the groups (n=399)									
Group	**MN**	**MIN**	**DL**	**NUM ABR**	**FRG**	**CHR ASSC**	**PCD**	**PP**	**PUL**
MIC Exp (n = 93)	11 (11.8%)	1 (1%)	03 (3.2%)	0	02 (2.1%)	23 (24.7%)	0	0	0
Non Exp (n = 72)	04 (5.5%)	1 (1.3%)	0	0	1 (1.3%)	6 (8.3%)	1 (1.3 %)	0	0
1st Progeny (n = 59)	3 (5%)	1 (1.6%)	0	0	1 (1.6%)	4 (6.7%)	0	0	0
2nd Progeny (n =19)	1 (5.2%)	1 (5.2%)	0	0	0	0	0	0	0
Other Ailments/ W Exp (n = 19)	3 (15.7%)	3 (15.7%)	0	0	0	6 (31.5%)	0	0	0
Other Ailments/ WO Exp (n = 50)	18 (36%)	7 (14%)	0	2 (4%)	1 (2%)	14 (28%)	0	0	0
CM W Exp (n = 37)	1 (2.7%)	0	0	37 (100%)	0	7 (18.9%)	0	0	0
CM/ WO Exp (n = 50)	4 (8%)	0	0	50 (100%)	1 (2%)	16 (32%)	0	0	0

Legend: **CM W** Exp- Congenital Malformed with exposure, **CM/WO** Exp- Congenital Malformed without exposure, **MN**- Micronucleus, **MIN**- Minute, **DL** - Deletion, **NUM ABR**- Numerical Aberration, **FRG**- Fragment, **CHR ASSC**- Chromosomal Association **PCD**- Premature Centromeric Division, **PP**- Polyploidy, **PUL**- Pulverized Chromosomes.

Table 8: Frequency of Micronucleus in Males of the MIC and Non-Exposed group

Type of Aberrations	Healthy Control MIC Exposed Males (n=100) Mean ± SE	Healthy Control Non-Exposed Males (n=100) Mean ± SE
Micronucleus	0.60 ± 0.123	0.130 ± 0.039

p-value = 0.0004 (P<0.05) P***



Graph 1: Showing Mean Percentage of Micronucleus in Males of HC (Healthy Control) of MIC Exposed and Non-Exposed Group.

Table No. 8 revealed that the frequency of micronucleus in males of MIC exposed individuals is highly significant ($p < 0.05$) than the non-MIC exposed individuals.

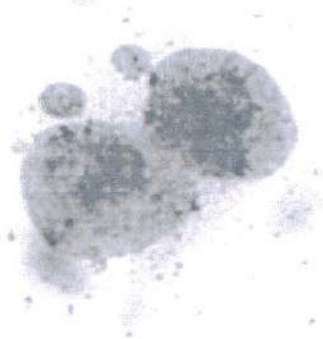
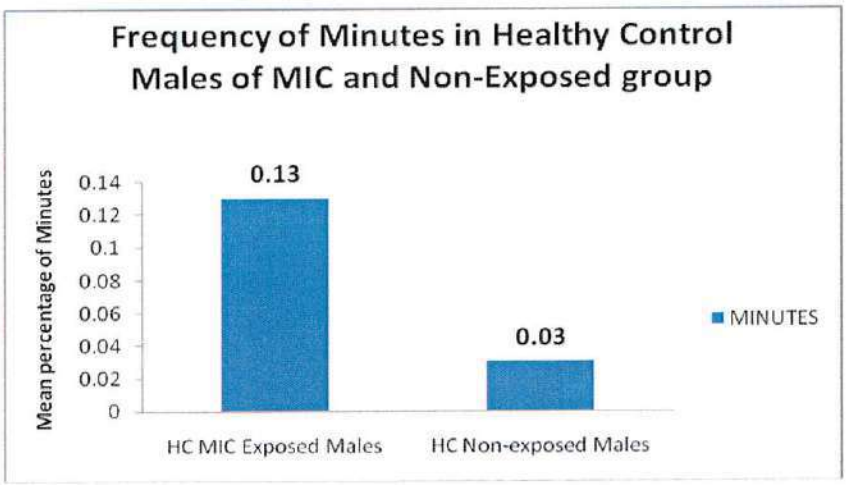


Fig. 2: Showing Micronucleus

Table 9: Frequency of Minutes in Males of the MIC-Exposed group

Type of Aberrations	Healthy Control MIC Exposed Males (n=100) Mean ± SE	Healthy Control Non-Exposed Males (n=100) Mean ± SE
Minutes	0.130 ± 0.0393	0.030 ± 0.0223

p-value = 0.0281 (P<0.05) P*



Graph 2: Showing Mean Percentage of Minutes in Males of HC (Healthy Control) of MIC Exposed and Non-Exposed Group

Minutes were evidenced in the exposed group; it's another type of chromatid type of aberrations (**Fig 3**). On comparison with the non-exposed group (0.030±0.0223) it was found to be higher (0.130± 0.0393) in the exposed and the difference between the mean was significant (p= 0.0281)

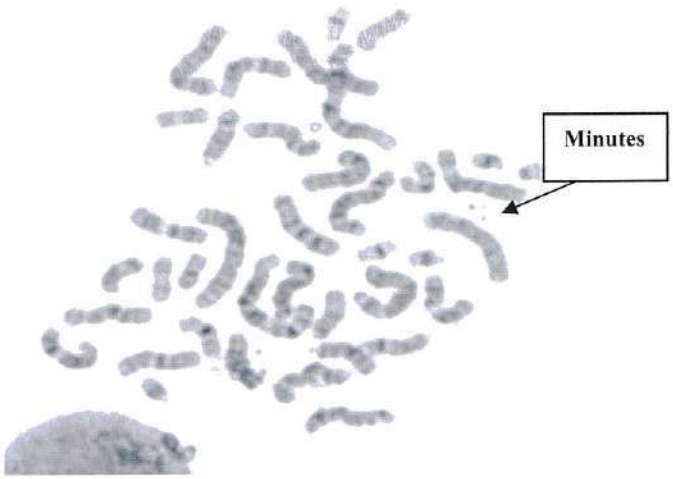
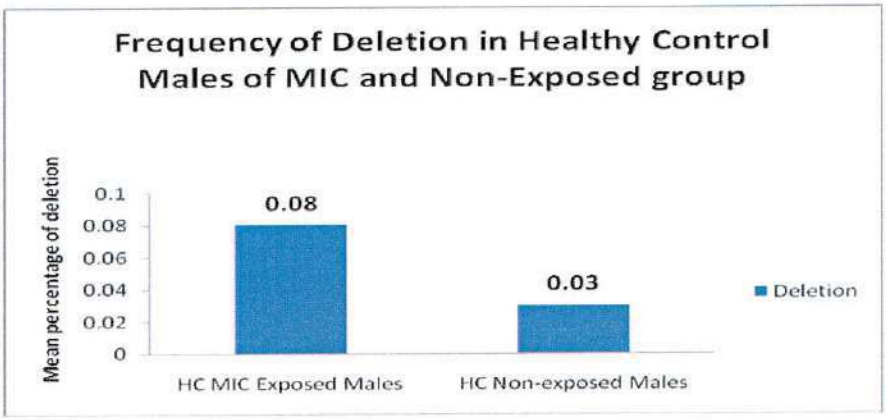


Fig. 3: Showing Minutes

Table 10: Frequency of Deletion in Males of the MIC and Non-Exposed group

Type of Aberrations	Healthy Control MIC Exposed Males (n=100) Mean ± SE	Healthy Control Non-Exposed Males (n=100) Mean ± SE
Deletion	0.080 ± 0.030	0.030 ± 0.010

p-value = 0.0316 (P<0.05) P*



Graph 3: Showing Mean Percentage of Deletion in Males of HC (Healthy Control) of MIC Exposed and Non-Exposed Group.

The frequency of deletion (chromosome type aberrations) in the exposed male (0.080±0.030) was found to be significant than the non-exposed male (0.030±0.010)

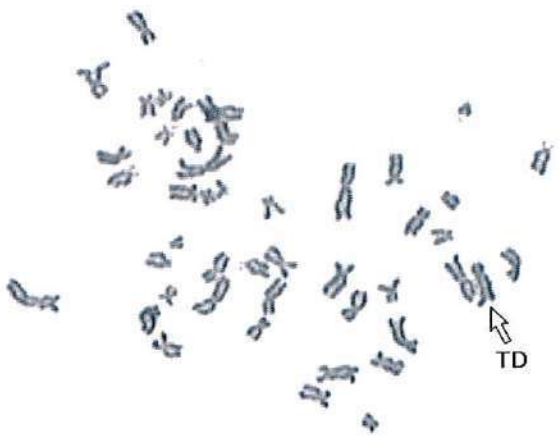
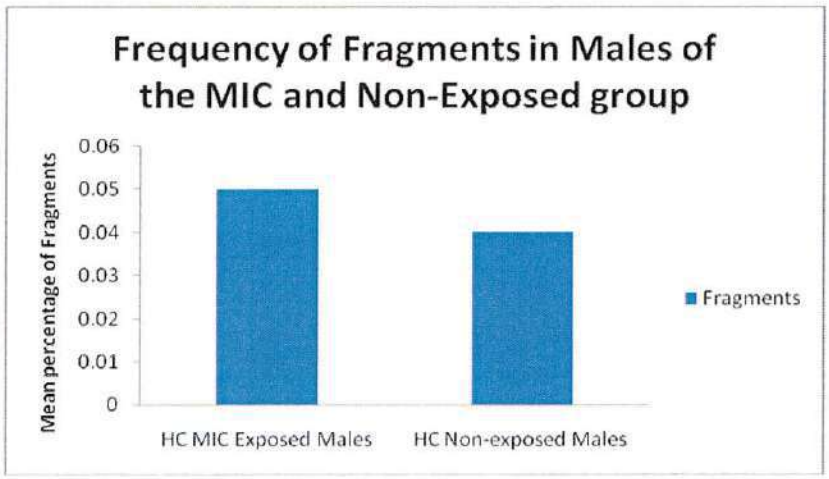


Fig. 4: Showing Terminal Deletion

Table11: Frequency of Fragments in Males of the MIC and Non-Exposed group

Type of Aberrations	Healthy Control MIC Exposed Males (n=100) Mean ± SE	Healthy Control Non-Exposed Males (n=100) Mean ± SE
Fragments	0.050 ± 0.026	0.040 ± 0.024

p-value = 0.779 (P<0.05) NS



Graph 4: Showing Mean Percentage of Fragments in Males of HC (Healthy Control) of MIC Exposed and Non-Exposed Group.

The fragments chromatid type of aberrations in male exposed population has not shown any significant value when compared with non-exposed male of this group. The p value is > the expected.

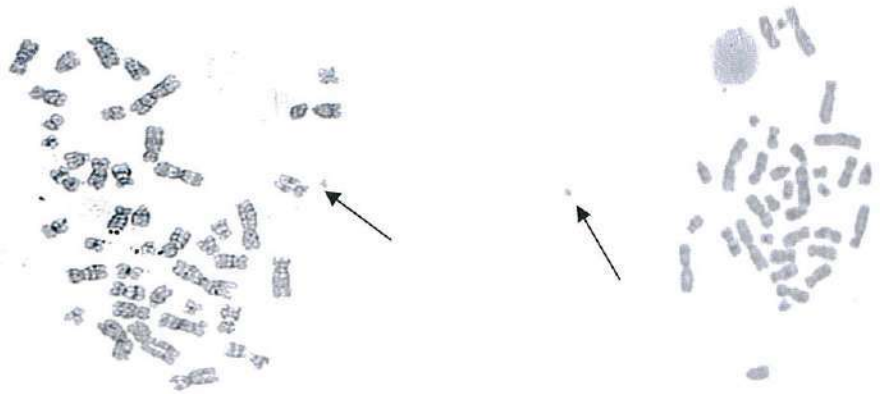
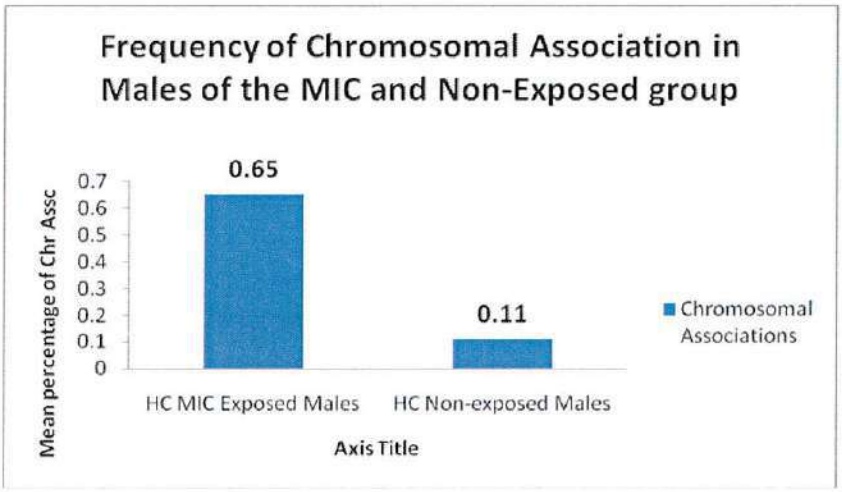


Fig. 5: Showing Fragments

Table 12: Frequency of Chromosomal Association in Males of the MIC and Non-Exposed group

Type of Aberrations	Healthy Control MIC Exposed Males (n=100) Mean ± SE	Healthy Control Non-Exposed Males (n=100) Mean ± SE
Chromosomal Associations	0.650 ±0.145	0.110 ± 0.039

p-value = 0.0004 (P<0.05) P***



Graph 5: Showing Mean Percentage of Chromosomal Associations in Males of HC (Healthy Control) of MIC Exposed and Non-Exposed Group.

Occurrence of cells exhibiting chromosomal associations (chromosome type aberrations) was observed in exposed and non-exposed individuals (Table No 12). The mean % frequency of such cells in the exposed group was recorded to be 0.650 ± 0.145 which was significantly higher than that in non-exposed individuals (0.110 ± 0.039).

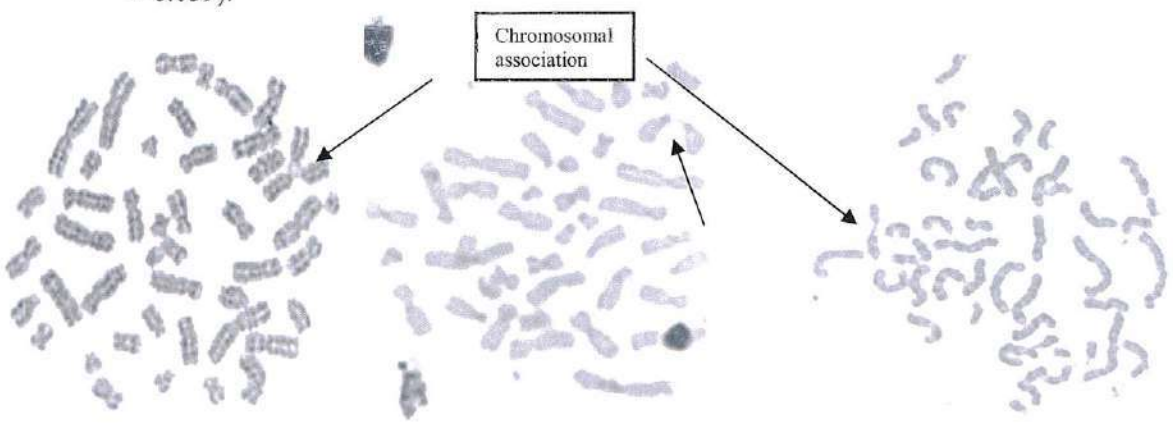
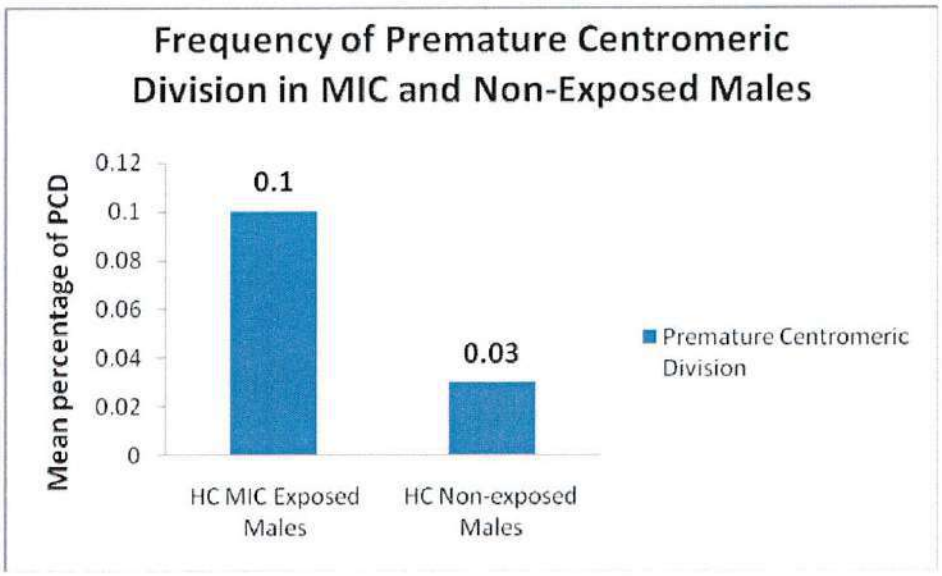


Fig. 6: Showing Chromosomal Associations

Table 13: Frequency of Premature Centromeric Division in MIC and Non-Exposed Males

Type of Aberrations	Healthy Control MIC Exposed Males (n=100) Mean ± SE	Healthy Control Non-Exposed Males (n=100) Mean ± SE
Premature Centromeric Division	0.100 ± 0.033	0.030 ± 0.022

p-value = 0.0823 (P>0.05) NS



Graph 6: Showing Mean Percentage of PCD (Premature Centromeric Division) in Males of HC (Healthy Control) of MIC Exposed and Non-Exposed Group.

