FINAL PROJECT REPORT

PROJECT TITLE

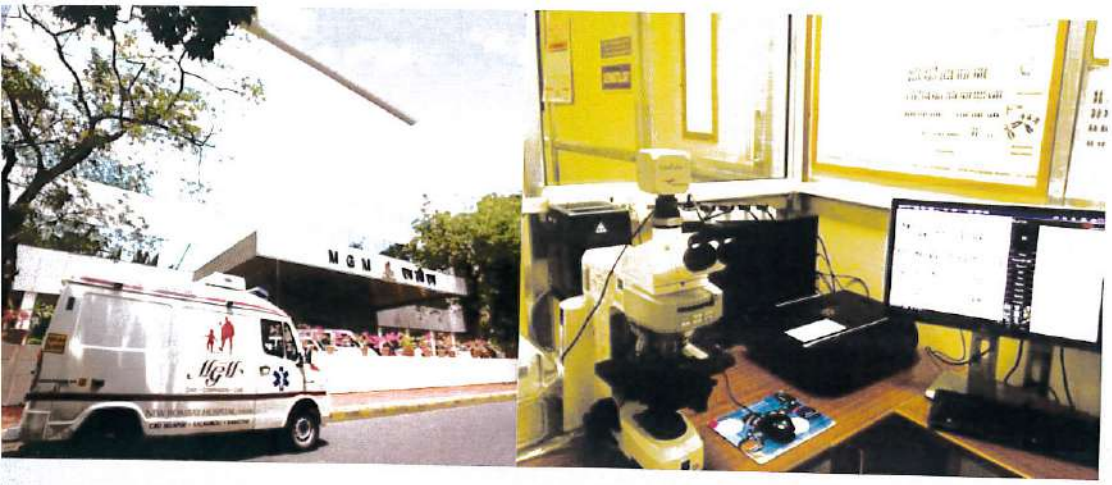
'LONG TERM GENETIC EFFECT(S) OF MIC GAS, IF ANY, ON THE BHOPAL POPULATION EXPOSED IN DECEMBER 1984'

NO. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

DR. BANI BANDANA GANGULY, Ph.D., FICMCH

PRINCIPAL INVESTIGATOR

MGM CENTER FOR GENETIC RESEARCH & DIAGNOSIS
MGM NEW BOMBAY HOSPITAL
VASHI, NAVI MUMBAI 400703, MAHARASHTRA, INDIA
TEL. 91 22 61526527; 61526666 Extn. 216/227; FAX: 91 22 27824618
MOBILE: 91 9869214680
E-MAIL: mgmgeneticlab@yahoo.com; center.genetics@gmail.com



New Bombay Hospital, Vashi, the preferred health care destination in Navi Mumbai

ma Gandhi had said "**The best way to find yourself, is to lose yourself in service of** " and this has inspired the founder members to establish MGM New Bombay Hospital a multi-specialty tertiary care hospital with a mission to create affordable, sustainable and healthcare for the community. MGM New Bombay Hospital provides medical services are & compassion under the banner of **Mahatma Gandhi Mission Trust**. **Mahatma i Mission Trust** runs hospitals with around 2000 serving beds and feels proud to have more than 21 million patients in the state of Maharashtra, in all specialties.

e years, MGM has constantly endeavored to bring together the best of expertise, skills, icture, technology, equipments, and processes under one roof to fulfill objectives of the ation. MGM is the first Hospital in Navi Mumbai to get accredited with both prestigious and NABL certifications, and also to establish Robotics surgery which assures the quality of service. MGM Hospital is first in Navi Mumbai to set up **MGM Center for Research and Diagnosis**, a state of art facility, for catering diagnostic and counseling in the field of clinical and onco-cytogenetics to reputed hospitals within and outside htra, since 2004.

pital's motto: ***"WE HOLD THE DIGNITY OF PATIENT'S TO BE PARAMOUNT".***

"This report is dedicated to my beloved late parents, who always wanted me to utilize my education and skills for the good of mankind"

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Technological contribution of Poonam Pawar, field work of Raza Ansari, and cytogenetic data analysis by Dr. Shouvik Mandal are also acknowledged.

Last, but not the least, sincere acknowledgements are due to my family members, including husband Mr. Prodosh Ganguly and son Mr. Shameek Ganguly, who is a Ph.D. student of Stanford University, California, USA for their continuous support, encouragement and guidance but for which the execution of the entire project would have been difficult.

Bani Bandana Ganguly, Ph.D., FICMCH
The Principal Investigator
June 28, 2017

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FINAL REPORT OF THE PROJECT

1. **Project title:** 'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'

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

   No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

3. **Principal Investigator (PI) and Co-Investigators (CI)**

   3.1. Principal Investigator (name & address):
   Dr. Bani Bandana Ganguly, Ph.D., FICMCH
   MGM Centre for Genetic Research and Diagnosis, MGM New Bombay Hospital, Vashi, Navi Mumbai 400703, India
   Tel. 91 22 61526527; 91 9869214680
   E-mail: mgmgeneticlab@yahoo.com; center.genetics@gmail.com

   3.2. Co-Investigator (name & address):
   Dr. N. Banerjee
   Head, National Institute of Research on Environmental Health (NIREH)
   Bhopal, MP
   Tel. 91 755 2533106
   E-mail: nirehbhopal@yahoo.in

   (Involvement of a Co-investigator from NIREH was suggested by the expert committee during final discussion of the project for facilitating field work and sample collection at Bhopal. Otherwise, the project was singly submitted by the PI)

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

   4.1. Implementing Institution

   MGM Center for Genetic Research & Diagnosis
   MGM New Bombay Hospital
   Vashi Sector 3, Navi Mumbai 400703, India
   Tel. 91 22 61526666, Extn. 216/227
   Fax: 91 22 27824618
   E-Mail: mgmgeneticlab@yahoo.com

   4.2. Other collaborating Institution
   National Institute for Research in Environmental Health (NIREH)
   Indian Council of Medical Research
   Kamla Nehru Hospital Building, Gandhi Medical College Campus
   Bhopal, MP, India.
   Tel. 91 755 2533106

5.**Date of commencement:**

October 23, 2013 (as per letter of approval).
However, the project could not be started due to some administrative issues regarding availability of fund and staff to PI for the project work. Actually, the project has started in August, 2016 on regular basis after fund and staff were made available to PI.

6.**Duration:** One year as a pilot study

7.**Date of completion:** Duration was one year from November 2013 to October 2014. However, since there was delay in receiving fund and staff, it has been extended till June 30, 2017 due to the reason mentioned in point no. 5.