The PCD which is a chromosome type of aberrations in male exposed population have not shown any significant value when compared with non-exposed male of this group. The p value is > the expected.

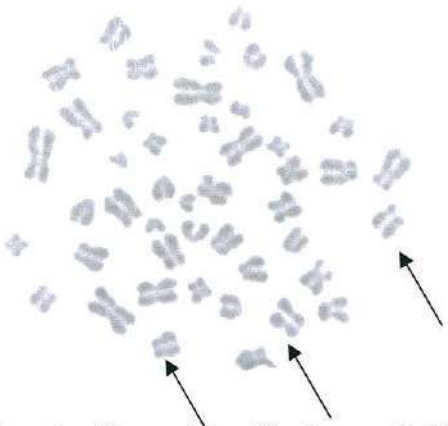
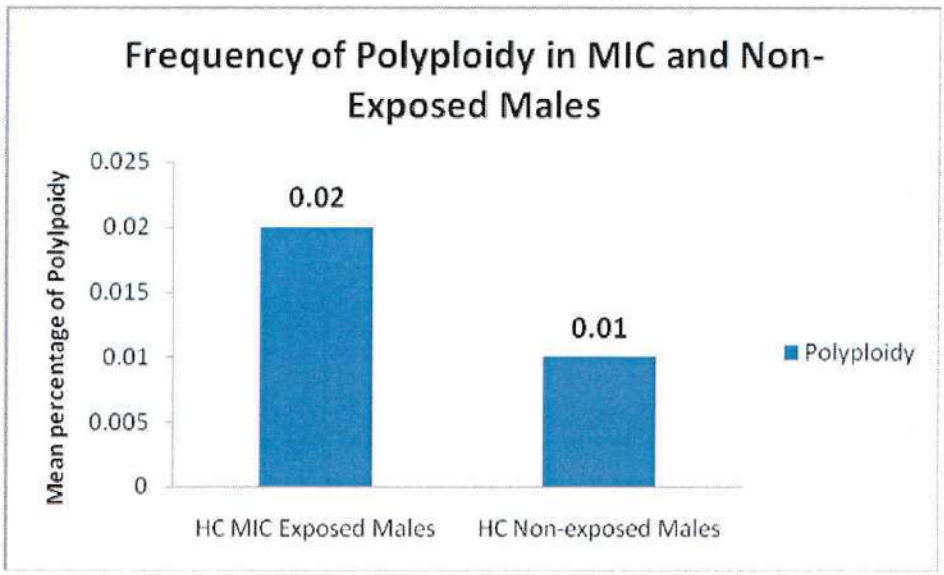


Fig. 7: Showing Premature Centromeric Division

Table14: Frequency of Polyploidy in MIC and Non-Exposed Males

Type of Aberrations	Healthy Control MIC Exposed Males (n=100) Mean ± SE	Healthy Control Non-Exposed Males (n=100) Mean ± SE
Polyploidy	0.020 ± 0.014	0.010 ± 0.010

p-value = 0.563 (P>0.05) NS



Graph 7: Showing Mean Percentage of Polyploidy in Males of HC (Healthy Control) of MIC Exposed and Non-Exposed Group.

Occurrence of polyploid cells in the exposed (0.020 +- 0.014) and non-exposed (0.010 +- 0.010) MIC males was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 14**)

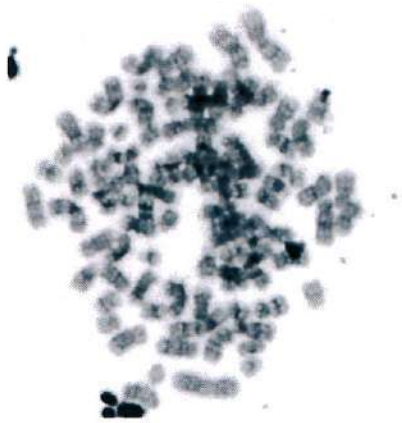
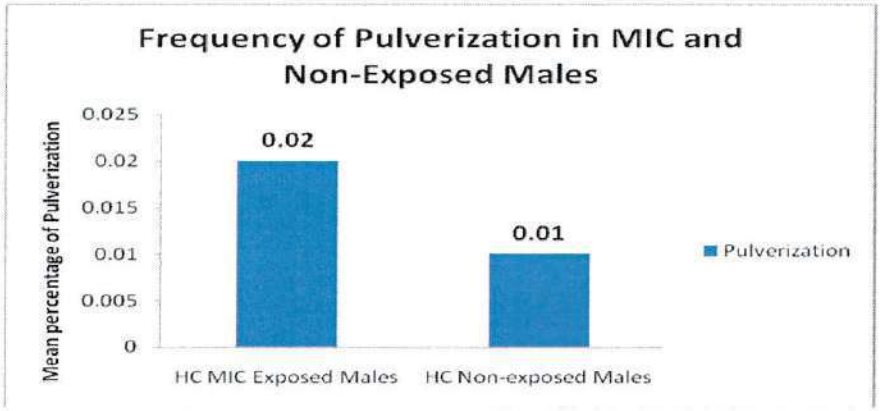


Fig 8: Showing Polyploidy

Table 15: Frequency of Pulverization in MIC and Non-Exposed Males

Type of Aberrations	Healthy Control MIC Exposed Males (n=100) Mean ± SE	Healthy Control Non-Exposed Males (n=100) Mean ± SE
Pulverization	**0.020 ± 0.014**	**0.010 ± 0.010**

p-value = 0.563 (P>0.05) NS



Graph 8: Showing Mean Percentage of Pulverization in Males of HC (Healthy Control) of MIC Exposed and Non-Exposed Group.

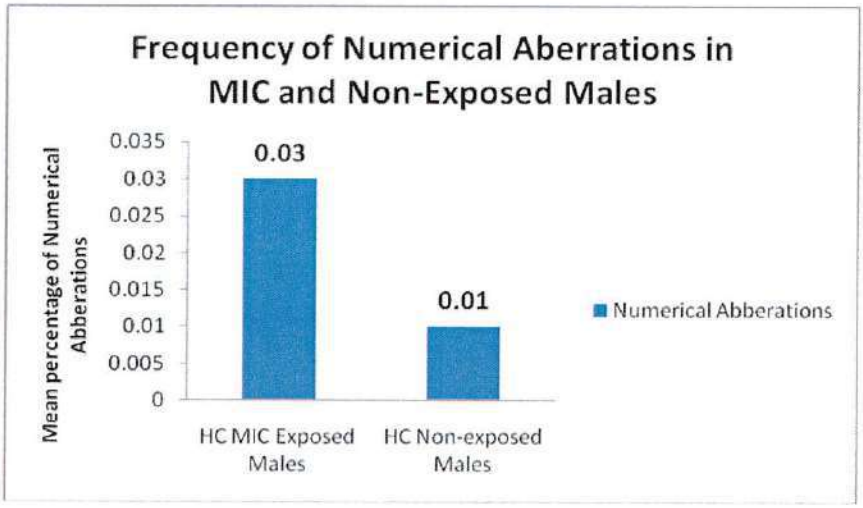
Occurrence of pulverization cells in the exposed (**0.020 ± 0.014**) and non-exposed (**0.010 ± 0.010**) MIC males was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 15**)

Fig. 9: Showing Pulverized Chromosome

Table16: Frequency of Numerical Aberrations in MIC and Non-Exposed Males

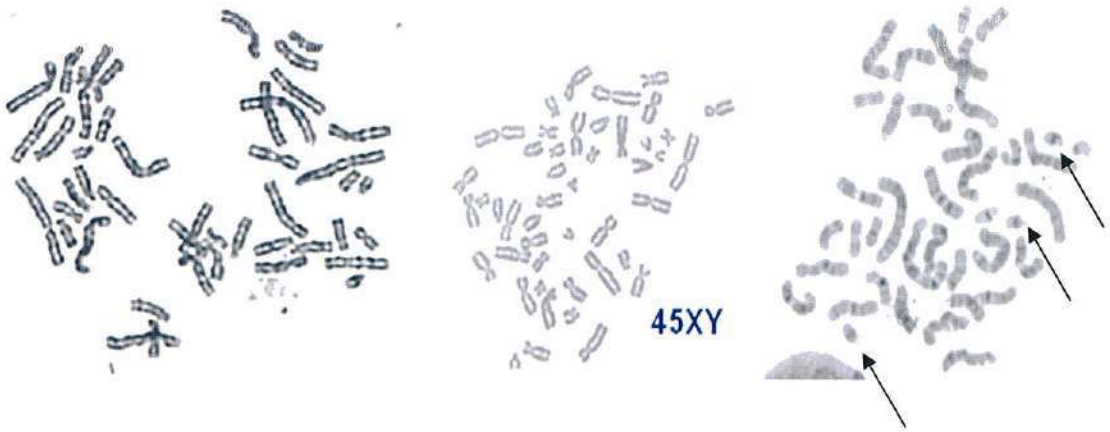
Type of Aberrations	Healthy Control MIC Exposed Males (n=100) Mean ± SE	Healthy Control Non-Exposed Males (n=100) Mean ± SE
Numerical Aberrations	0.030 ± 0.017	0.010 ± 0.010

p-value = 0.314>0.05 NS



Graph 9: Showing Mean Percentage of Numerical Aberrations in Males of HC (Healthy Control) of MIC Exposed and Non-Exposed Group

Occurrence of Numerical Aberrations cells in the exposed (**0.030 ± 0.017**) and non-exposed (**0.010 ± 0.010**) MIC males was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 16**)



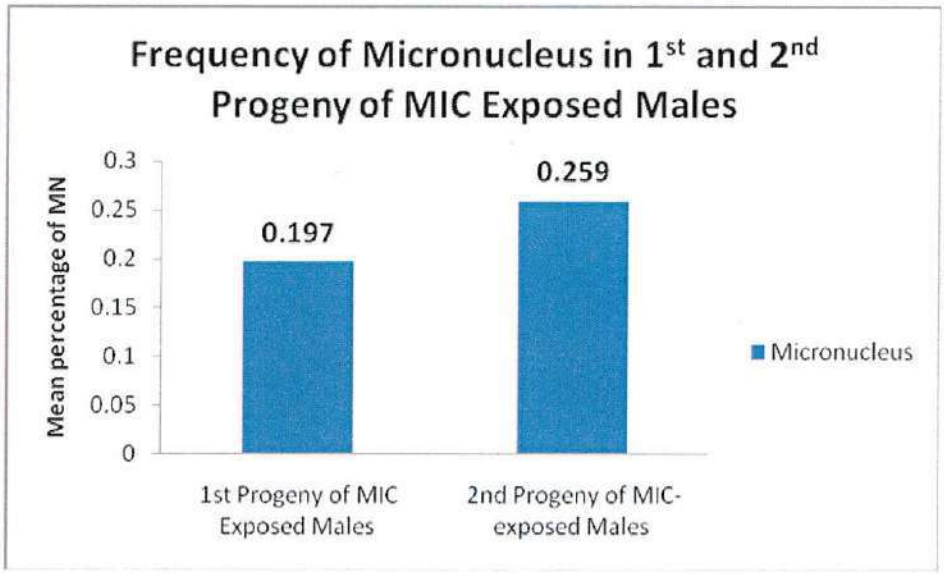
Hyperdiploid **Hypodiploid** **Hyperdiploid**

Fig.10: Showing Numerical Aberrations

Table 17: Frequency of Micronucleus in 1st and 2nd Progeny of MIC Exposed Males

Type of Aberrations	Healthy Control MIC Exposed 1st Progeny (n=72) Mean ± SE	Healthy Control MIC Exposed 2nd Progeny (n=27) Mean ± SE
Micronucleus	0.197± 0.061	0.259 ± 0.137

p-value = 0.635 (P>0.05) NS



Graph 10: Showing Mean Percentage of Micronucleus in 1st and 2nd Progeny of MIC Exposed Males.

Occurrence of Micronucleus in the 1st Progeny (**0.197± 0.061**) and 2nd Progeny (**0.259 ± 0.137**) of MIC exposed males was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 17**)

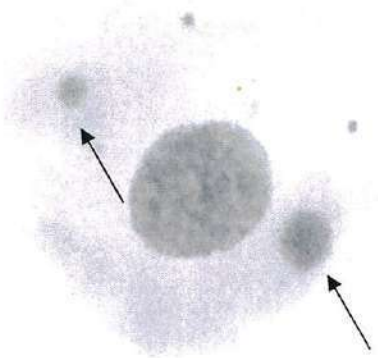
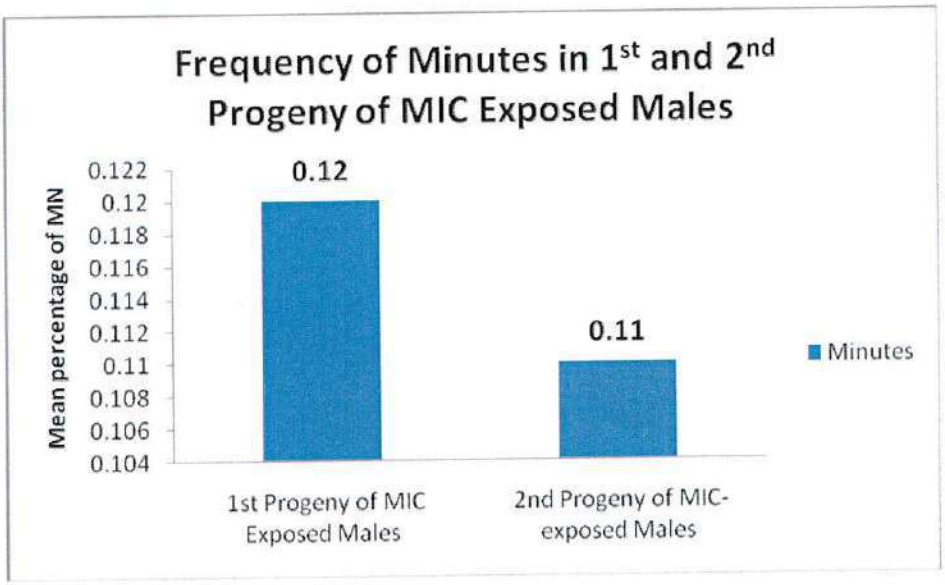


Fig 11: Showing Micronucleus

Table 18: Frequency of Minutes in 1st and 2nd Progeny of MIC Exposed Males

Type of Aberrations	1st Progeny of MIC Exposed Males (n=72) Mean ± SE	2nd Progeny of MIC Exposed Males (n=27) Mean ± SE
Minutes	0.127 ± 0.055	0.111 ± 0.081

p-value = 0.872 (P>0.05) NS



Graph 11: Showing Mean Percentage of Minutes in 1st and 2nd Progeny of MIC Exposed Males.

Occurrence of Minutes in the 1st Progeny (**0.127 ± 0.055**) and 2nd Progeny (**0.111 ± 0.081**) of MIC exposed males was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 18**)

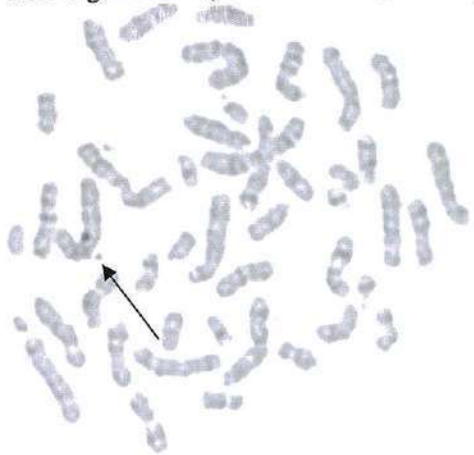
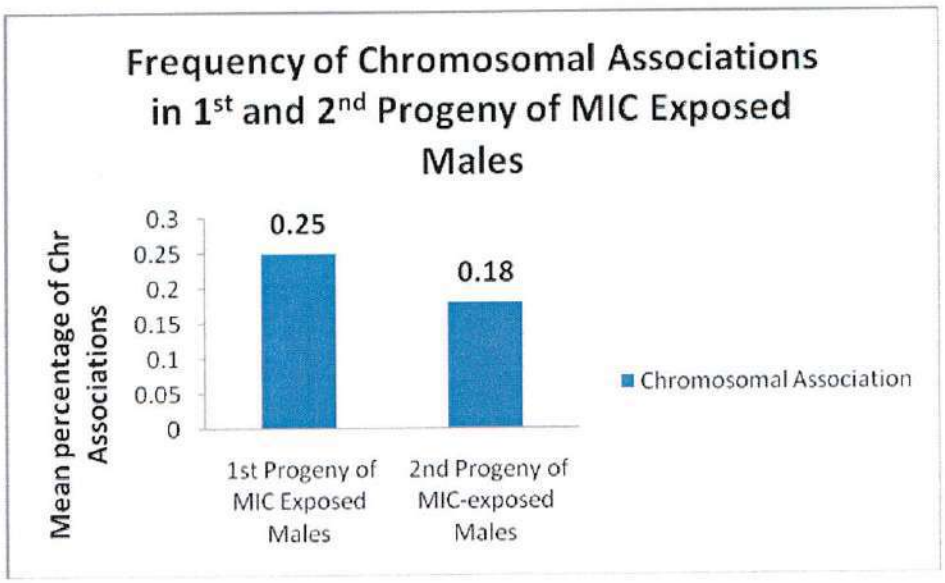


Fig 12: Showing Minutes

Table 19: Frequency of Chromosomal Associations in 1st and 2nd Progeny of MIC Exposed Males

Type of Aberrations	1st Progeny of MIC Exposed Males (n=72) Mean ± SE	2nd Progeny of MIC Exposed Males (n=27) Mean ± SE
Chromosomal Association	0.250 ± 0.078	0.1852 ± 0.107

p-value = 0.654 (P>0.05) NS



Graph 12: Showing Mean Percentage of Chromosomal Associations in 1st and 2nd Progeny of MIC Exposed Males.

Occurrence of Chromosomal Associations in the 1st Progeny **(0.250 ± 0.078)** and 2nd Progeny **(0.1852 ± 0.107)** of MIC exposed males was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 19**)

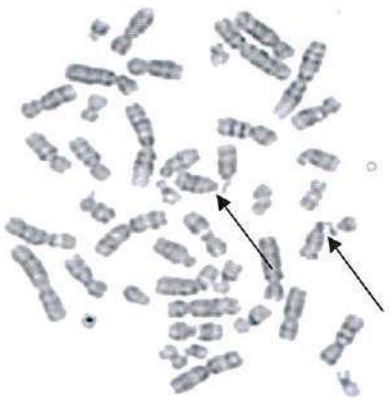
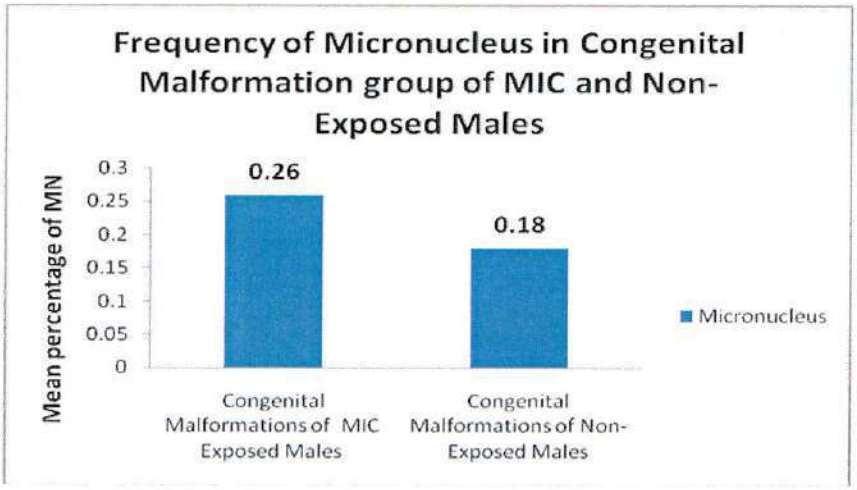


Fig 13: Showing Chromosomal Associations

Table20: Frequency of Micronucleus in Congenital Malformation group of MIC and Non-Exposed Males

Type of Aberrations	Congenital Malformations of MIC Exposed Males (n=50) Mean ± SE	Congenital Malformations of Non-Exposed Males (n=50) Mean ± SE
Micronucleus	0.260 ± 0.093	0.180 ± 0.073

p-value = 0.504 (P<0.05) NS



Graph 13: Showing Mean Percentage of Micronucleus in Congenital Malformations group of MIC and Non-Exposed Males.

Occurrence of Micronucleus in the congenital Malformations group of MIC exposed **(0.260 ± 0.093)** and Non-Exposed Males **(0.260 ± 0.093)** was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 20**)

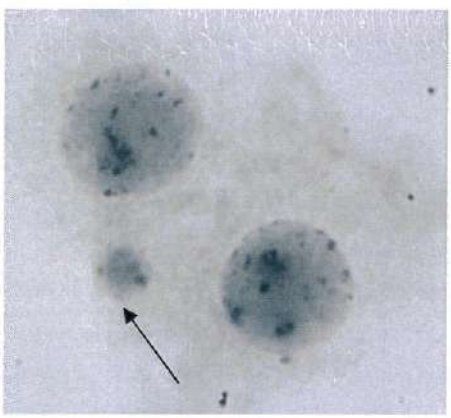
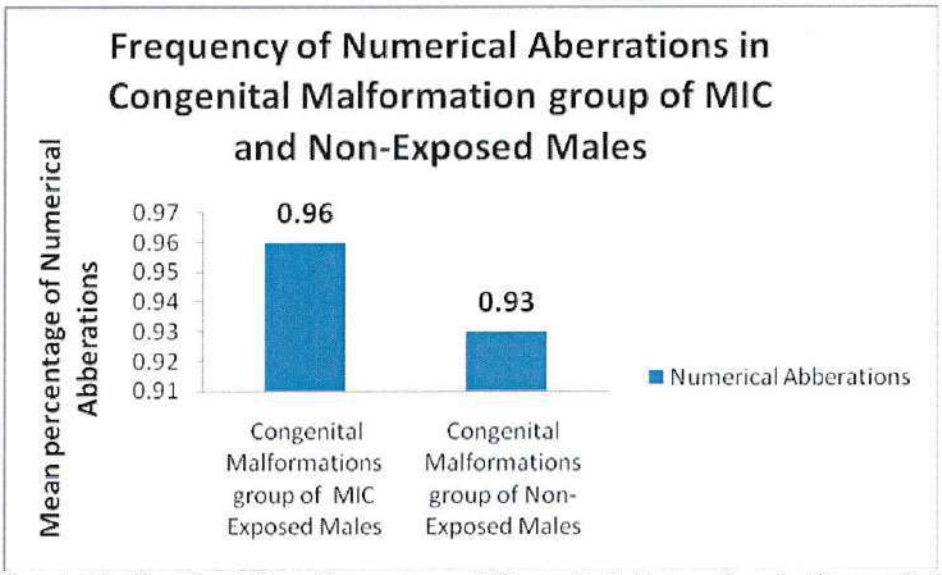


Fig 14: Showing Micronucleus

Table 21: Frequency of Numerical Aberrations in Congenital Malformation group of MIC and Non-Exposed Males

Type of Aberrations	Congenital Malformations Group of MIC Exposed Males (n=50) Mean ± SE	Congenital Malformations Group of Non-Exposed Males(n=50) Mean ± SE
Numerical Aberrations	0.980 ± 0.020	0.930 ± 0.019

p-value = 0.988 (P>0.05) NS



Graph 14: Showing Mean Percentage of Numerical Aberrations in Congenital Malformations group of MIC and Non-Exposed Males.

Occurrence of **Numerical Aberrations** in the Congenital Malformations group of MIC exposed **(0.980 ± 0.020)** and Non-Exposed Males **(0.930 ± 0.019)** was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 21**)

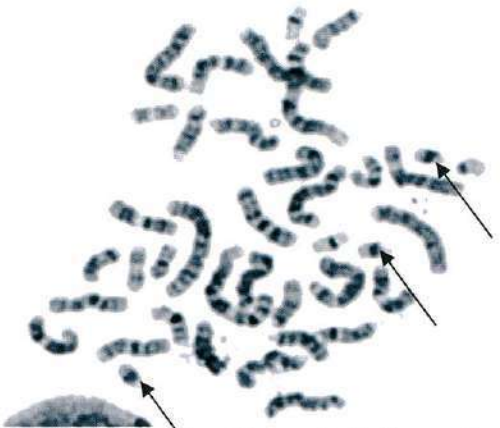
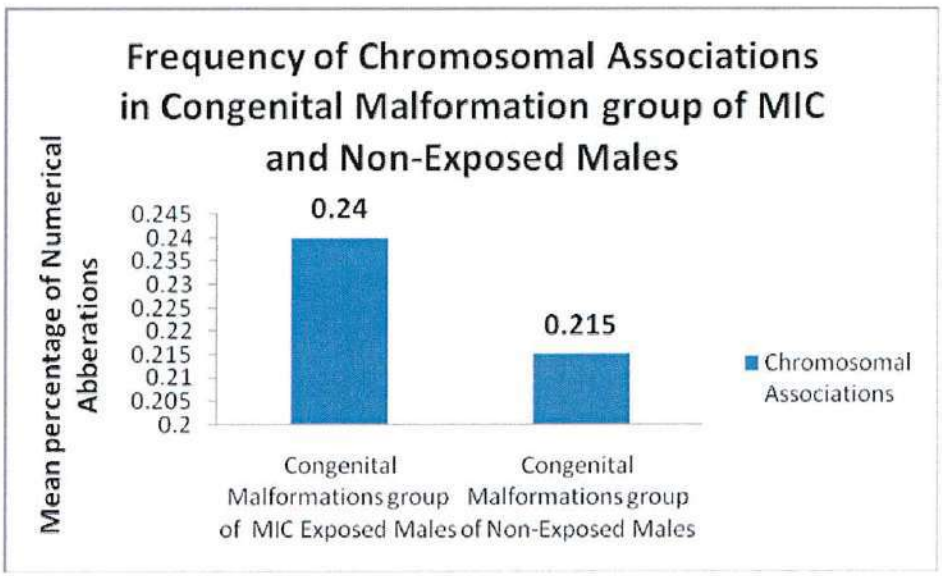


Fig 15: Showing Numerical Aberrations

Table 22: Frequency of Chromosomal Associations in Congenital Malformation group of MIC and Non-Exposed Males

Type of Aberrations	Congenital Malformations of MIC Exposed Males (n=50) Mean ± SE	Congenital Malformations of Non-Exposed Males (n=50) Mean ± SE
Chromosomal Association	0.240 ± 0.07318	0.215 ± 0.058

p-value = 0.7949 (P>0.05) NS



Graph 15: Showing Mean Percentage of Chromosomal Associations in Congenital Malformations group of MIC and Non-Exposed Males.

Occurrence of **Chromosomal Associations** in the Congenital Malformations group of MIC exposed **(0.980 ± 0.020)** and Non-Exposed Males **(0.215 ± 0.058)** was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 22**)

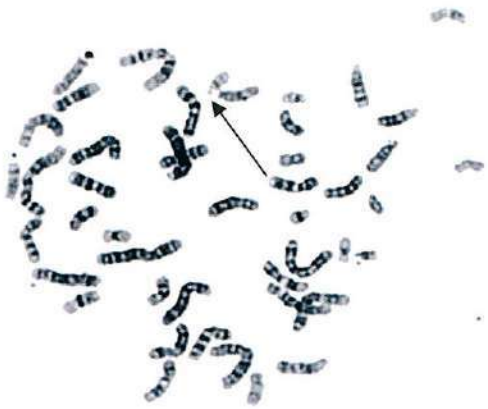
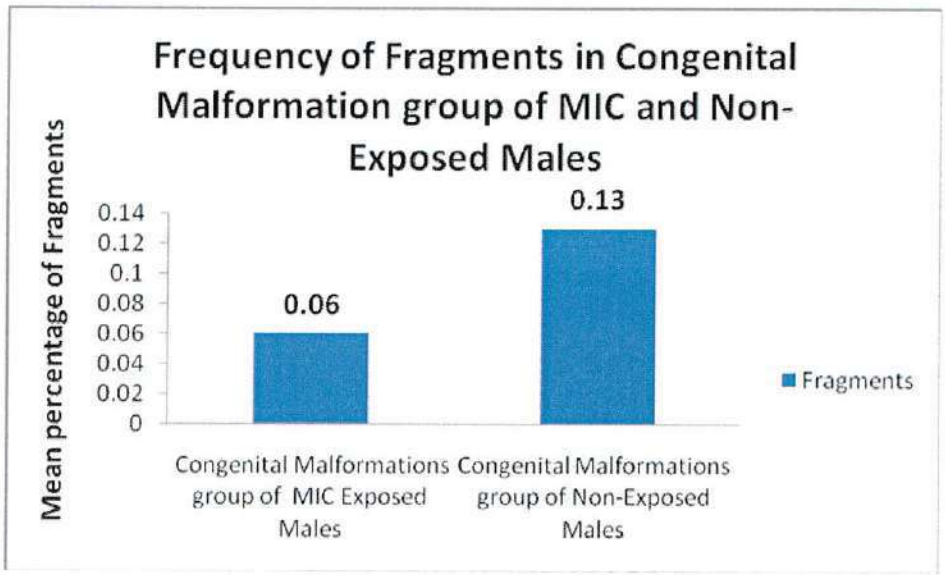


Fig 16: Showing Chromosomal Associations

Table 23: Frequency of Fragments in Congenital Malformation group of MIC and Non-Exposed Males

Type of Aberrations	Congenital Malformations of MIC Exposed Males (n=50) Mean ± SE	Congenital Malformations of Non-Exposed Males (n=50) Mean ± SE
Fragments	0.060 ± 0.044	0.137± 0.048

p-value = 0.244 (P>0.05) NS



Graph 16: Showing Mean Percentage of Fragments in Congenital Malformations group of MIC and Non-Exposed Males.

Occurrence of **Fragments** in the Congenital Malformations group of MIC exposed (**0.060 ± 0.044**) and Non-Exposed Males (**0.137± 0.048**) was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 23**)

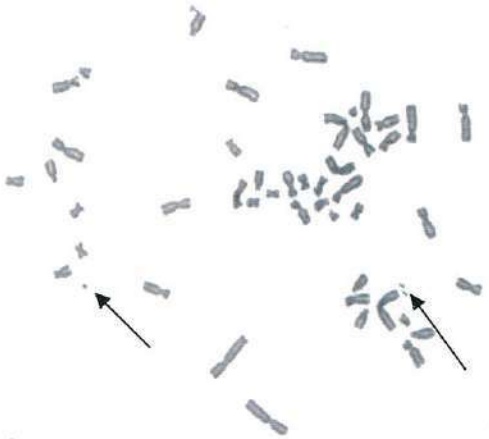
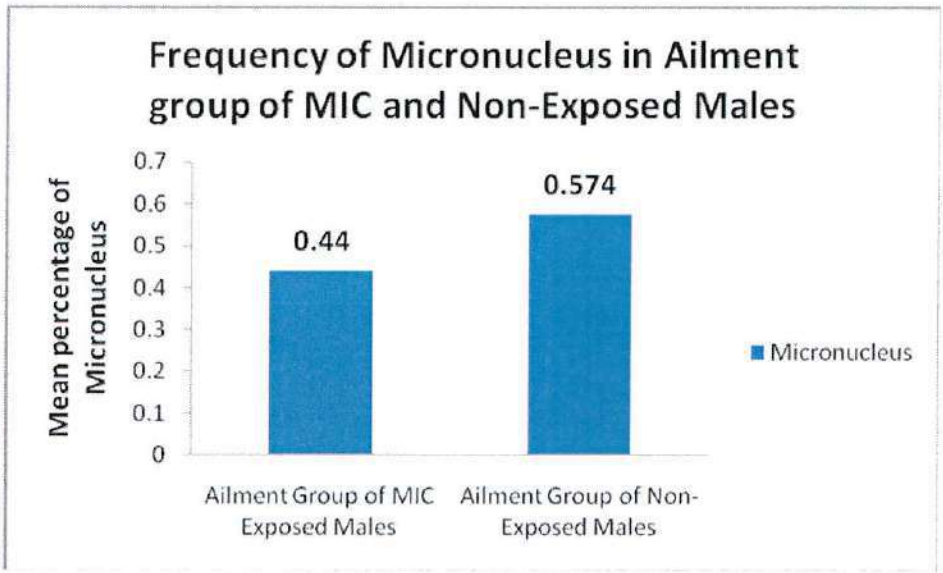


Fig 17: Showing Congenital Malformation

Table 24: Frequency of Micronucleus in Ailment group of MIC and Non-Exposed Males

Type of Aberrations	Ailment group of MIC Exposed Males (n=38) Mean ± SE	Ailment group of Non-Exposed Males (n=47) Mean ± SE
Micronucleus	0.447 ± 0.117	0.574 ± 0.112

p-value = 0.440 (P>0.05) NS



Graph 17: Showing Mean Percentage of Micronucleus in Ailment group of MIC and Non-Exposed Males.