8.**Objectives as approved**

i. To study cytogenetic status of 100 gas exposed and 100 unexposed individuals, who were previously screened during 1986-1990 by the PI (from Calcutta center) under the multi-center genetic screening program of ICMR, and its comparison with their previous genetic status to exclude the possibility of long-term effects of MIC gas, if any, by employing conventional cytogenetic technique.

ii. To study the current health status from 3-generation pedigree.

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

Deviations are described at the end of this report in point no. 18.

10. **Abstract**

Genotoxic potential of MIC has been tested *in vivo* and *in vitro* after the MIC gas-leak accident took place in Bhopal in 1984. Initial genetic screening carried out on small groups of gas-victims by different institutions shortly after the MIC-disaster had variations in study design, sample selection, testing methodology, etc. The multi-center genetic screening program of ICMR had followed uniform double-blinded protocol and stringent quality assurance; however, the data generated on chromosome aberrations, sister chromatid exchanges and cell cycle kinetics were not analyzed and reported. However, aftermath of MIC-disaster on health of MIC-exposed population has been continuously measured by NIREH (ICMR) through epidemiological survey. Owing to lack of knowledge on genetic changes, ICMR launched projects for measuring the long-term effect of MIC on gas-exposed population. The present pilot study was conceived to study cytogenetic status of 100 gas exposed and 100 unexposed individuals, who were previously screened during 1986-1990 by the present PI (from Calcutta center) under the then multi-center genetic screening program of ICMR, and to compare the present outcome with their previous genetic condition, and also to record their health status through 3-generation pedigree. Old records of genetic screening were retrieved from NIREH's archive, of which 236 records were selected for the present study. Altogether, 174 individuals/families, including 44 unexposed subjects, could be traced and recruited through counseling and informed consent. However, peripheral blood could be collected from a total of 143 (35 unexposed and 108 exposed) individuals since remaining consenters declined or not available for blood collection in 2-3 attempts.

Peripheral blood lymphocyte culture was performed following the standard protocol for chromosome analysis. For each participant, ~100 G-banded metaphases were studied following ISCN classification of at least 10 metaphases. Numerical and structural aberrations were recognized as aneuploidies, hypo- and hyperdiploidies, translocation, deletion, inversion, etc., which was finally pooled as abnormal cell (Abc), aberrations (Abn) and number of aberrations per abnormal cell (Abn/Abc) for final analysis and comparison with that of the previous screening. The previous data on chromosome aberrations was

generated following solid-staining. Out of 143, 130 cases finally could be considered for data piling and analysis because ~10% cases were excluded due to inadequate metaphase-yield from the final analysis and interpretation.

The incidence of aberration was higher in MIC-exposed subjects over the unexposed individuals in the present as well as previous screening with predominance in the severely exposed groups. However, the aberration frequency appeared higher in the present study carried out after 30 years than the previous screening, which has been reflected in one-to-one comparison of past and present screening for each individual. The power of G-banding for recognition of structural rearrangements has facilitated identification of more recombinant chromosomes in the present assay, whereas solid-staining had previously allowed scoring of only breaks and chromatid exchanges. Inter-individual variation of MIC-effect was highly remarkable in both the screening.

Stable rearrangements such as translocation, ring, inversion, deletion, fragile sites, complex karyotypes with ≥3 aberrations, endoreduplication, monosomal karyotype, etc. were detected irrespective of MIC-exposure. Also >8% subjects were observed with constitutive abnormality, which was higher in severely exposed area. Stable rearrangements, which could represent clonal mutation in the hematopoietic stem cells; constitutive genomic abnormalities; hypocellularity or peripheral blood aplasia; abnormalities of genetic instability, etc. certainly raise serious concerns for future monitoring of the identified cases at frequent interval, and also their blood-linked relatives. Since spontaneously acquired clonal aberrations can facilitate clonal expansion and acquisition of gene mutations, screening of age-related epigenetic and RNA-splicing mechanism would be meaningful for MIC-exposed elderly groups. Normal karyotype in the congenitally malformed and mentally retarded children of severely exposed parents also urge for such molecular screening, at least for copy number variations and interstitial deletions and duplications. However, further exercise on G-banding screening at a larger scale would be meaningful for tracking of more balanced rearrangements in circulating lymphocytes on more MIC-exposed individuals. The present pilot study has identified stable chromosomal rearrangements, and recommends for further follow up of these

individuals for examining its persistence. The study also recommends extending similar screening on larger sample size of differently exposed areas, including migrated unexposed controls. Chromosomal involvement in acrocentric association would be interesting to throw some light on its biology of cyclical arrangement of the chromosomes. The present spectrum of chromosome aberrations cannot be directly correlated to MIC-exposure due mainly to interaction of environmental admixture with living/life-style and individual genetic composition over the last three decades.

11. Methodology

The present pilot study was planned to check the genetic condition three decades after the disaster. The aim was to compare the past and present genetic condition of each of 100 exposed and 100 unexposed subjects on one-to-one basis for understanding their present health status following chromosome analysis, and to exclude the possibility of long-term health effects of MIC in Bhopal population. To fulfill the proposed target, the primary task was to locate the individuals, who have expanded families with members of at least two new generations during 30 years post-exposure, and also split families and relocated in different areas.

11.1. Brief history of the previous genetic screening of MIC-disaster

Immediately following the disaster, a multi-center genetic screening was carried out during 1985-1989 on Bhopal population under supervision and financial support of ICMR, and overall co-ordination of King George's Medical College (KGMC), Lucknow. A complete blinded study was carried out by six participating labs, including Gandhi Medical College (GMC), Bhopal; Center for Advanced Studies in Cell and Chromosome Research (CAS), Kolkata; Cytology Research Centre (CRC) (presently Institute of Cytology and Preventive Oncology, ICPO), Delhi; King George's Medical College (KGMC), Lucknow; St. John's Medical College, Bangalore; and Vanaras Hindu University (BHU), Varanasi. Basic laboratory facility was established at GMC, Bhopal. Genetic screening on exposed and unexposed population of Bhopal was carried out by all six participating laboratories (PL).

The operation, of the screening was defined in a unified and consorted manner by all six PLs, which was endorsed by the scientific committee of ICMR. Basic sample processing including blood culture till slide preparation used to be carried out at GMC, Bhopal, and microscopic analysis at the home center of each PL. PLs used to visit GMC as per the schedule communicated by GMC with full preparation from their parent lab for carrying out technological processing at GMC. Following every cycle of sampling, the screening result was submitted to the coordinator at KGMC for every individual sample as well as summary

of the total samples processed by all PLs. Also two chromosome slides were submitted to another PL (as communicated by the coordinator at KGMC) for a double (blind)-check and monitoring the performance of the PL (inter-laboratory comparison, ILC) with a view to maintaining quality assurance (QA). Thus, the coordinator at KGMC had collected laboratory-data from all six PLs of all the cases recruited and studied during 1985-1990. The coordinator had also collected the result of ILC after every sampling scheduled during 1986-1990. The screening program was ended by ICMR in March 1990.