Occurrence of Micronucleus in the Ailment group of MIC exposed (**0.447 ± 0.117**) and Non-Exposed Males (**0.574 ± 0.112**) was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 24**)

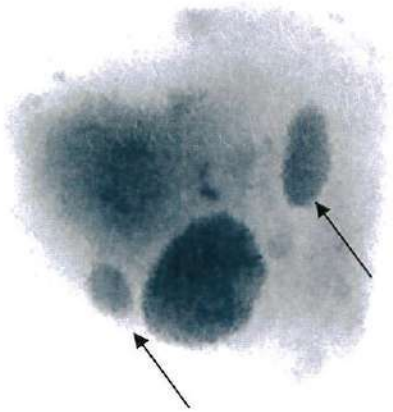
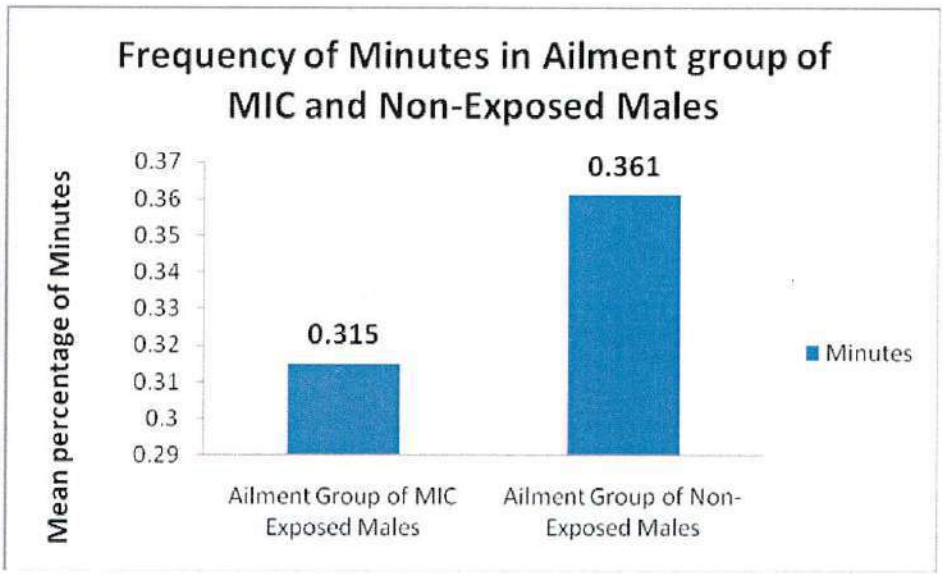


Fig 18: Showing Micronucleus

Table 25: Frequency of Minutes in Ailment group of MIC and Non-Exposed Males

Type of Aberrations	Ailment group of MIC Exposed Males (n=38) Mean ± SE	Ailment group of Non-Exposed Males (n=47) Mean ± SE
Minutes	0.315 ± 0.107	0.361 ± 0.088

p-value = 0.739 (P>0.05) NS



Graph 18: Showing Mean Percentage of Minutes in Ailment group of MIC and Non-Exposed Males.

Occurrence of Minutes in the Ailment group of MIC exposed **(0.315 ± 0.107)** and Non-Exposed Males **(0.361 ± 0.088)** was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 25**)

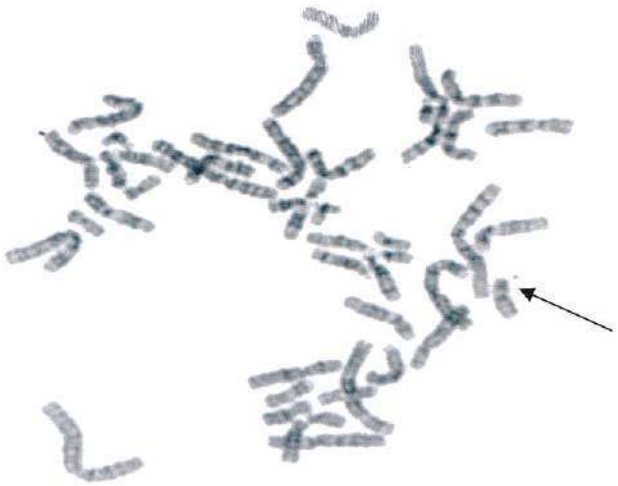
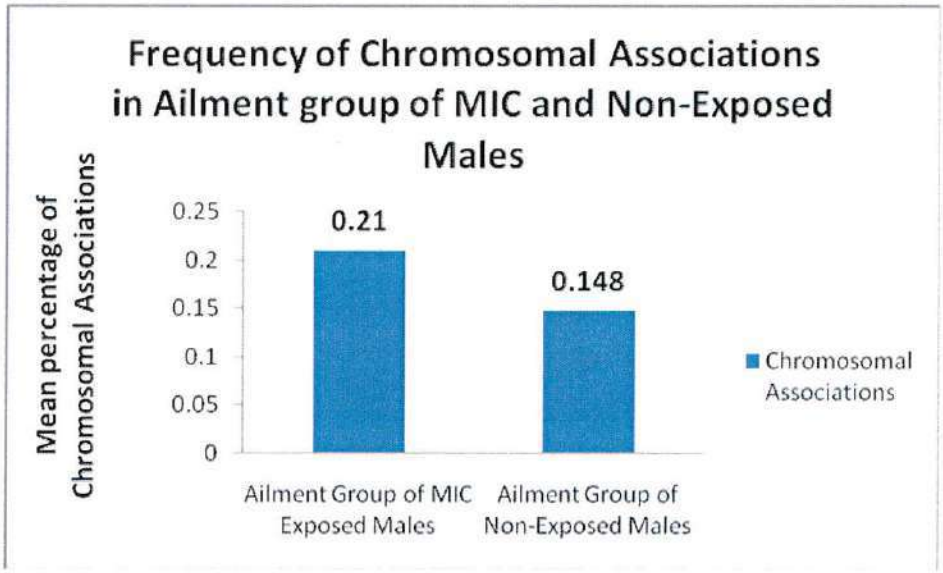


Fig 19: Showing Minutes

Table 26: Frequency of Chromosomal Associations in Ailment group of MIC and Non-Exposed

Type of Aberrations	Ailment group of MIC Exposed Males (n=38) Mean ± SE	Ailment group of Non-Exposed Males (n=47) Mean ± SE
Chromosomal Associations	0.210 ± 0.076	0.148 ± 0.052

p-value = 0. 4979 (P>0.05) NS



Graph 19: Showing Mean Percentage of Chromosomal Associations in Ailment group of MIC and Non-Exposed Males.

Occurrence of Chromosomal Associations in the Ailment group of MIC exposed (**0.210 ± 0.076**) and Non-Exposed Males (**0.148 ± 0.052**) was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 26**)

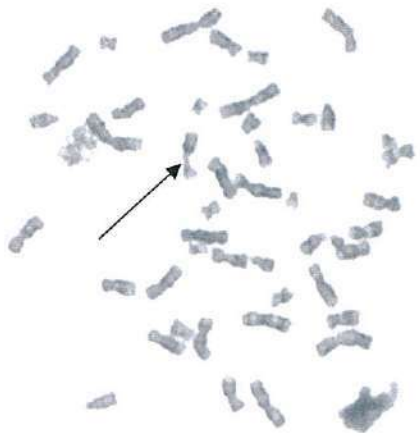
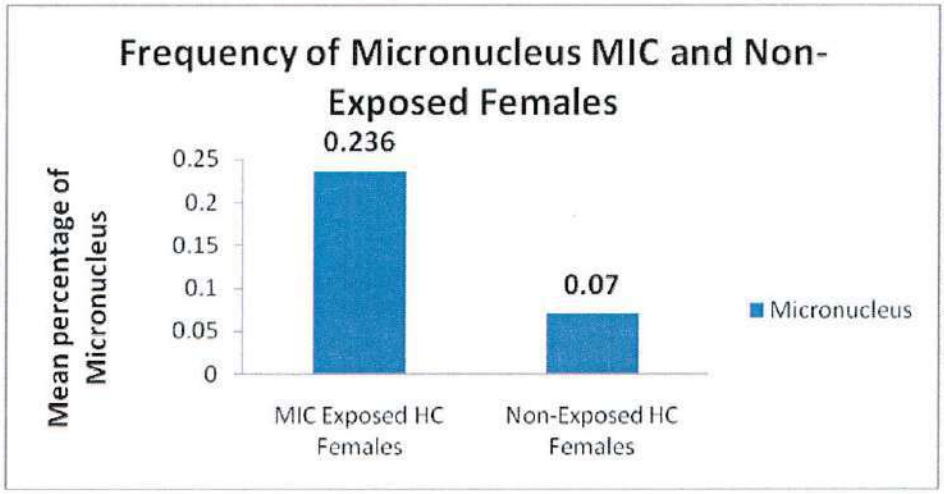


Fig 20: Showing Chromosomal Associations

Table 27: Frequency of Micronucleus in MIC and Non-Exposed Healthy Control Females

Type of Aberrations	Healthy Control MIC Exposed Females (n=93) Mean ± SE	Non-Exposed Healthy Control Females (n=71) Mean ± SE
Micronucleus	0.236 ± 0.075	0.070 ± 0.036

p-value = 0. 073 (P>0.05) NS



Graph 20: Showing Mean Percentage of Micronucleus in MIC and Non-Exposed HC (Healthy Control) Females.

Occurrence of Micronucleus in the MIC exposed (**0.236 ± 0.075**) and Non-Exposed Females (**0.070 ± 0.036**) was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 27**)

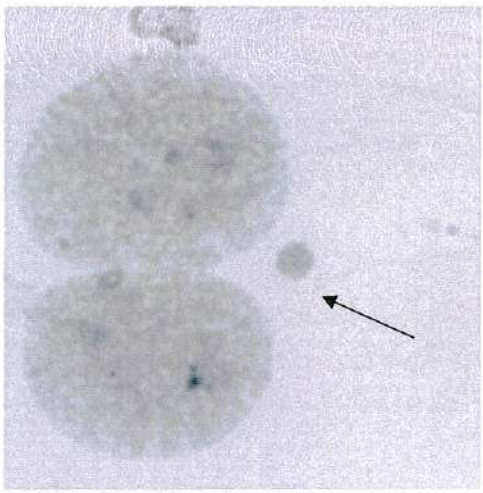
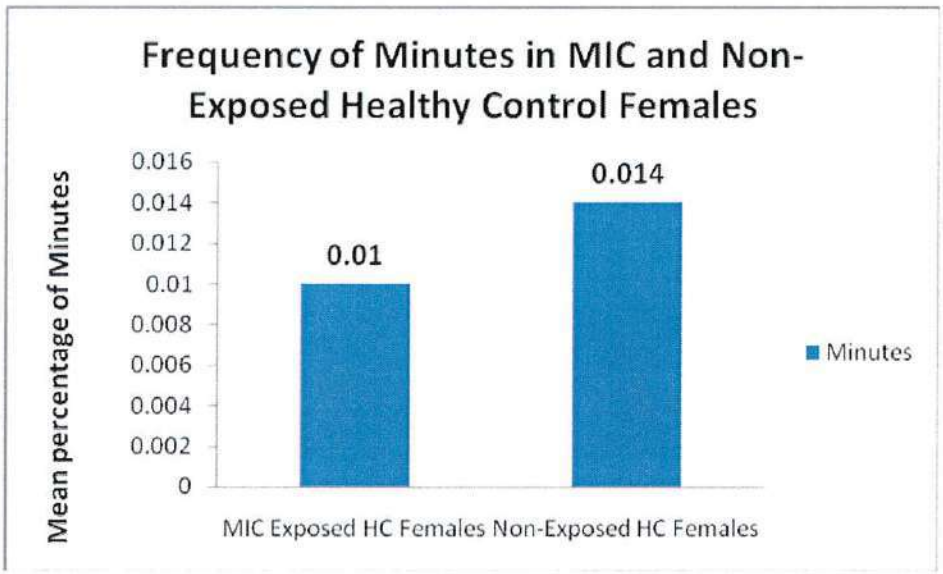


Fig 21: Showing Micronucleus

Table 28: Frequency of Minutes in Healthy MIC and Non-Exposed Healthy Females

Type of Aberrations	MIC Exposed Healthy Control Females (n=93) Mean ± SE	Non-Exposed Healthy Control Females (n=71) Mean ± SE
Minutes	0.010 ± 0.010	0.014 ± 0.014

p-value =0.848 (P>0.05) NS



Graph 21: Showing Mean Percentage of Minutes in MIC and Non-Exposed HC (Healthy Control) Females.

Occurrence of Minutes in the MIC exposed **(0.010 ± 0.010)** and Non-Exposed Females **(0.014 ± 0.014)** was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 28**)

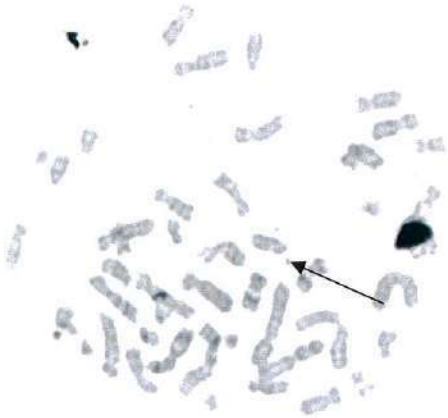
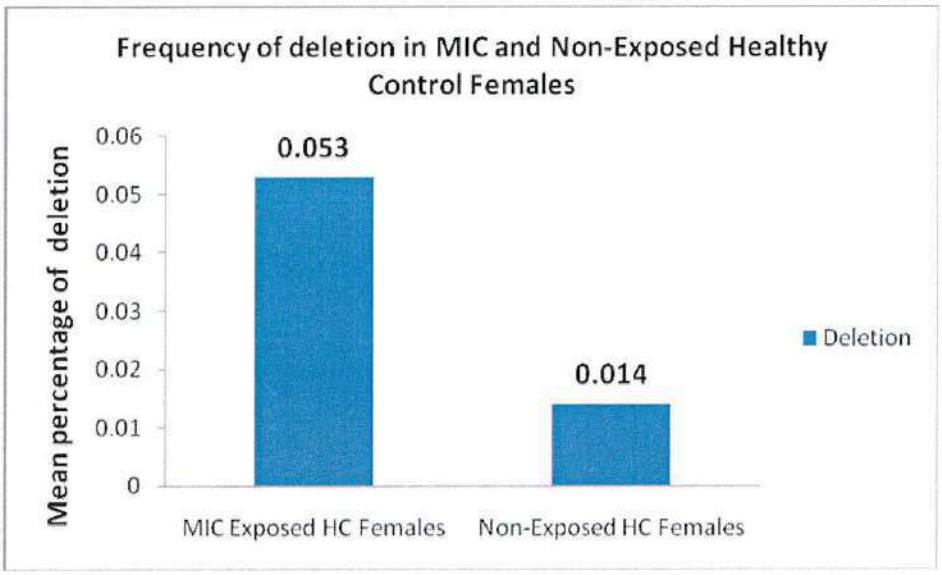


Fig 22: Showing Minutes

Table 29: Frequency of deletion in MIC and Non-Exposed Healthy Control Females

Type of Aberrations	MIC Exposed Healthy Control Females (n=93) Mean ± SE	Non-Exposed Healthy Control Females (n=71) Mean ± SE
Deletion	**0.053 ± 0.031**	**0.014 ± 0.014**

p-value =0.3055 (P>0.05) NS



Graph 22: Showing Mean Percentage of deletion in MIC and Non-Exposed HC (Healthy Control) Females.

Occurrence of deletion in the MIC exposed (**0.053 ± 0.031**) and Non-Exposed Females (**0.014 ± 0.014**) was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 29**)

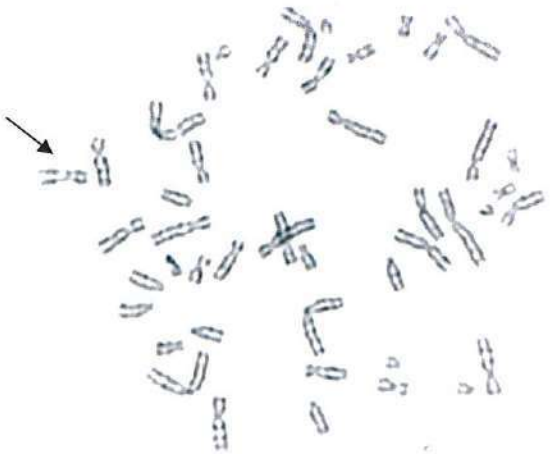
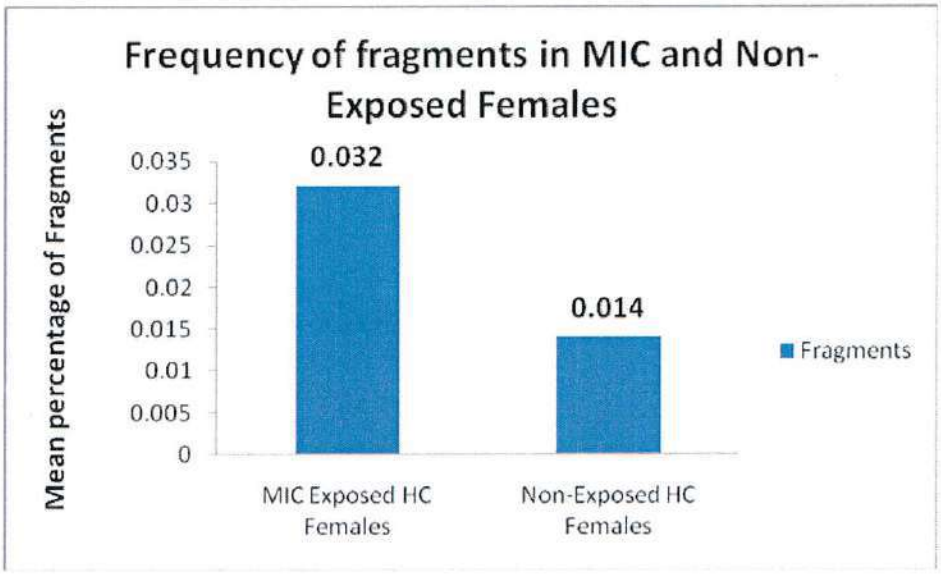


Fig 23: Showing Deletion

Table 30: Frequency of fragments in MIC and Non-Exposed Females

Type of Aberrations	MIC Exposed Healthy Control Females (n=93) Mean ± SE	Non-Exposed Healthy Control Females (n=71) Mean ± SE
Fragments	0.032 ± 0.023	0.014 ± 0.014

P value=0.546 (P>0.05) NS



Graph23: Showing Mean Percentage of Fragments in MIC and Non-Exposed HC (Healthy Control) Females.

Occurrence of Fragments in the MIC exposed (**0.032 ± 0.023**) and Non-Exposed Females (**0.014 ± 0.014**) was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 30**)

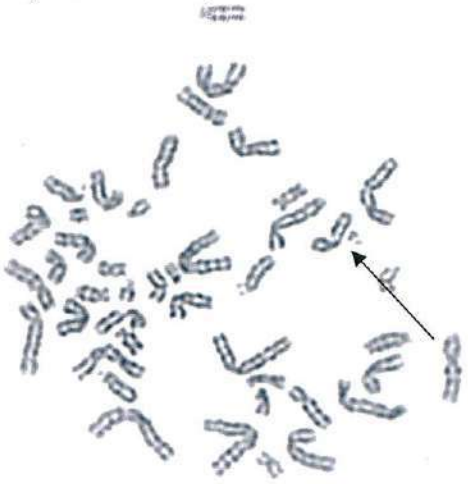
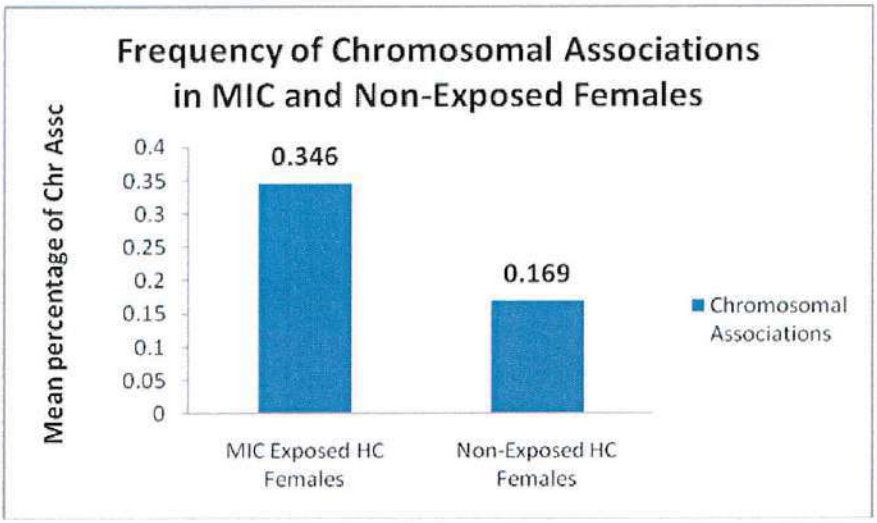


Fig 24: Showing Fragments

Table 31: Frequency of Chromosomal Associations in MIC and Non-Exposed Females

Type of Aberrations	MIC Exposed Healthy Control Females (n=93) Mean ± SE	Non-Exposed Healthy Control Females (n=71) Mean ± SE
Chromosomal Associations	0.346 ± 0.078	0.169 ± 0.072

P value=0.109 (P>0.05) NS



Graph 24: Showing Mean Percentage of Chromosomal Associations in MIC and Non-Exposed HC (Healthy Control) Females

Occurrence of Fragments in the MIC exposed (**0.346 ± 0.078**) and Non-Exposed Females (**0.169 ± 0.072**) was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 31**)

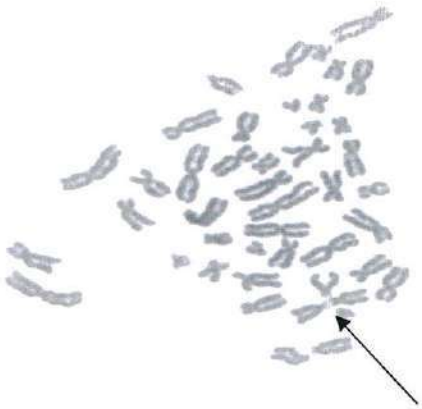
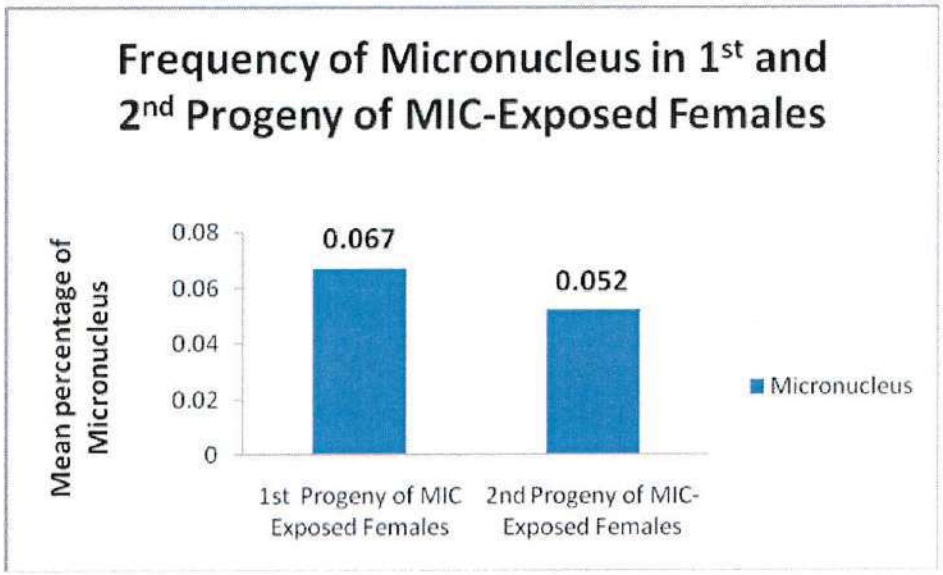


Fig 25: Showing Chromosomal Associations

Table 32: Frequency of Micronucleus in 1st and 2nd Progeny of MIC-Exposed Females

Type of Aberrations	1st Progeny of MIC Exposed Females (n=59) Mean ± SE	2nd Progeny of MIC-Exposed Females (n=19) Mean ± SE
Micronucleus	0.067± 0.040	0.052 ± 0.052

p-value =0.8467 (P>0.05) NS



Graph 25: Showing Mean Percentage of Micronucleus in 1st and 2nd Progeny of MIC-Exposed Females

Occurrence of Micronucleus in the 1st Progeny (**0.067± 0.040**) and 2nd Progeny (**0.052 ± 0.052**) of MIC Exposed Females was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 32**)

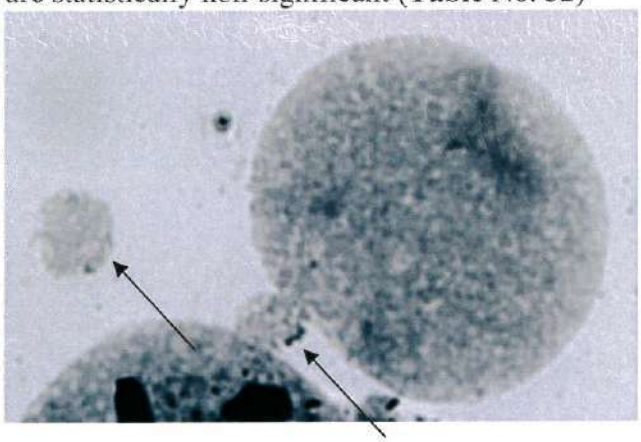
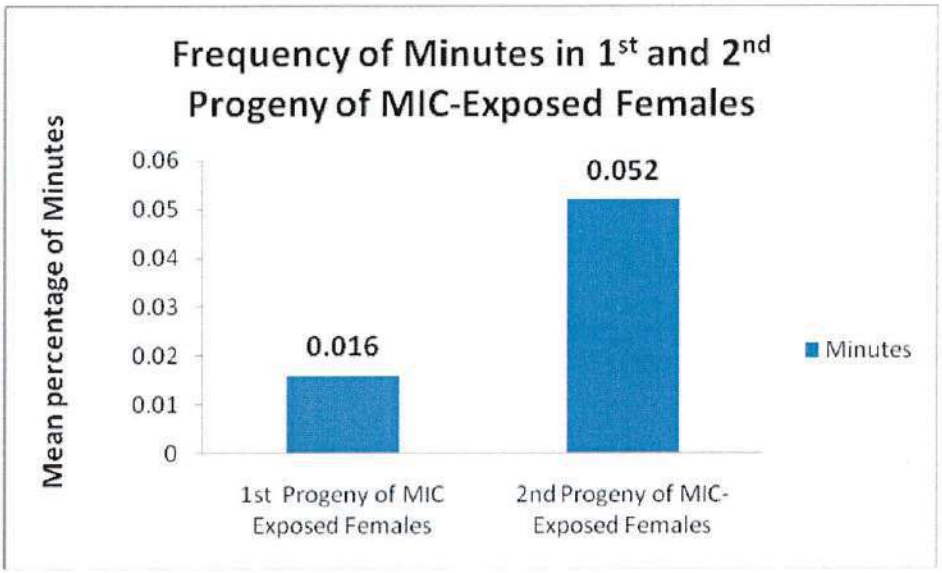


Fig 26: Showing Micronucleus

Table 33: Frequency of Minutes in 1st and 2nd Progeny of MIC-Exposed Females

Type of Aberrations	1st Progeny of MIC Exposed Females (n=59) Mean ± SE	2nd Progeny of MIC-Exposed Females (n=19) Mean ± SE
Minutes	0.016 ± 0.016	0.052 ± 0.052

p-value =0.398 (P>0.05) NS



Graph 26: Showing Mean Percentage of Minutes in 1st and 2nd Progeny of MIC-Exposed Females

Occurrence of Minutes in the 1st Progeny (**0.016 ± 0.016**) and 2nd Progeny (**0.052 ± 0.052**) of MIC Exposed Females was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 33**)

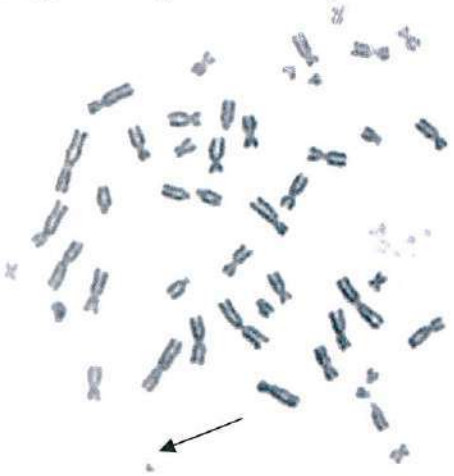
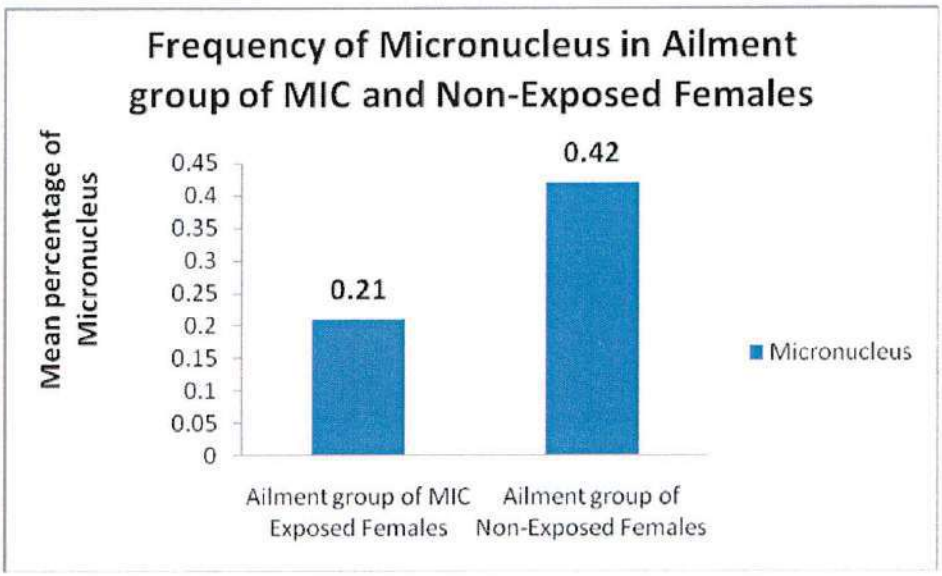


Fig 27: Showing Minutes

Table 34: Frequency of Micronucleus in Ailment group of MIC and Non-Exposed Females

Type of Aberrations	Ailment group of MIC Exposed Females (n=19) Mean ± SE	Ailment group of Non-Exposed Females (n=50) Mean ± SE
Micronucleus	0.210 ± 0.122	0.420 ± 0.086

p-value =0.192 (P<0.05) NS



Graph 27: Showing Mean Percentage of Micronucleus in Ailment group of MIC and Non-Exposed Females

Occurrence of Micronucleus in the Ailment Group of Exposed **(0.210 ± 0.122)** and Non-exposed **(0.420 ± 0.086)** Females was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 34**)

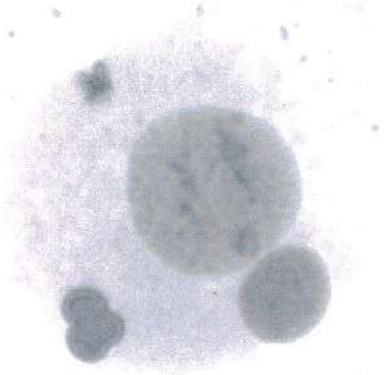
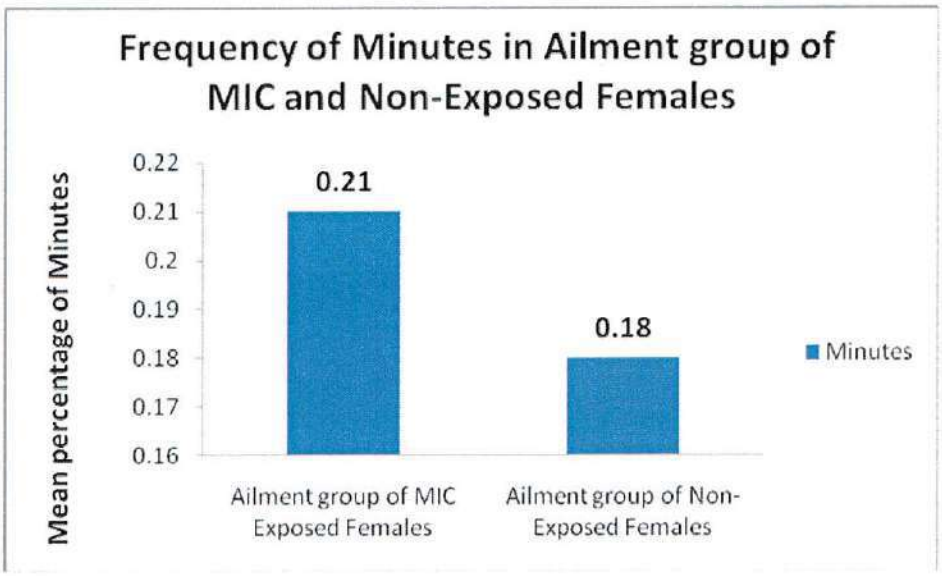