Identification of the case/family, case/family history, exposure-status, gravity of illness following exposure and the degree of cytogenetic alterations were kept blinded to all PLs. Although, the PIs of the respective centers had participated in discussion forum/scientific meeting convened by ICMR, the exposure-status/case-details and performance of PL or outcome/result of screening were never made available to PIs. The PIs were unaware about the relationship between exposure status and cytogenetic result. PIs had processed the given blood samples and collected cytogenetic results of the individuals irrespective of the knowledge of the exposure status. The PIs were also not informed about the final outcome of the genetic screening for estimation of the genetic damage caused by accidental exposure to MIC. Since ICMR was the sponsor of the screening program, final genetic analysis and correlation with exposure-status were expected to be published by ICMR as per the protocol and as happened with other health parameters studied on MIC-exposed population following the accident (ICMR Technical Report 2008; 2010). The double-blinded study was made triple-blinded to PIs and public in general.

The Principal Investigator (PI) of the present investigation was involved in that multi-center screening from Center for Advanced Study in Cell and Chromosome Research, University of Calcutta, Kolkata and stated the above information from the experience gained through her participation in the previous study. Late Dr. Geeta Talukder was the PI of the then screening from Calcutta Center. The present PI remembers Dr. Talkuder's contribution and encouraging guidance with heartfelt respect and honor.

11.2. Retrieval of old records of the earlier study

Following the final review meeting of the present project held in March, 2013 at NIREH, Bhopal, and based on the aim of the pilot project, the PI was suggested to retrieve some of the old records on genetic data. Records of the previous genetic screening conducted on MIC-exposed population were archived at the NIREH (ICMR), Bhopal in both hard and soft form. However, there was no index available for hard copies and magnetic tapes were not retrievable due to outdated operating system. As per the suggestion of the then review committee, randomly ~8-10 bags were picked up from the NIREH's archive after that review meeting. The bags were opened by authorized persons of NIREH. PI herself segregated the laboratory records generated by six PLs of the multi-center genetic screening program of '86-'90. After center-wise segregation and filtration of the records, a total of 174 cases could be located for the present study, in place of projected 200 cases due mainly due to the fact that many have left Bhopal or not traceable or declined. More such records of the previous genetic screening are expected in the archive of NIREH, which could be retrieved for further analysis and drawing interpretation on the initial level of genetic damage occurred due to MIC-exposure in Bhopal population.

11.3. Field work for identification of cases

It was difficult to locate the exposed individuals due to in and out migration of Bhopal population since MIC-disaster. However, since unique identification numbers assigned by ICMR to the gas-victims were retained, it was helpful for tracing the individual families, although splitting of expanded families and isolation from joint families created trouble, and thus, locating them was really time-consuming. Another serious problem was to catch hold of them for counseling and collection of consent for participation in the study since most of them are engaged in transport-truck driving or in building construction as day-laborers. Many of the individuals could not be located.

11.4. Collection of informed consent and recruitment of subjects for the present study

Initial field work was conducted by the PI along with the project staff for extending training to staff and explaining the purpose of the project to participants/subjects of the present study. The targeted individual was explained about genetics, benefit of participation and risk or adverse effects. The participants were counseled for genetic study by the PI herself in the beginning. Thereafter, the project staff, based at NIREH, used to visit the field for locating the families and collection of pedigree and consent of participation. The consent form was made available in both Hindi and English for their understanding (**ANNEXURE I & II**). Three-generation pedigree and consent of participation was collected of 174 cases studied in past by Calcutta center during 1986-1990.

Only 44 unexposed/control individuals were traced. Thus, projected 100 numbers could not be accomplished. More numbers could have been enrolled from the list of cases studied by other centers of six PLs, which may be considered for future study based on the present analysis and result.

Participation exclusively included their family and health history and ~2ml peripheral blood. Hence, there was no risk or adverse effect from participation. The participants rather got G-banded karyotype and information on transmissible-risk of genetic defects. Karyotype indicates heritable abnormality and genetic damage indicates risk of future health and immunity. Thus, participation was beneficial for the families. There was no financial compensation for participation.

PI has observed highly positive response, though few of the consenters declined at the time of blood collection, and few could not be contacted for blood collection following repeated attempts because of their type of job. In all, 174 subjects could be contacted for history and consent collection for the present study; however, data of 168 subjects could be considered for final analysis of health status after 30 years.

11.5. Sample collection and processing for the present investigation

Since the project was approved as NIREH's intramural project and the PI was from Mumbai, a standard operating procedure was defined for execution of the task (**ANNEXURE III**).

As per the consents collected, 2ml of peripheral venous blood (PBL) was collected for total of 156 subjects from both exposed and unexposed groups during 2014-2016. The details of sample matrix, locality-wise distribution of subjects, information on additional samples without ICMR no., culture outcome of all blood samples and reporting status based on analytical completion are presented in Table 1-4. However, finally **143** participants could be considered for the present investigation based on the outcome of cultures, especially from a batch of 34 control samples initiated at NIREH in February, 2015. Repeat samples were collected for some of them; however, that was not possible for all cases appeared with inadequate culture outcome (Table 3). Also some of the peripheral blood samples were appeared with extremely low mitotic index, even in the repeat sample, and thus excluded from comparative analysis. Initial protocol of sample was followed as approved in the project (**ANNEXURE III**). However, the proposed protocol/standard operating procedure could not be followed for the entire laboratory processing for all the samples (**ref. point on 'Deviation'**). Since another aspect of the sample processing at NIREH was to facilitate setting up of the laboratory at NIREH, all samples were decided to be processed at NIREH. However, since NIREH didn't have image processing facility, analysis was decided to be carried out at PI's institute in Mumbai, and approved accordingly.

Table 1. Sample matrix

Total samples	Total samples	Final study samples	Total consents collected	Total proposed no.
Collected and processed	173	143	174	200
Total repeats	17	-	-	-
Total without ICMR#	13	-	-	-

Comments:

1. Individuals (26) could not be traced for cases whose documents were retrieved from the archive. Therefore, 200 consents could not be collected since 2013 November till May 2016 (during this period the project staff was placed at NIREH, Bhopal)
2. Out of 174 consents, 31 cases could not be contacted for sample collection due to their absence at all repeat visits. Few of them declined to give blood sample, though they consented at the time of pedigree collection.
3. Of the 13 cases without having ICMR no., 7 cases were considered for genetic screening because they were presented with congenital deformities, 4 were considered for validation and quality control and remaining 2 cases were spouses, whose ICMR no. could have been traced from the archive; however, further retrieval of records from archive was not done after March 2013 to trace their documents for assigning ICMR no. The expenses for all these cases were borne by the PI's institute, since no amount was given to the PI during the period.
4. Finally, 143 samples were registered under the present study, including 35 controls and 108 exposed cases.