Fig 28: Showing Micronucleus

Table 35: Frequency of Minutes in Ailment group of MIC and Non-Exposed Females

Type of Aberrations	Ailment group of MIC Exposed Females (n=19) Mean ± SE	Ailment group of Non-Exposed Females (n=50) Mean ± SE
Minutes	0.210 ± 0.122	0.180 ± 0.068

p-value =0.820 (P<0.05) NS



Graph 28: Showing Mean Percentage of Minutes in Ailment group of MIC and Non-Exposed Females

Occurrence of Minutes in the Ailment Group of Exposed (**0.210 ± 0.122**) and Non-exposed (**0.180 ± 0.068**) Females was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 35**)

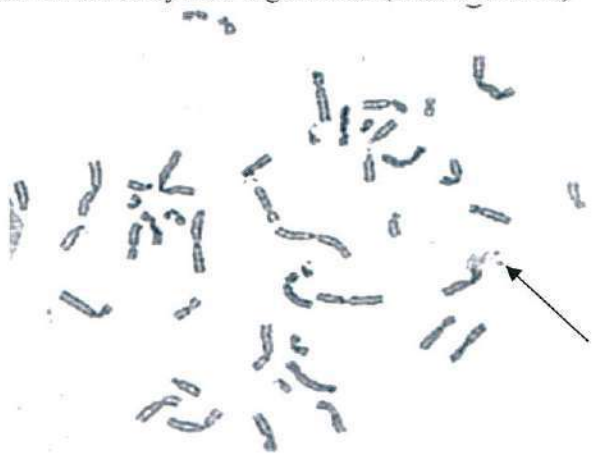
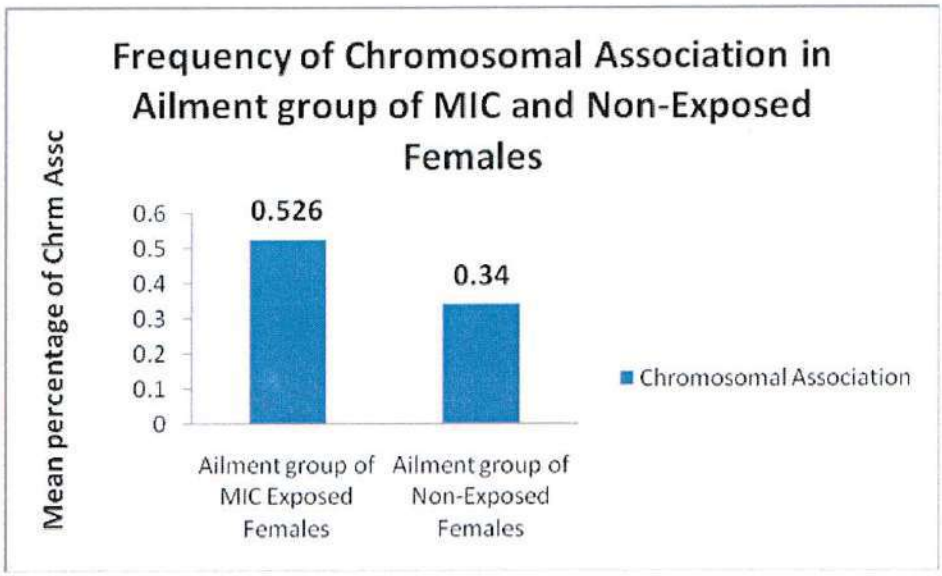


Fig 29: Showing Minutes

Table 36: Frequency of Chromosomal Association in Ailment group of MIC and Non-Exposed Females

Type of Aberrations	Ailment group of MIC Exposed Females (n=19) Mean ± SE	Ailment group of Non-Exposed Females (n=50) Mean ± SE
Chromosomal Association	0.526 ± 0.207	0.340 ± 0.080

p-value =0.3204 (P>0.05) NS



Graph 29: Showing Mean Percentage of Chromosomal Associations in Ailment group of MIC and Non-Exposed Females

Occurrence of Chromosomal Associations in the Ailment Group of Exposed (**0.526 ± 0.207**) and Non-exposed **(0.340 ± 0.080)** Females was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 36**)

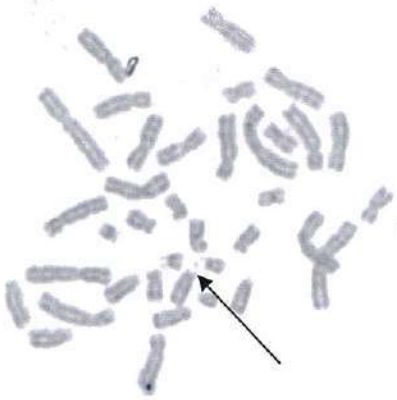
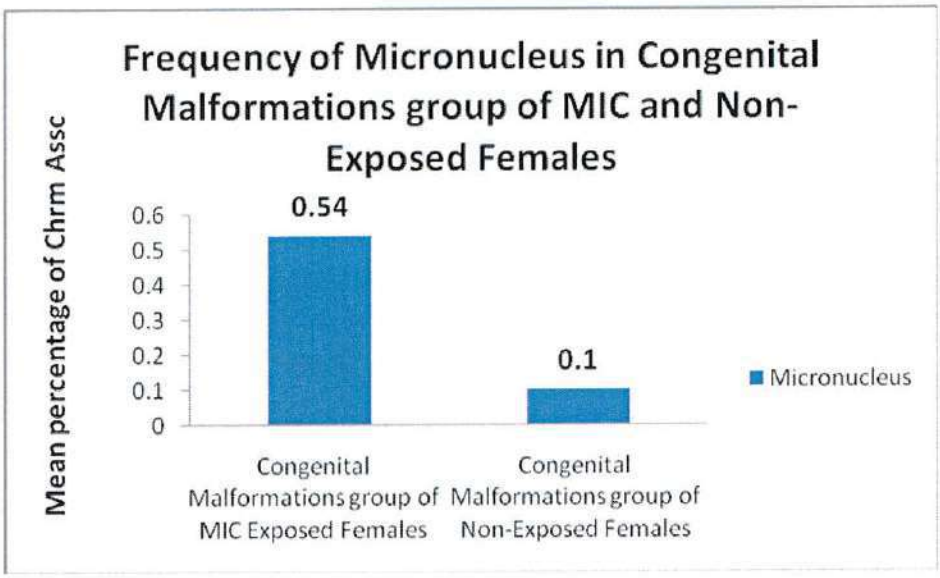


Fig 30: Showing Chromosomal Associations

Table 37: Frequency of Micronucleus in Congenital Malformations group of MIC and Non-Exposed Females

Type of Aberrations	Congenital Malformations group of MIC Exposed Females (n=37) Mean ± SE	Congenital Malformations group of Non-Exposed Females (n=50) Mean ± SE
Micronucleus	0.540 ± 0.540	0.100 ± 0.051

p-value =0.546 (P>0.05) NS



Graph 30: Showing Mean Percentage of Micronucleus in Congenital Malformations group of MIC and Non-Exposed Females

Occurrence of Micronucleus in the Congenital Malformations group of Exposed **(0.540 ± 0.540)** and Non-exposed **(0.100 ± 0.051)** Females was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 37**)

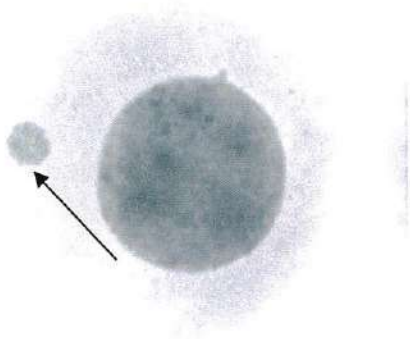
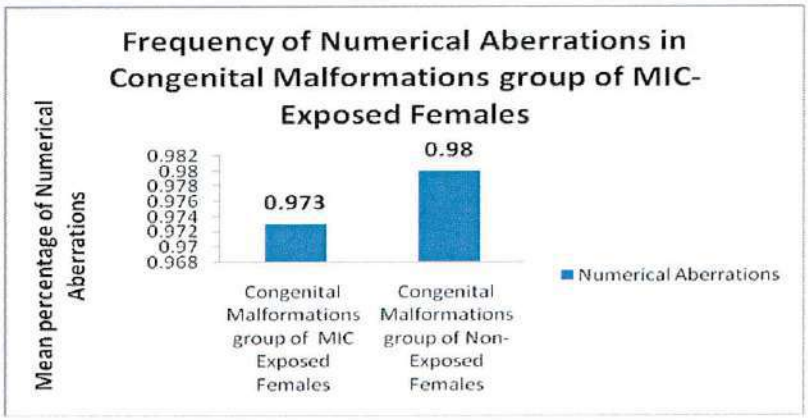


Fig 31: Showing Micronucleus

Table 38: Frequency of Numerical Aberrations in Congenital Malformations group of MIC-Exposed Females

Type of Aberrations	Congenital Malformations group of MIC Exposed Females (n=37) Mean ± SE	Congenital Malformations group of Non-Exposed Females (n=50) Mean ± SE
Numerical Aberrations	0.973 ± 0.026	0.980 ± 0.019

p-value =0.835 (P>0.05) NS



Graph 31: Showing Mean Percentage of Numerical Aberrations in Congenital Malformations group of MIC and Non-Exposed Females

Occurrence of Numerical Aberrations in the Congenital Malformations group of Exposed **(0.973 ± 0.026)** and Non-exposed **(0.980 ± 0.019)** Females was found to be non-significant and the difference between the means are statistically non-significant (**Table No. 38**)

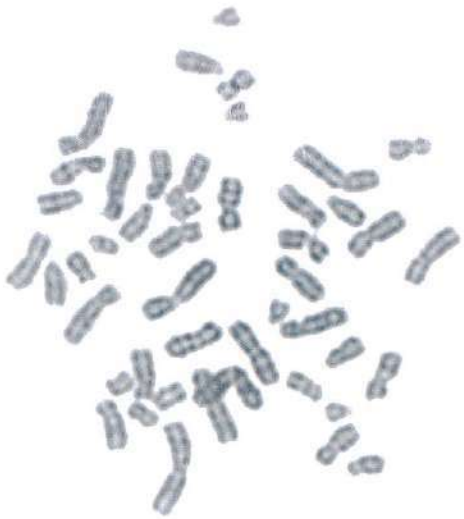
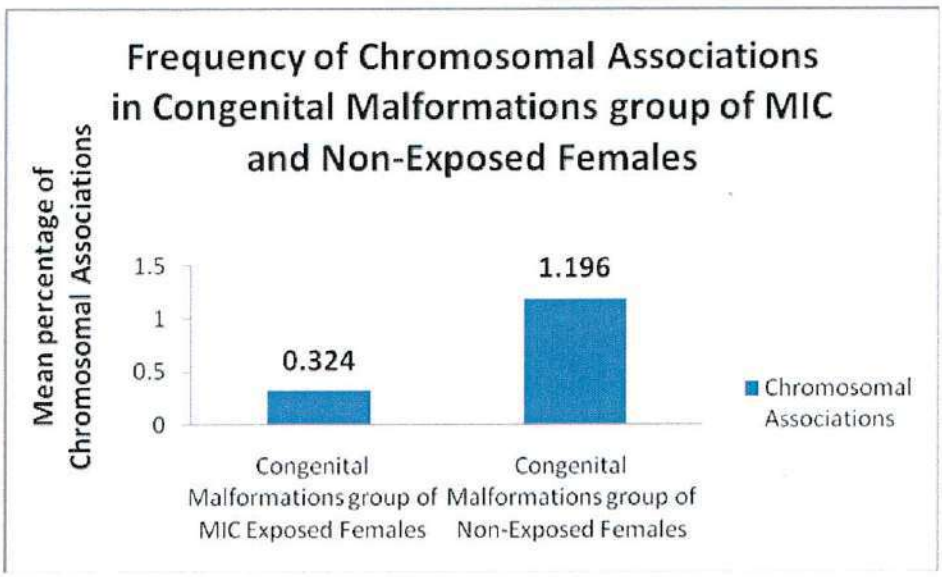


Fig 32: Showing Numerical Aberrations

Table 39: Frequency of Chromosomal Associations in Congenital Malformations group of MIC and Non-Exposed Females

Type of Aberrations	Congenital Malformations group of MIC Exposed Females (n=37) Mean ± SE	Congenital Malformations group of Non-Exposed Females (n=50) Mean ± SE
Chromosomal Associations	0.324 ± 0.122	1.196 ± 0.084

p-value =0.0001 (P<0.05) P***



Graph 32: Showing Mean Percentage of Chromosomal Associations in Congenital Malformations group of MIC and Non-Exposed Females

Table No. 39 revealed that the frequency of Chromosomal Associations in Congenital Malformation group of MIC exposed individuals is highly significant ($p < 0.05$) than the non-MIC exposed group.

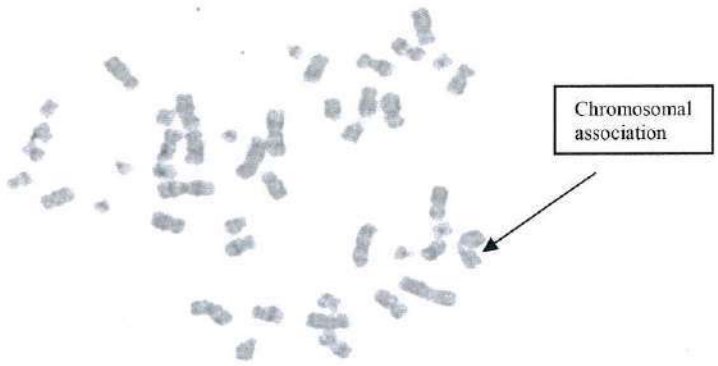


Fig 33: Showing Chromosomal Associations

The difference between mean percentage frequency of cells with acrocentric associations in the exposed males and the exposed females were recorded as highly significant (Table No. 12 and Table No. 39).

Comparing the groups on the basis of types of aberrations in males and females of exposed and non-exposed group were found to be highly significant in male population as compared with female population. Minutes, micronucleus, deletion, and acrocentric association is more significantly associated with MIC exposed male population than female population.

DISCUSSION

Structural chromosomal aberrations were found to be statistically higher in few of the exposed group than the non-exposed group. The role of some chemicals in inducing DNA double-strand breaks, if not repaired, results into structural chromosomal aberrations (Bryant, 1998; Natarajan, 1993; Obe *et al.*, 2002; Palitti, 1998 and Savage, 1998). The higher incidence of chromosomal aberrations in the exposed group than the control group is in concordance with the findings of Saxena *et al.*, (1988) and Ghosh *et al.*, (1990). Chromosome fragment and deletion, observed by them were also observed in the present study. In addition, micronucleus, minutes, pulverization, chromosomal associations, pre-centromeric division were also observed in the present study. However, the tri-radial and quadric-radial configurations of chromosomes, dicentric and rings as reported by Ghosh *et al.*, 1990) were not observed in the present study.

Measuring the frequency of chromosomal damage in humans exposed to environmental clastogens has been a priority in public health studies for decades and an increased level of chromosomal aberrations in population groups is currently interpreted as an evidence of genotoxic exposure and early biologic effects on DNA (Albertini *et al.*, 2000; Sram, 1981, Sram *et al.*, 1983 and Waters *et al.*, 1999). Significantly higher percentage of chromosomal aberrations in the exposed individuals can be attributed to their exposure to the toxic gases released at the time of the Bhopal gas tragedy. In general, the types and frequencies of induced chromosomal aberrations depend on the type of mutagen exposure and the cell cycle stage at the time of exposure.

Chromosome-type aberrations

Chromosome-type aberrations observed included chromosomal association, Premature Centromeric division and deletion. Chromosomal Association,

Premature Centromeric division and deletion were observed more in healthy male MIC exposed comparative with healthy female MIC exposed population; however, chromosomal association was significantly higher in Congenital Malformation of female non-exposed group than to female exposed group. In non-exposed population, it is insignificant. In first progeny of gas exposed male population, chromosomal associations is significantly high than first progeny of female exposed population. The percentage of chromosomal associations is more in congenital malformations of exposed and non-exposed than to other ailments group of male population. However, the female population of congenital malformation of non-exposed group has shown more percentage of chromosomal association than congenital malformation group of exposed group and there is no significant difference between the percentage of chromosomal association in female other ailments exposed and non-exposed group. There is no event of deletion and Premature Centromeric division observed in healthy female exposed and healthy non-exposed population (**Table No. 6 and 7**). A negligible percentage of deletions have been observed in MIC exposed individual and premature Centromeric division in non-exposed population. Chromosome-type aberrations are formed in the G_0 stage by a mechanism where apurinic or apyrimidinic sites are converted into strand breaks and misrepaired. (Natarajan and Obe, 1980; Carrano and Natarajan, 1988).