Table 2a. Locality-wise distribution of study-samples collected and processed

	Exposure status											
	Severe				Moderate				Unexposed			
	Area code				Area Code				Area code			Grand
Sex	1	2	7	**Total**	3	5	10	**Total**	14	16	**Total**	Total
Male	10	3	13	**26**	11	2	2	**15**	0	15	**15**	56
Female	24	3	21	**48**	15	1	3	**19**	3	17	**20**	87
Total	**34**	**6**	**34**	**74**	**26**	**3**	**5**	**34**	**3**	**32**	**35**	**143**

Table 2b. Additional samples processed from area code #1

Sex	Validation	Genetic diagnosis	Without ICMR #	Total
Male	2	3	1	6
Female	2	4	1	7
Total	**4**	**7**	**2**	**13**

Table 3. Summary of culture outcome

Collection date	No. of samples	Processed at	Outcome	Repeated/Not
28.02.14	03	Mumbai	Successful	No
08-09.05.14	23(10 of 23 samples with no ICMR #)	Mumbai	Successful	No
03-04.02.15	34	NIREH	Not satisfactory	17 repeated
16-17.02.16	26	Mumbai	Successful	No
23-24.02.16	24	Mumbai	Successful	No
09-11.03.16	12	Mumbai	Successful	No
27.04.16	28 (5 repeats)	Mumbai	Successful	No
30.04.16	17 (11 repeats)	Mumbai	Successful	No
07.05.16	6 (1 repeat)	Mumbai	Successful	No

Table 4. Completion status of all blood samples

Sl. No.	Dt. of receipt	Total samples	Traceability of ICMR No.	% analytical completion	Reporting status
1	28.02.14	03	N, 2 for validation and quality control, 1 for diagnosis	100%	Distributed
2	08-09.05.14	23(10 of 23 samples with no ICMR #)*	24x2, 185x2, 188, 254x2, 1072, 1138x2, 1009, 1308x2	100%	Distributed
3	03-04.02.15	34	Y	17.6%* unsuccessful (hypocellular)	Distributed
4	16-17.02.16	26	Y No age for 10 cases	100%	Distributed
5	23-24.02.16	24	Y	100%	Distributed
6	09-11.03.16	12	Y	100%	Distributed
7	27.04.16	28 (5 repeats)	Y	100%	Distributed
8	30.04.16	17 (11 repeats)	Y	100%	Distributed
9	07.05.16	6 (1 repeat)	5 (1 does not have ICMR no.)	100%	Distributed
	Total	**173**			

Y - Yes; N – No; * 6 of 34 cases could not be studied due to inadequate yield of metaphases. Ideally repeat analysis should have been carried out for another 11 cases, whose karyotype has been prepared but could not be included in the final analysis due to less number of metaphases studied. Therefore, altogether 50% of the samples collected on 3-4 February, 2015 could not be considered for comparative analysis, and thus should have been repeated. The cultures were set up at NIREH.

Short term (72h) peripheral blood lymphocyte culture technique was employed for preparation of chromosomes for various analytical endpoints (Carrano and Natarajan, 1988; EHC 1985; Ghosh 1988). Conventional solid staining and G-banding techniques were followed for all the cases. To comply with the requirements of the proposed project, three samples (without having ICMR no.) were processed for validation and quality control. (The PI is a NABL (National Accreditation Board for Calibration & Testing Laboratories, Quality Council of India) Auditor for Genetic testing (ISO 17025) and Medical genetics (ISO 15189) and an Expert Committee Member of NABL). A serious effort was paid to record the stable and unstable aberrations and recognize and analyze participation of chromosomes with their breakpoints and bands altered.

11.5.1. Chromosome analysis: present and previous screening

a. ***Short term peripheral blood lymphocyte culture and chromosome preparation:***

Present:

2-3 ml peripheral blood sample was collected in sterile sodium heparin vacutainer by vein-puncture by the Technician for chromosome analysis and karyotyping. Technician had D.M.L.T. certificate.

Peripheral blood (0.5 ml) was added to 4 ml RPMI 1640 medium (GIBCO, USA) supplemented with 20% fetal bovine serum and phytohaemagglutinin (mitogen) and maintained at 37°C for 72 hours. A total 2 cultures were maintained for each sample. Initially, 2 additional cultures were processed for chromosome analysis on first cycle mitotic cells with BrdU 2 for differential fluorescence plus Giemsa (FPG) staining; however, that was discontinued due to non-availability of staff for handling the technical operation, and shortened project duration (**due to wastage of time in the beginning as specified in point on 'Deviation'**).

Harvesting was followed as per standard colchicine-hypotonic-fixative technique. Metaphase chromosomes were prepared on chilled slides from a height of one foot. The slides were dried on hot plate. Chromosome analysis was carried out following solid-staining and GTG-banding of 100 metaphases. For establishing genomic karyotype, 25 G-banded metaphases were analyzed following ISCN classification (ISCN 2016).

Karyotyping was performed by the PI for the samples collected in 2014 (**ANNEXURE IV**), and their reports were distributed by the PI in February 2015 (receipt attached as **ANNEXURE V**). Laboratory processing of the remaining samples and microscopic screening of metaphase-chromosomes was started from September 2016 after recruiting new staff and training them for the purpose.

Differential FPG-staining was extensively employed by PI for previous genetic screening of Bhopal population and assessment of genotoxicity of tin (Sn) *in vitro*. Fluorescence in situ hybridization (FISH) is also routinely followed by PI's department for aneuploidies and

oncogenes. PI has extensive experience in whole chromosome painting that was employed for investigation of chromosomal participation in low-dose radiation-induced mutagenesis. However, FPG and FISH could not be carried out due to paucity of staff (for sample processing), fund remained and available to PI for procuring FISH probes, and availability of time left.

Note: 1. Sample was collected from few more individuals whose ICMR no. could not be assigned due to unavailability of the records (not retrieved from NIREH's archive). For example, in some cases husband's no. was assigned, but wife's no. could not be assigned, and not considered in the study. However, both husband and wife were exposed and studied during 1986-1990. Thus, record was expected to be available at the archive.

2.Samples of seven physically and mentally challenged children were also collected for karyotyping on request of the parents. Being a Geneticist, PI understood the necessity of karyotyping for their siblings and extended the facility at free of charge.

Past:

In the past screening program, PBLs were collected from exposed and unexposed individuals in coded vials and cultured in RPMI 1640 medium supplemented with FBS and PHA. The methodology of sample processing was uniform for the past and present investigation and the reagents were used from the same brand. In the past screening, genetic parameters were selected as chromosome aberrations (CA), sister chromatid exchange (SCE) and replicative index (RI). In the beginning, micronuclei was also considered as a study parameter; however, discarded after first cycle of screening due mainly to the fact that additional cultures were required for cytokinesis-blocking. A total of four cultures were maintained at 37°C in absence and presence of 5-Bromo-2-Deoxy-uridine (BrdU) for differentiation of cell cycles in replicate sets and terminated at 48h and 72h respectively.

b. Data handling

In the present study, the chromosomal investigation included all kinds of numerical and structural alterations. Every metaphase with numerical or structural changes was karyotyped for recognition of the chromosome affected, its breakpoints and type of aberration, using IKAROS imaging system (MetaSystems, Germany). A serious thought was paid for recognition of stable or unstable rearrangements. Finally, the abnormalities were presented as chromatid (chtd) and chromosome (chm) breaks, translocations (t) and dicentrics (dic), complex (CK) and monosomal (MK) karyotypes, and a large number of rearrangements were grouped as others, including trisomy, deletions (del), inversions (inv), ring (r), fragile sites (frag), endoreduploication (endo), etc. Tetraploid (tetra) cells were also recoded. All types of alterations were pooled in terms of total abnormal cells (Abc), aberrations (Abn) and aberration per abnormal cell (Abn/Abc). On an average, 10 metaphases were karyotyped for generating the genomic karyotype for every individual following ISCN (2016) classification system (**ANNEXURE VI**).

In the previous screening, different cell generation metaphases were recognized as first (M1), second (M2) and third (M3) generation cells by FPG-staining (Ghosh 1988; Ganguly 1995a). CA was exclusively recorded in M1, SCE in M2 cells and RI was estimated from frequencies of M1, M2 and M3 cells as described in Ghosh (1988) and Ghosh et al. (1990). However, CA was screened from 100 cells following solid Giemsa-staining. For each parameter, uniform numbers of cells were screened by all PLs such as 100 cells for CA, 50 cells for SCEs, and 200 cells for RI (EHC 1985; IAEA, 1986). SCE and RI were not considered, since the present investigation didn't include them.

In past, uniformity was strictly maintained in all respects in that screening consortium. Importantly, all PLs had procured RPMI 1640 nutrient medium, FBS and PHA from GIBCO, Grand Island, USA, from the same batch and lot number to maintain the integrity of culture ingredients, and also other reagents from the same manufacturing company to avoid conflicts of reagent-performance. **The present PI had studied ~>1500 individuals from different stratified zones from UCIL plant from which an excerpt of the data on 129 individuals was published in 1990 (Ghosh et al. 1990).**

Among CA, SCE, and RI of the previous study, only Abc, Abn and Abn/Abc has been segregated for the present analysis from the records retrieved. Karyotypic classification of chromosomes could not be performed since result was generated on solid-stained metaphases, and karyotype was not recorded. From the assorted cases studied by the present PI through Calcutta center, a total of 236 cases could be considered, including 78 controls, 62 moderately exposed and 96 severely exposed individuals. The individual data was calculated and pooled for Abc, Abn and Abn/Abc in the present report.

c. Statistical analysis

On the whole, 946 records from the previous study, including unexposed (257), moderately exposed (298) and severely exposed (391) were retrieved from NIREH's archive. However, the data of the present consented individuals have been included in the present analysis, including 78 controls, 62 moderately and 96 severely exposed individuals. From the present sampling, a total of 130 cases, including unexposed (19), moderately exposed (34) and severely exposed (77) could be considered for final analysis. Some of the samples collected from area no.1 of the spouses, who could not be identified with ICMR no., and have also considered for final comparison, and one control from Bhopal without ICMR no. has also been considered to increase control no. He walked into PI's center for karyoting as required for treatment of infertility. Hypoplastic condition and inadequate culture outcome in 26 cases led to exclusion from analysis. Individuals with a minimum of 25 metaphases were considered for final analysis. The baseline information on the analytical aspect is presented in Table 5. Frequencies of Abc, Abn and Abn/Abc were considered for comparison among the moderately exposed, severely exposed and unexposed groups in the past and present investigation separately. The three groups were also considered for comparison of the past and present situation. For all, t-test was carried out for comparison of two groups on all parameters.

12. List of annexure, tables and figures

The following tables, figures and photographs have been inserted suitably in the running test.

13. Detail analysis and results

13.1. Assay of past and present genetic status as chromosome aberrations in MIC-exposed and unexposed population

The result was compiled for the past and present investigation separately based on exposure status. One-to-one comparison was possible only for few cases since all of the present individuals could not be identified from the previous list. Present subjects have been identified from ICMR no., but their serial number in the family was possible only the cases selected for one-to-one analysis. On the whole, totally the present observation has not been compared with the exposed groups studied immediately after the exposure in the present report, due mainly to wide variation in sample number of the two studies. Baseline information of the present investigation is presented in Table 5.