Chromosomal Associations (Acrocentric associations)

The occurrence of cells exhibiting acrocentric associations (ACA) was observed to be significantly higher in the healthy MIC-exposed group of male (**0.650 ±0.145**) than the healthy non-exposed female (**0.346 ± 0.078**). Significantly higher frequency of satellite associations (SA) of acrocentric chromosomes can be correlated to the Robertsonian translocations as has been reported by Goswami *et al.* (1990).

Tjio and Levan, 1956 first reported the satellites on human chromosomes. All D and G group chromosomes i.e. chromosome numbers 13, 14, 15, 21 and 22 have satellites. The satellite chromosomes tend to associate with their satellites directed towards each other and this phenomenon is known as satellite association (SA) or acrocentric association. These associations were first of all observed in the human metaphase chromosomes by Ferguson-Smith and Handmaker in 1961. The acrocentric chromosomes associate together due to the sticky nucleolar material.

Hassold and Jacob (1984) reported that one third of trisomies observed in spontaneous abortions and live births involve acrocentric chromosomes. A significant increase of the acrocentric associations was observed in the aborter couples and the recurrent spontaneous aborter couples by Anuradha *et al.*, (2002) and Kalpana *et al.,* (2004) respectively. Besides, Hansson, 1970 and Hansson and Mikkelsen, 1974 have reported evidence of an increased SA tendency in mothers of Down's syndrome children. The idea that high SA tendency may influence the risk of non-disjunction was strongly supported by the fact that the SA tendency of chromosome 21 of the parents with non-disjunction was significantly increased when compared with a control group. Correspondingly, the high frequency of satellite associations evidenced in the exposed individuals of the present study may also predispose them to non-disjunction resulting in abortions and birth of syndromes.

Chromatid-type aberrations

Chromatid type of aberrations observed in the study included minutes, fragments, micronucleus, etc. It is in agreement with the record of higher incidence of micronuclei in male mice subjected to MIC *in-vitro* (Kligerman *et al.,* 1987; Tice *et al.,* 1987). This could be due to the reason that genotoxic chemicals induce a wide variety of lesions in the DNA of lymphocytes in different proportions. Most of the chemically induced aberrations are formed only during the DNA synthesis

phase, probably due to replication errors. Exposure to chemical mutagens induces lesions in the DNA of lymphocytes, most of which are removed by cellular repair processes. The non-repaired fraction of lesions gives rise to chromatid-type aberrations during S phase, when the lymphocytes are treated with mitogen *in vitro.* (Natarajan and Obe, 1980; Carrano and Natarajan, 1988). The frequency of chromatid type aberrations like micronucleus, minutes, in the healthy MIC exposed male group (0.60±0.123) was observed to be higher than the healthy non-exposed male group (0.130±0.039).

Double minutes

Double minutes were recorded as significantly higher in the healthy male exposed individuals. These are probably small deletions which may be interstitial or terminal. These may be associated with carcinogenesis if deletions include tumor suppressor genes if any.

Acentric fragments

Incidence of acentric fragments was found to be higher in the exposed group than the controls. As these fragments do not have centromeres, they will not be attached to the spindle in the metaphase and will be consequently lost in the subsequent cell divisions.

Both the chromosome-type and the chromatid-type aberrations were found to be significantly increased in the exposed males than the exposed females. Unstable chromosomal aberrations induced in lymphocytes of atom bomb survivors of Hiroshima and Nagasaki could be detected even after about 40 years (Awa, 1983) indicating the presence of long-lived lymphocytes in their peripheral blood. Likewise, there is every possibility of the presence of such lymphocytes circulating in the peripheral blood of Bhopal gas tragedy survivors indicating a long term genotoxic effect.

Chromosomal aberrations and gender

Considering the healthy non-exposed and other group of non-exposed as well as the healthy exposed group and other group of exposed population on the basis of gender, the incidence of chromosomal aberrations in the exposed male of all the group was found to be statistically elevated as compared to the control females. Although, the incidence of chromosomal association was higher in non-exposed congenital females than males, the difference between the means was also statistically significant. These findings are supported by study done by Ghosh *et al.,* 1990. However, the trend is different in case of the healthy exposed male individual to healthy exposed female where the chromosomal association is significantly higher in the healthy exposed male individuals.

When compared to the overall aberrations, the observations suggest that males are more susceptible to chromosomal damage than the females.

Aberrant cell divisions

Early centromeric divisions were recorded as aberrant cell divisions and were observed to be statistically higher in the male group as compared to the females. Premature centromeric division (PCD) of chromatids has been described by Fitzgerald (1975), Galloway and Buckton (1978) in aged woman in association with aneuploidy of X-chromosome. Vig (1984) proposed a hypothesis that PCD may result in non-disjunction by impairing the attachment of prematurely separated centromeres to spindle fibers. Miller *et al.*, (1990) reported association of PCD with various aneuploidies in a high percentage, supporting a functional relationship between disturbances in the mechanism of centromere separation and chromatid separation at cell division.

The increased percentage of early centromeric divisions in the exposed group may result in spontaneous abortions and birth of syndromes (Bajnoczky and Meher, 1988).

Slightly higher percentage of chromosomal aberrations in the exposed population is a validation of the chromosomal instability. The chromosomal aberrations may act as the intermediate processes in the pathway of the progression of any genetic disorder like cancer. An increased risk of cancer in healthy individuals with high levels of chromosomal aberrations in peripheral blood lymphocytes has been described in recent epidemiological studies carried out in Nordic countries and Italy by Bonassi *et al.,* (2000) and Bonassi *et al.,* (2008).

CONCLUSION

Circulating lymphocytes are the functional indicator of the genotoxic effects induced by chemical exposures. Many type of diseases like cancers are due to lymphocyte-specific instability. Several biological endpoints may be monitored to study the consequences of chemical exposures. The cytogenetic endpoints were demonstrated long lived lymphocytes existed among the chemical disaster survivors of Bhopal gas tragedy even after 33 years.

Significantly elevated chromosomal association, micronucleus, minutes and deletion in the healthy exposed male individuals actually emerged as a noteworthy cytogenetic endpoints that indicates link between MIC exposure and possibility of disease risk. This study highlights the genotoxic affect of MIC exposure healthy male and female 1st progeny 2nd progeny etc. and suggests the urgent need for health surveillance and risk management to reduce the genetic damage in Bhopal MIC affected population. As a prophylaxis, the affected population can be counseled and motivated through interventional programs for healthy life styles, dietary habits to minimize the disease risk and tobacco cessation programs to withdraw the tobacco habits.

Though the 1st progeny and 2nd progeny have not shown any statistically significant chromosomal aberration, but the major strength of the present study is the inclusion of the offspring of two generations of the MIC exposed survivals, which allows us to predict the impending risk and trans-generational effects. However, this study has several limitations. A limitation was its enrollment of 1st and 2nd generation individuals, their un-willingness to participate in the study, excuses of religious reasons and many of the survivors are non responders, and not allowed their progeny to participate in the study. It was also found that during the time of counseling and pedigree charting many of the individuals who carry gas victim's cards found to be fake.

Another possible bias is the selection criterion used in the study did not classify the subjects according the exposure status and affected area such as severely and moderately exposed to MIC.

To support our findings there must be population based monitoring specially for 1st and 2nd generation so that a larger subject of such offspring's can be studied to conclude the cytogenetic endpoints. Which are needed for the two substantiates trans-generational effects in the offspring's. This study offers future insight into clinical setting which could be useful to researchers and clinicians for disease risk management, particularly among the MIC affected population.

Taking into account of all above results biomonetering study conclude long term effects specially on the basis of elevated chromosomal associations, micronucleus, minutes, and deletion frequency suggest disease risk including cancer in the MIC affected population.

We also conclude that the exposed population is more vulnerable to the genetic diseases and must be counseled for dietary and lifestyle changes to minimize the risk of developing any kind of disease/genetic disorder. Continued monitoring of the MIC affected population with cytogenetic follow-up protocols will be helpful in the disease risk management.

SUMMARY

1. The present study aimed at investigating the cytogenetic status in methyl isocyanate exposed population and their progeny. Bhopal gas tragedy of 1984 is the world's worst chemical industrial disaster that resulted in the death of thousands of residents within days due to their exposure to the toxic gases released from the Union Carbide's pesticide plant. There have been many studies reporting the genotoxicity and mutagenicity in the exposed population.

2. Besides genotoxicity, studies have been carried out regarding the reproductive disorders in the exposed population revealing still births and fetal loss. The peri-natal and the neonatal mortalities have also been found to be significantly higher in the affected area in earlier studies. However, there has been no study regarding the long term genotoxicity of this disaster. Hence, the present study was undertaken to assess the cytogenetic damage in the survivors of gas exposed victims as well in the offspring.

3. The study was approved for two years and extension had been given for one year (Oct 2013 to Oct 2016).

4. Total target of subjects to be completed were 1200 individuals. Total 2169 individuals (male = 1150, female = 1019) were identified out of which 1029 were randomized (male = 483, female = 399) . These subjects were identified from the Jawaharlal Nehru Cancer Hospital Registration followed by Pedigree charted. The exposed individuals age group between 29-59 years and non-exposed individuals age between 18-59 years. The exposed individual living within radius of 1.5 km around the Union Carbide's pesticide plant at Bhopal. The non-exposed individuals were from Bhopal and its neighboring districts.

5. Total 100 males and 93 females samples were processed from normal healthy MIC-exposed group, 100 males and 72 females were from normal healthy non-exposed group, 72 males and 59 females from 1st Progeny of MIC-exposed individuals, 27 males and 19 females from second progeny of MIC-exposed individuals, 50 males and 37 females from Congenital Malformations, MIC exposed group, 50 male and 50 female from non-

exposed group, 38 males and 19 females from other ailments MIC-exposed group, 46 males and 56 females from non-exposed group. Therefore, total 882 subjects samples processed out of 1200. All the subjects were non-smokers non-smokers and not employed in any type of chemical industry or a radiation department per their declaration.

6. Heparinized blood samples were collected from the study subjects as well as the controls after their informed consent. The chromosomal anomalies were assessed on the metaphase plates of 72 hour standard lymphocyte cultures. Fifty well spread metaphases per sample were observed. For the study of acrocentric associations, the criteria used by Hansson (1970) were applied. The statistical analysis was done by applying Student's't' test.

7. The aberrant metaphases in mean percentage of male in the MIC exposed healthy individuals were observed to be significantly higher than the non-exposed healthy male individuals. Structural as well as numerical chromosomal aberrations were recorded in the study subjects. Structural aberrations observed comprised of both chromosome-type and chromatid-type aberrations. The chromosomal type aberrations included chromosomal association, deletion and precentromeric division. The chromatid type aberrations included fragments, micronucleus, and minutes. Both the chromosome-type and the chromatid-type aberrations were found to be significantly higher in healthy exposed male individual as compared to the non-exposed healthy male individuals.

8. On the basis of gender the male individuals of all the groups of healthy MIC exposed males, first progeny and second progeny and other ailments of exposed and non-exposed groups have shown chromosomal aberrations statistically higher as compare to the female. The incidence of chromosome type aberrations especially chromosomal association is more in female non-exposed congenital malformation than male exposed and non-exposed congenital malformation.

9. The frequency of chromatid type of aberrations in the healthy exposed males is significantly higher than in the healthy non-exposed males.

10. The frequency of chromosomal association, micronucleus, minutes and deletion are significantly higher in healthy male exposed individuals than non-exposed healthy males.

11. Premature Centromeric division, polyploidy, pulverization was negligible in female subjects as compared to male individuals.

12. The mean percentage of chromosomes showing early centromeric divisions was statistically observed in male population.

13. The Chromosomal anomalies have been observed in the healthy exposed individuals in the present study which indicates that the genomic instability persists as a long term effect of the Bhopal gas tragedy. Higher percentage of chromosomal anomalies like chromosomal association, micronucleus, minutes and deletion in the exposed healthy male population may act as the intermediate processes in the pathway of the progression of any genetic disorder like cancer. The results of the present study demonstrate long-term genotoxicity owing to the Bhopal gas tragedy persisting even after more than three decades. The incidence of chromosomal aberrations after such a long period indicates persistent clastogenic effects of the exposure.

14. The exposed individuals having higher incidence of chromosomal aberrations can be counseled for dietary and life style changes to minimize the risk of developing any kind of genetic disorder.

15. Such studies will be useful in counseling of the affected people so as to avoid genetic risks.

16. In summary, although this study has some limitations our results suggest that MIC has performed genotoxic affect in the healthy exposed individual which may enhance long term disease risk in the affected population. Several life style habits, particularly tobacco may also strengthen the risk. The affected population investigated in this study is highly

vulnerable to cancer, thus need to change their lifestyle habits. There is a need for disease prevention and control strategies can be achieved further counseling the affected population for healthy dietary and lifestyle habits to minimize the disease risk.

References

Klaassen, C.D. (2001). Casarett and Doull's Toxicology: the Basic Science of Poisons, *Sixth edition.* Mc. Graw-Hill, New York.

Greenberg, M.I., Hamilton, R.J., Philips, S.D. and McCluskey, G.J. (Eds). (2003). Occupational, *Industrial and Environmental Toxicology,* Second edition. Mosby, Inc, Philadelphia, Pennsylvania.

Chauhan, A.J. and Jhonston, S.L. (2003). Air pollution and infection in respiratory illness. *British Medical Bulletin*, 68:1, 95-112.

Johnson, S., Sahu, R., Jadon, N. and Duca, C. (2003). Contamination of soil and water inside and outside the Union Carbide India Limited, Bhopal. Centre for Science and Environment, New Delhi.

Winder, C. (2004). Toxicology of gases, vapours and particulates. In: *Occupational Toxicology, Second edition.* Winder, C. and Stacey, N.H. (Eds). CRC Press, Boca Raton, 399-424.

Singh, M.P. and Ghosh, S. (1987). Bhopal Gas Tragedy: Model Simulation of the Dispersion Scenario. *Journal of Hazardous Materials*, 17:1-22.

Dhara, V.R. and Gassert, T.H. (2002). The Bhopal syndrome: persistent questions about acute toxicity and management of gas victims. *International Journal of Occupational and Environ Health*, 8: 380-6.

Stringer, R., Labunska, I., Brigden, K. and Santillo, D. (2002). Chemical stockpiles at Ubion Carbide India Limited in Bhopal: an investigation. Greenpeace Research Laboratories Technical Note.

Department of Relief & Rehabilitation. (1989). Bhopal gas tragedy. Government of Madhya Pradesh. Bhopal, India.

Sriramachari, S. (2004) The Bhopal gas tragedy: an environmental disaster. *Current Science*, 86: 905-20.

Indian Council of Medical Research. (2004). Report on Health Effects of the Toxic Gas Leak from the Methyl Isocyanate Plant in Bhopal.

Andersson, N., Muir, M.K., Mehra, V. and Salmon, A.G. (1988). Exposure and response to MIC: results of a community based survey in Bhopal. *BJIM,* 45:469-475.

Goswami, H.K. (1986). Cytogenetic effects of methyl iso cyanate exposure in Bhopal. *Hum. Genet*, 74: 42-45.

Varma, D.R. (1987). Epidemiological and experimental studies on the effects of methyl isocyanate on the course of pregnancy. *Environ Health Perspect,* 72:151-55.

Saxena, A.K., Singh, K.P., Nagle, S.L., Gupta, B.N., Ray, P.K., Srivastav, R.K., Tewari, S.P. and Singh, R. (1988). Effect of exposure to toxic gas on the population of Bhopal: Part IV-Immunological and Chromosomal Studies. *Ind J Exp Biol.* 26:173-176.

Ghosh, B.B., Sengupta, S., Roy, A., Maity, S., Ghosh, S., Talukdar, G. and Sharma, A. (1990). Cytogenetic studies in human populations exposed to gas leak at Bhopal, India. *Environmental Health Perspectives*, 86: 323-326.

Ganesh, N., Sanyal, B., Panday, R. K., Aarti, K. and Gupta, N. (2005). Cancer patterns among MIC gas survivors and their offsprings. *Health Administrator*, XVII (1): 50-58.

Varma, D.R., Ferguson, J. and Alarie, Y. (1987). Reproductive toxicity of methyl isocyanate in mice. *J Toxicol Env Hlth.* 21:265-275.