13.1.1. Chromosome aberrations detected in the present subjects 30-years post disaster

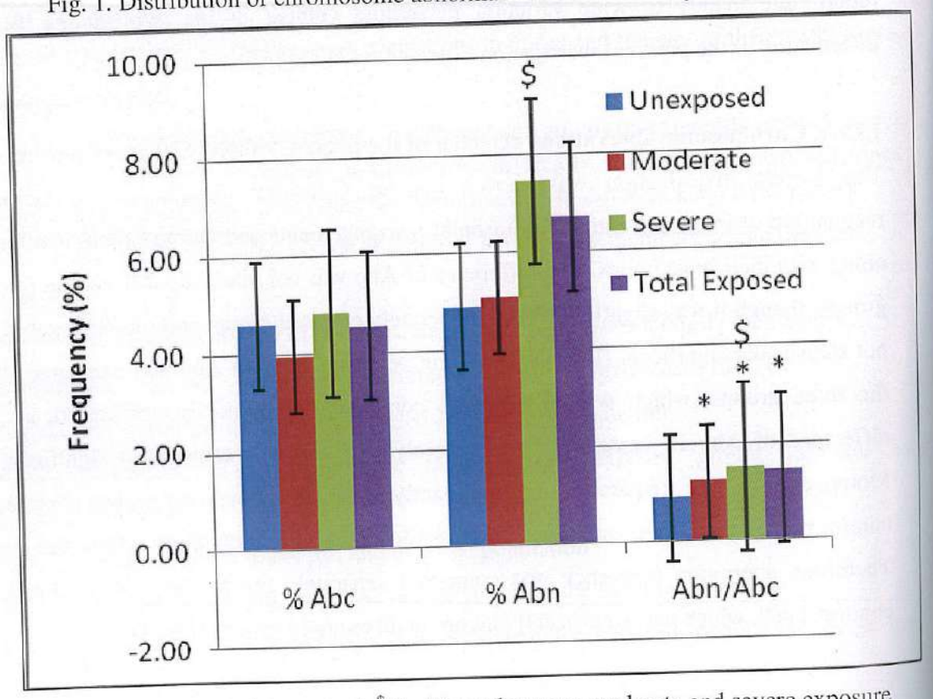
The present investigation was carried out on G-banded metaphases for facilitating recognition of inter- and intra-chromosomal rearrangements and chromosomal involvement along with their breakpoints. The difference of Abc was not markedly different in the three groups, though it was slightly lower in moderately exposed group, and the differences were not statistically significant (Fig. 1). The result was similar when Abn was compared among the three groups, which was higher than control but statistically not significant. The difference of Abn in moderately and severely exposed was statistically significant ($). Moreover, Abn/Abc frequency was significantly higher in all exposed groups compared to control population. Wide variation was observed among the individuals within the groups. Therefore, aberration frequency was computed separately on the ratio of aberration per abnormal cell, which was significantly higher in all exposed groups (Fig. 1).

Table 5. Baseline information about the present study

Variables	30-years post exposure			Total exposed
Exposure	unexposed	moderate	severe	
Samples	019	034	077	111
Mean age (yrs)	50.42±13.43	51.24±12.16	52.84±11.04	52.35±11.36
Range of age (yrs)	32-70	32-86	32-72	32-86
Cells studied	1986	4511	7916	12427
Mean cells	104.53±43.42	132.68±39.41	102.81±42.24	111.95±43.47
Range of Abc	0-18.18	0-12.67	1-23.33	1-23.33
Range of Abn	0-18.18	0-18.67	1-15.79	0-18.67
Ratio of Abn	0-1.5	0-2.5	1.0-3.3	0-3.3

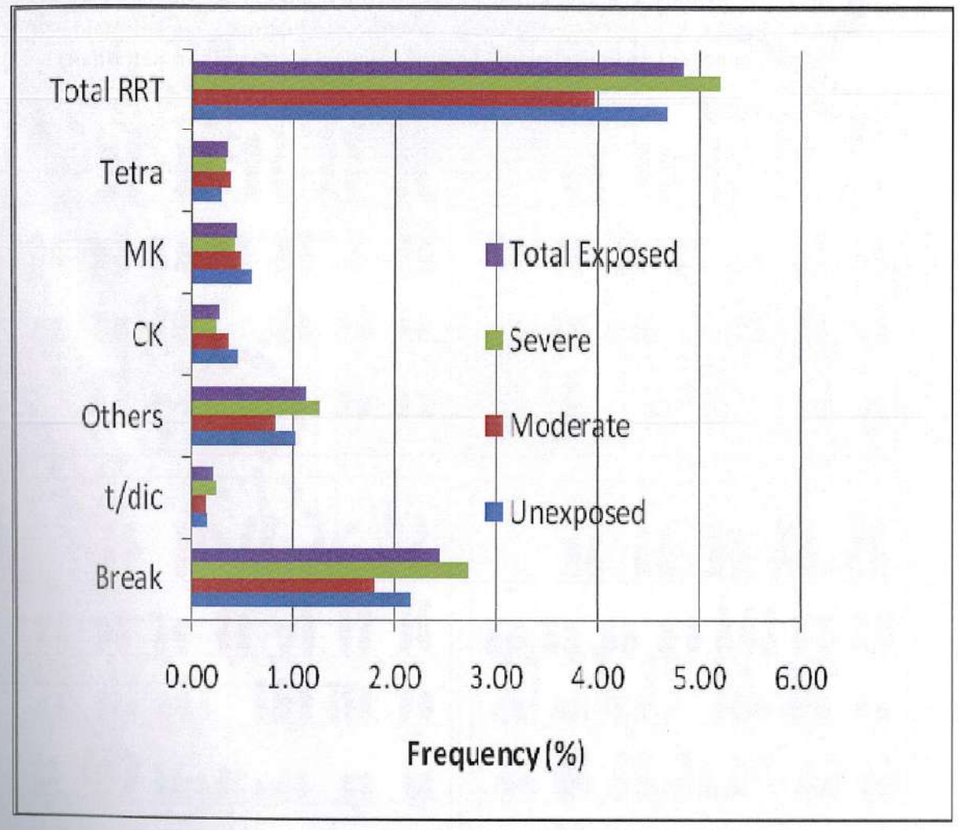
Abc Abnormal cells; Abn aberrations; yrs Years; NE Not extracted

Fig. 1. Distribution of chromosome abnormalities collected 30 years post disaster



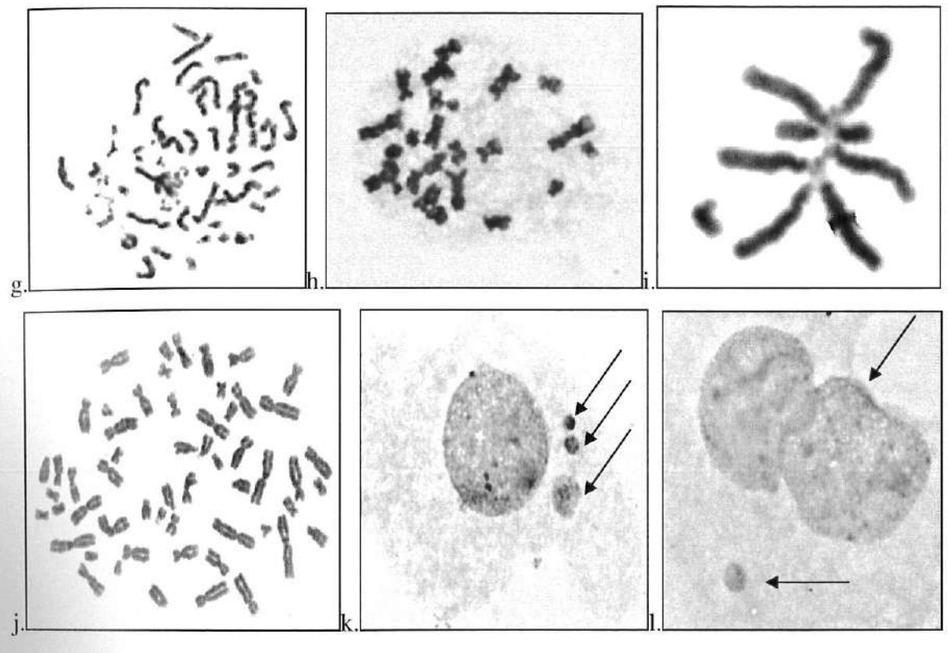
*Significant at <0.05 compared to control; $significant between moderate and severe exposure

Fig.2. Distribution of chromosomal rearrangements (RRT) in different study groups



t Translocations; dic Dicentrics; CK complex karyotype; MK monosomal karyotype; Tetra Tetraploid; RRT rearrangements; Others include trisomy, deletions (del), inversions (inv), ring (r), fragile sites (frag), endoreduploication (endo), etc.

Fig.3. Types of chromosomal alterations detected in the present study: a. del(16q22); b. monosomy 7; c. trisomy 8; d. del(Xq); e. dic(1;13); f. tetraploidy; g. complex karyotype with multiple breaks; h. hypodiploid cell; i. acrocentric association; j. hyperdiploid cell; k. micronuclei; l. binucleated cell with micronucleus; m. t(7;15); n. t(10;14)



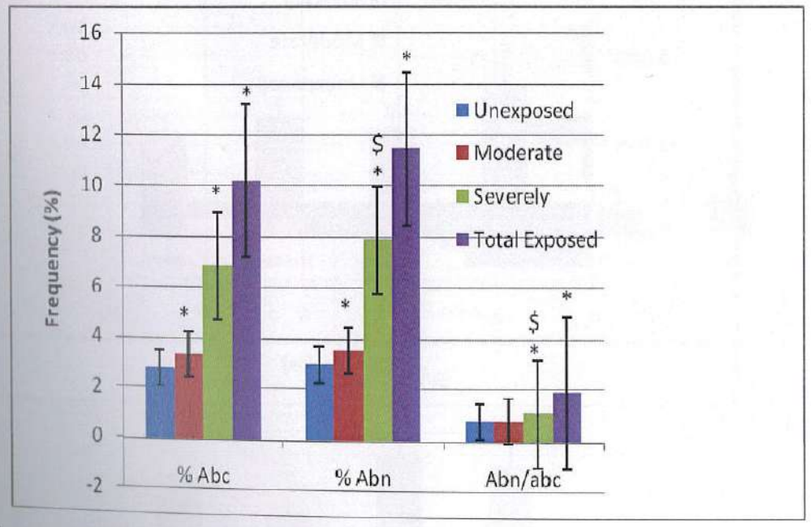
G-banded metaphases were critically analyzed for numerical and structural alterations and classified as breaks, translocations and dicentrics, complex and monosomal karyotype, tetraploidy, wherein deletions, inversions, trisomy, endoreduplication, fragile sites, etc grouped as 'others' (Fig. 2, 3). Among the different types of aberrations, breaks (chromatid + chromosome types) were prevalent in all groups compared to other aberrations (Fig. 2), which was higher in the severely exposed group among all. Control population also showed

markedly higher frequency of breaks compared to exposed groups. 'Other' abnormalities appeared with the second rank among all abnormalities, wherein CK and MK were detected almost in a similar range in all groups; though the frequency was higher in unexposed. Higher frequency of t/dic appeared in severely exposed group and tetraploidy was uniform in all classes. Altogether, the severely exposed group was presented with highest degree of chromosomal rearrangements (RRT) in the present investigation. The kind of structural aberrations recorded in the present study indicated genetic instability (Fig. 3).

13.1.2. Genetic changes assessed immediately after MIC-disaster

CA measured in the previous study is presented in Fig.4 as Abc, Abn and Abn/Abc. It is important to mention that such analysis has been carried out first time these parameters. This data was collected during 1985-1989. The frequency of Abc, Abn and degree of aberrations were significantly higher in both the exposed groups compared to control population (Fig 4). The differences between moderately and severely exposed groups on Abc, Abn and Abn/Abc were also statistically significant. The abnormalities were collected on solid stained metaphases, and distribution of abnormalities were recorded (Fig. 5). Mainly Abcs were observed with a single aberration, where four or more abnormalities were noticed only in a small number of cells. There was a gradual decrease of cells with more than one aberration; however, abnormal cells with varying number of aberrations were always higher in the exposed population compared to control group (Fig. 5).

Fig. 4. Spectrum of chromosome abnormalities detected during 1985-1989



*Significant at <0.05 compared to control; $significant between moderate and severe exposure

Though methodology of culture and chromosome analysis was similar in past and present investigation, chromosome analysis following solid staining and G-banding has extracted substantially different picture in

the two investigation program. Therefore, a comparative analysis is not totally justified between the two screening results. However, Abc, Abn and Abc/Abn parameters were kept constant for the two studies. Apparently, the average frequency of Abc and Abn were higher in the present investigation in control and moderately exposed population than the previous study, and the difference was statistically significant for Abc (Fig. 6a,b). However, the frequency of Abc was significantly higher in the severely exposed groups of the previous study carried out immediately following the accident (Fig. 6c). In all, Abn/Abc appeared higher in moderate and severely groups of the present study.