Bhandari, N.R., Syal, A.K., Kambo, I., Nair, A., Beohar, V., Sexena, N.C., Dabke, A.T., Agarwal, S.S. and Saxena, B.N. (1990). Pregnancy outcome in women exposed to toxic gas at Bhopal, *Indian J Med. Res.,* 92, 28-33.

Fortun, K. (2001). Advocacy after Bhopal. Chicago, University of Chicago Press. 259.

Shrivastava, P. (1987a). Bhopal: Anatomy of a Crisis. Cambridge, MA, Ballinger Publishing. 184.

Sapna Johnson, Ramakant Sahu, Nimisha Jadon,s Clara Duca (2009). Contamination of soil and water inside and outside the Union Carbide India Limited, Bhopal.

Chouhan, T.R. (2004). Bhopal: The Inside Story. Other India Press, India and The Apex Press, USA.

Shrivastava, P. (1987b). Managing Industrial Crisis. New Delhi , Vision Books; 1987:196.

MacKenzie, D. (2002). Fresh evidence on Bhopal disaster. *New Scientist*, 19(1).

Subramanian, A. (1985). The dangers of diagnostic delay. BusinessIndia August 12-25:128-133.

Worthy, W. (1985). Methyl isocyanate: the chemistry of hazard. *Chem. Eng. News*, Chicago: 27-33

Anonymous. (2005). Ontarion air standards for methyl isocyanate. Standards Development Branch.

Indian Council of Medical Research. Annual Report, Bhopal Gas Disaster Research Centre. Bhopal: India; 1991.

Andersson, N., Muir, M.K., Ajwani, M.K., Mahashabde, S., Salmon, A. and Vaidyanathan, K. (1986). Persistent eye watering among Bhopal survivors. *Lancet,* 2:1152.

Raizada, J.K. and Dwiwedi, P.C. (1987). Chronic ocular lesions in Bhopal gas tragedy. *Ind J Ophthalmol.* 35:453-455.

Khurrum, M.A. and Ahmad HS. (1987). Long term follow-up of ocular lesions of methyl isocyanate gas disaster in Bhopal. *Ind J Ophthal,* 35(3):136-7.

Salmon, A.G., Muir, M.K. and Andersson, N. (1985). Acute toxicity of methyl isocyanate: a preliminary study of the dose response for eye and other effects. *Br J Ind Med.* 42:795-798.

Andersson, N., Ajwani, M.K., Mahashabde, S., Tiwari, M.K., Muir, M.K., Mehra, V., Ashiru, K. and Mackenzie, C.D. (1990). Delayed eye and other consequences from exposure to methyl isocyanate: 93% follow up of exposed and unexposed cohorts in Bhopal. *Br J Ind Med,* 47:553-558.

Mishra, U.K. and Nag D. (1988). A clinical study of toxic gas poisoning in Bhopal, India. *Ind J Exp Biol,* 26:201-204.

Rastogi, S.K., Gupta, B.N., Husain, T., Kumar, A., Chandra, S., Ray, P.K. (1988). Effect of exposure to toxic gas on the population of Bhopal: Part II-Respiratory Impairment. *Ind J Exp Biol,* 26:161-4.

Irani, S.F. and Mahashur, A.A. (1986). A survey of Bhopal children affected by methyl isocyanate gas. *J Postgrad Med (India),* 32(4):195-8.

Kamat, S.R., Patel, M.H., Kolhatkar, V.P., Dave, A.A. and Mahashur, A.A. (1987). Sequential respiratory changes in those exposed to the gas leak at Bhopal. *Ind J Med Res*, 20- 38.

Vijayan, V.K. and Sankaran, K. (1989). Relationship between lung inflammation, changes in lung function and severity of exposure in victims of the Bhopal gas tragedy. *Eur Respir J,* 9:1977-82.

Karol, M.H., Taskar, S., Gangal, S., Rubanoff, B.F. and Kamat, S.R. (1987). The antibody response to methyl isocyanate: Experimental and clinical findings. *Environ. Health Perspect.* 72:167-173.

Boorman, G.A., Uraih, L.C., Gupta, B.N., Bucher, J.R. (1987). Two hour methyl isocyanate inhalation and 90 day recovery study in B6C3F1 mice. *Environ Health Perspect,* 72:5-11.

Fowler, E.H. and Dodd, D.E. (1987). Respiratory tract changes in guinea pigs, rats and mice following a single six-hour exposure to methyl isocyanate. *Environ Health Perspect,* 72:107-114.

Tice, R.R., Luke, C.A. and Shelby M.D. (1987). Methyl isocyanate: An evaluation of in vivo cytogenetic activity. *Environmental Mutagenesis*, (9)1: 37–58.

Mason, J.M., Zeiger, E., Haworth, S., Ivett, J. and Valencia, R. (1987). Genotoxicity studies of methyl isocyanate in salmonella, Drosophila and cultured Chinese hamster ovary cells. *Environ Mutagen,* 9:19-28.

Meshram, G.P. and Rao. K.M. (1988). Cytogenetic activity of methyl isocyanate in vivo in the mouse micronucleus test. *Toxicology Letter,* (42) 1: 65-71.

Shelby, M.D., Allen, J.W., Caspary, W.J., Haworth, S., Ivett, J., Kligerman, A., Luke, C.A., Mason, J.M., Myhr, B., Tice, R.R., Valencia, R. and Zeiger, E. (1987). Results of in vitro and in vivo genetic toxicity tests on methyl isocyanate. *Environ Hlth Perspect,* 72:181-185.

Mishra, P.K., Bhargava, A., Raghuram, G.V., Gupta, S., Tiwari, S., Upadhyaya, R., Jain, S.K. and Maudar, K.K. (2009a). Inflammatory response to isocyanates and onset of genomic instability in cultured human lung fibroblasts. *Genetics and Molecular Research,* 8 (1): 129-143.

Mishra, P.K., Samarth, R.M., Pathak, N., Jain, S.K., Banerjee, S. and Maudar, K.K. (2009c). Bhopal Gas Tragedy: review of clinical and experimental findings after 25 years. *International Journal of Occupational Medicine and Environmental Health*, 22(3):193 – 202.

Stahl, W., Lenhardl, S., Przybylski, M. and Eisenbrand, G. (1992) Mechanism of glutathione-mediated DNA damage by the antineoplastic agent 13-bis(2-chloroethyl)-N-nitrosourea. *Chem. Res. Toxicol.,* 5:106-109.

Tamura, N., Aoiki, K. And Lee, M.S. (1992). Selective reactivities of isocyanates towards DNA bases and genotoxicity of methylcarbamoylation of DNA. *Mutat Res*; 283:97-106.

Bonassi, S., Hagmar, L., Stromberg, U., Montagud, A., Tinnerberg, H., Forni, A., Heikkila, P., Wanders, S., Wilhardt, P., Hansteen, I., Nudsen, L.E. and Norpa, H. (2000). Chromosomal aberrations in lymphocytes predict human cancer independently from exposure to carcinogens. *Cancer Res,* 60, 1619–25.

Hagmar, L., Stromberg, U., Bonassi, S., Hansteen, I.L., Knudsen, L.E., Lindholm, C., and Norppa, H. (2004). Impact of types of lymphocyte chromosomal aberrations on human cancer risk results from Nordic and Italian Cohorts. *Cancer Research,* 64: 2258–2263.

Rossner, P., Boffetta, P., Ceppi, M., Bonassi, S., Smerhovsky, Z., Landa, K., Juzova, D. and Sram, R.J. (2005). Chromosomal aberrations in lymphocytes of healthy subjects and risk of cancer. *Environmental Health Perspective,* 113 (5): 517-520.

Bonassi, S., Norppa, H., Ceppil, M., Stromberg, U., Vermeulen, R., Znaor, A., Antonina, C.W., Fabianova, Fucic, A., Gundy, S., Hansteen, I.L., Knudsen, L.E., Lazutka, J., Rossner, P., Sram, R.J. and Boffettal, P. (2008). Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: results from a pooled cohort study of 22 358 subjects in 11 countries. *Carcinogenesis,* 29(6): 1178–1183.

Awa, A.A. (1997). Analysis of chromosome aberrations in atomic bomb survivors for dose assessment: Studies at the radiation effects research Foundation

from 1968 to 1993. *Radiation Injury and the Chernobyl Catastrophe.* Stem Cells;15 (suppl 2):163-173.

Ichimaru, M., Tomonaga, M., Amenomori, T. and Matsuo, T. (1991). Atomic bomb and leukemia: A Review of forty-five years study of Hiroshima and Nagasaki atomic bomb survivors. *J. Radiat. Res.*: 162-169.

Bloom, A.D., Neriishi, S., Kiamada, N., Iseki. T. and Keehn, R. J. (1966) Cytogenetic investigation of survivors of the atomic bombings of Hiroshima and Nagasaki. *Lancet* II: 672.

Bloom, A.D., Awa, A.A., Neriishi, S, Honda, T. and Archer, P.G. (1967). Chromosome aberrations in leucocytes of older survivors of the atomic bombings of Hiroshima and Nagasaki. *Ibid.* II :802.

Lazutka, J.R. and Rimdeika, G.J. (2006). Influence of the Chernobyl accident on the frequency of chromosomal damage and health status of Lithuanian clean-up workers. *Biolgija*, (Suppl 1:36–40.

Prakash, P. (1986). Effect of Bhopal gas leak on women's reproductive health. Published by Medico Friend Circle at IBCS. Bombay, India.

Schwetz, B.A., Adkins, B. Jr., Harris, M., Moorman, M. and Sloan, R. (1987). Methyl Isocyanate: Reproductive and developmental toxicology studies in Swiss mice. *Environ Health Perspect.* 72:147-150.

Varma, D.R., Guest, I., Smith, S. and Mulay, S. (1990). Dissociation between maternal and fetal toxicity of methyl isocyanate in mice and rats. *J Toxicol Env Hlth,* 30:1-14.

Mishra, P.K., Jatawa, S.K., Raghuram, G.V., Pathak, N., Jain, A., Tiwari, A., Varshney, S. and Maudar, K.K. (2009b). Correlation of aberrant expression of p53, Rad50, and cyclin-E proteins with microsatellite instability in gallbladder adenocarcinomas. *Genet Mol Res.*, 6;8(4):1202-10.

Dikshit, R.P. and Kanhere, S. (1999). Cancer patterns of lung, oropharynx and oral cavity cancer in relation to gas exposure at Bhopal. *Cancer Causes & Control,* 10:627-636.

Moorhead, P.S, Nowell, P.C, Mellman, W.J, Battips, D.M. and Hungerford, D.A. (1960). Chromosome preparation of leukocytes cultured from human peripheral blood, *Exp Cell Res*, 20: 613-616.

Seabright, M. (1971). A rapid banding technique for human chromosomes. *Lancet II*, 971- 972.

Gustashaw K.M. (1991). Chromosome stains. In *The ACT Cytogenetics Laboratory Manual* 2nd ed. M.J. Barch. The Association of Cytogenetic Technologists, Raven Press, New York.

Hansson, A. (1970). Difference in the satellite association's pattern in human population, Hereditas. 66 21-30.

Bryant, P.E. (1998). Mechanisms of radiation-induced chromatid breaks, *Mutat Res*, 404: 107–111.

Obe, G., Pfeiffer, P., Savage, J.R., Johannes, C., Goedecke, W. and Jeppesen, P. (2002). Chromosomal aberrations: formation, identification and distribution. *Mutat Res,* 504, 17–36.

Palitti, F. (1998). Mechanisms of the origin of chromosomal aberrations. *Mutat Res,* 404, 133–137

Savage, J.R. (1998). A brief survey of aberration origin theories. *Mutat Res,* 404, 139–147.

Albertini, R.J, Anderson, D., Douglas, G.R., Hagmar, L., Hemminki, K., Merlo, F., Natarajan, A.T., Norppa, H., Shuker, D.E., Tice, R., Waters, M.D. and Aitio, A. (2000). IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. International Programme on Chemical Safety. *Mutat Res,* 463, 111–172.

Sram, R.J. (1981). Cytogenetic analysis of peripheral lymphocytes as a method for monitoring environmental levels of mutagens. In: Industrial and Environmental Xenobiotics: Metabolism and pharmacokinetics of Organic Chemicals and Methods. (Gut I, Cikrt M, Plaa GL, eds). *Berlin:Springer Verlag*, 187–193.

Sram, R.J., Landa, L. and Samkova, I. (1983). Effect of occupational exposure to epichlorohydrin on the frequency of chromosome aberrations in peripheral lymphocytes. *Mutat Res,* 122, 59–64.

Waters, M.D., Stack, H.F. and Jackson, M.A. (1999). Genetic toxicology data in the evaluation of potential human environmental carcinogens. *Mutat Res,* 437:21–49.

Natarajan, A.T. and Obe, G. (1980). Screening human population for mutations induced by environmental pollutanats, use of human lymphocyte system. *Ecotoxicol. Environ. Safety*, 4: 468.

Carrano, A.V. and Natarajan, A.T. (1988). International considerations for population monitoring using cytogenetic techniques, Commission for Protection against Environmental Mutagens and Carcinogens, *Mutat. Res*, 204, 379-406.

Goswami, H.K., Chandolkar, M., Bhattacharya, K., Vaidyanath, J., Parmar, D., Sengupta, S., Patidar, S.L., Sengupta, L.K., Goswami, R., and Sharma, P.N. (1990). Search for chromosomal variations among gas exposed persons in Bhopal. *Hum. Genet*, 84: 172-176.

Tjio, J.H. and Levan, A. (1956). The chromosome number of man. *Hereditas*, 42:1-6.

Ferguson-Smith, M., Handmaker, S.D. (1961). Observations on the satellited human chromosomes. *Lancet*, I: 638-640.

Hassold, T.J. and Jacob, P.A. (1984). Trisomy in man. *Ann Rev Genet*, 18, 69-97.

Anuradha, N., Satyanarayana, M.and Manjunatha, K.R. (2002). Satellite associations in recurrent aborters. *Int J Hum Genet,* 2(1): 61-64.

Kalpana, V.L., Satyanarayana, M., Prabhaker, S. and Sarvani R. (2004). Chromosomal Instability in Recurrent Spontaneous Aborters. *Int J Hum Genet,* 4(1): 31-36.

Hansson, A. and Mikkelson, M. (1974). An increased tendency to satellite association of human chromosome 21: a factor in the etiology of Downs Syndrome – IRCS. *Anat Pediat Psychiat*, 2, 1617.

Kligerman, A. D., Campbell, J. A., Erexson, G. A., Allen, J. W., and Shelby, M. D. (1987). SCE in lung and peripheral blood lymphocytes of mice exposed to MIC by inhalation. *Environ. Mutagen,* 9:29-36.

Awa, A.A. (1983). Chromosome damage in atomic bomb survivors and their offspring-Hiroshima and Nagasaki. In: *Radiation Induced Chromosome damage in Man* (Ishihara, T. and Sasaki, M.S., eds.).Alan R. Liss Inc., New York, 235-275.

Fitzgerald, P.H. (1975). A mechanism of X chromosome aneuploidy in lymphocytes of aging women. *Humangenetik,* 28: 153-158.

Galloway, S.M. and Buckton, K.E. (1978). Aneuploidy and aging: Chromosome studies on a random sample of the population using G-banding. *Cytogenet Cell Genet,* 20: 78-95.

Vig, B.K. (1984). Sequence of centromere separation another mechanism for the origin of nondisjunction. *Hum Genet,* 66: 239-243.

Miller, K., Muller, W. and Winkler, L. (1990). Mitotic disturbance associated with mosaic aneuploides. *Hum Genet,* 84: 361-364.

Bajnoczky, K. and Meher, K. (1988). Parental Centromere separation sequence and aneuploidy in the offspring. *Hum Genet,* 78: 286-288.

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List of Abbreviations

WHO	**World Health Organization**
ACA	**Acrocentric association**
WBC	**White Blood Corpuscles**
MIC	**Methyl Isocyanate**
INF	**Infertility**
HI	**Healthy Individual**
DDW	**Double Distilled Water**
CM	**Congenital Malformations**
PP	**Polyploidy**
CM W Exp.	**Congenital Malformed with exposure**
MN	**Micronucleus**
MIN	**Minute**
DL	**Deletion**
NUM ABR	**Numerical Aberration**
FRG	**Fragment**
CHR ASSC	**Chromosomal Association**
PCD	**Premature Centromeric Division**
PP	**Polyploidy**
PUL	**Pulverized Chromosomes**
ECD	**Early centromeric Division**
CMI	**Cell Mediated Immunity**
PHA	**Phytohemagglutinin**
ICMR	**Indian Council of Medical Research**
RC	**Ring Chromosome**
PCD	**Premature centromeric Division**
SA	**Satellite Association**
TAM	**Total Aberrated Metaphase**
ISCN	**International System of Chromosomal Nomenclature**

DNA	**Deoxyribose nucleic acid**
ACF	**Acentric Fragments**
UCC	**Union Carbide Corporation**
UCIL	**Union Carbide India Limited.**