Fig. 5. Distribution of cells with frequency of aberrations

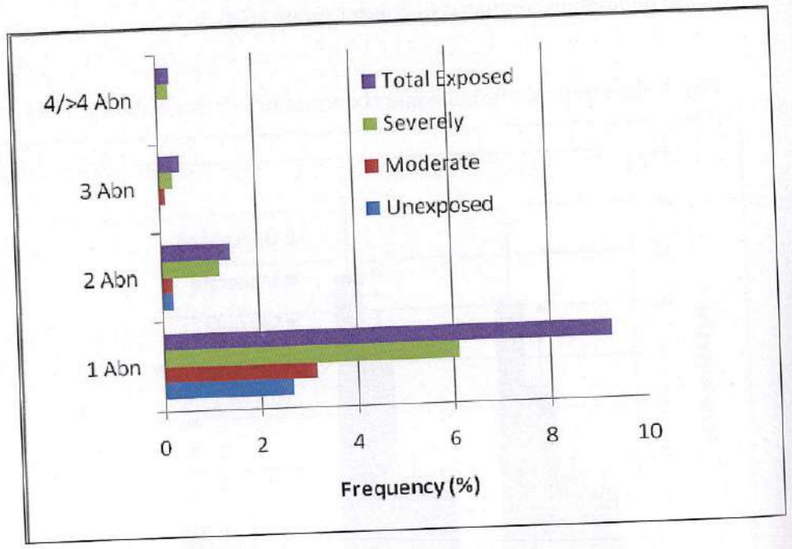
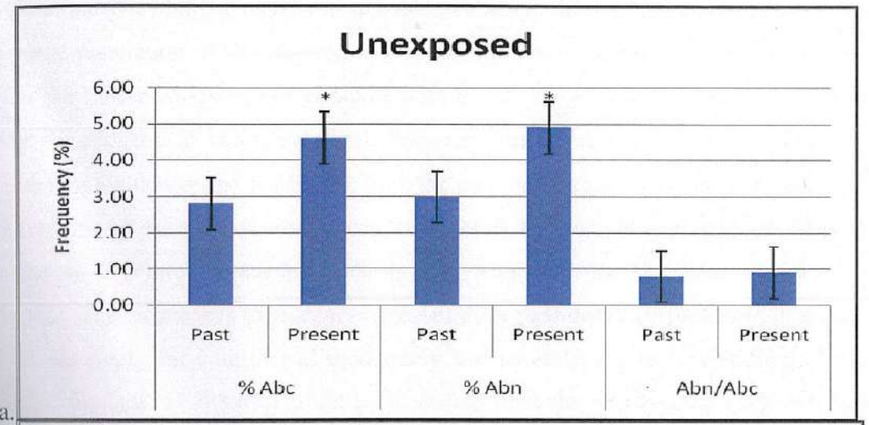
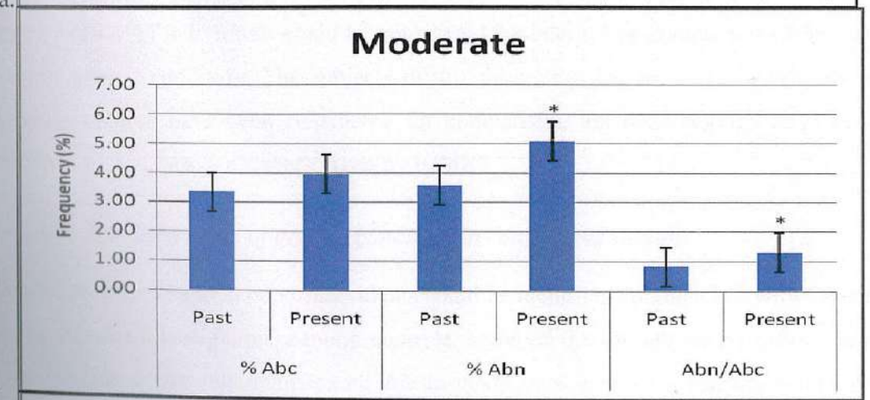


Fig.6. Comparison of abnormalities in unexposed and moderately and severely exposed groups of the previous and present genetic screening: a. unexposed; b. moderately exposed; c. severely exposed.

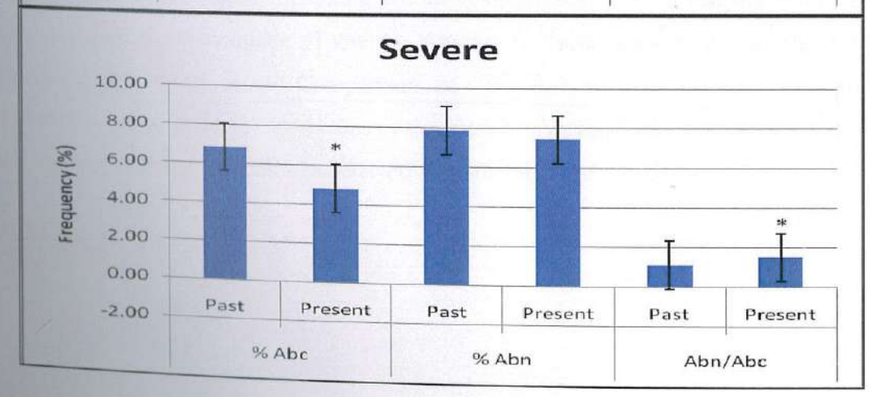
a.



b.



c.



In general, aberration frequencies have been detected higher in the present study compared to that of the previous study. Employment of banding has facilitated recognition of translocation, deletion, inversion, etc, which was not considered in the previous investigation.

'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'
No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

13.1.3. One-to-one comparison of past and present status of genetic alteration at individual level

The present pilot study was conceived to check the present genetic status in MIC-exposed population. The ultimate target was to evaluate the long-term effect of MIC, if any, on MIC-exposed population. It was decided to evaluate the present genetic condition after 30 years from the time of disaster, and compare with that of the previous screening carried shortly after the disaster, at individual level. Subjects were identified accurately based on serial number in the family and ICMR No. from the previous records. The subjects recruited in the present investigation have only been considered for comparison with previous genetic condition. The three parameters such as Abc, Abn and Abn/Abc were considered for the purpose. The differences (**difference=present data-past data**) are presented in at individual level separately for control and moderately and severely exposed population. Among 35 control subjects, 31 (88.6%) could be identified from the past records retrieved; however, only 19 (**54.3%**) individuals could be considered for one-to-one comparison of the past and present genetic condition. The subjects of this discussion are the same individuals whose previous records have been considered for comparison, not their siblings other members belonged to that family numbers given by ICMR.

13.1.3.1. The differences of genetic condition in control population

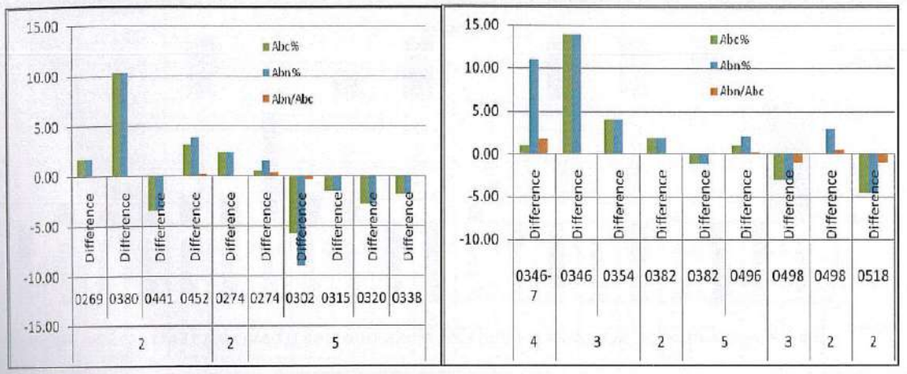
Genetic records of a total of 31 individuals could be identified and matched with the subjects of the present investigation. Among controls, some of the present investigations have not been considered for this comparison, due to inadequate yield of metaphases, though their previous data were available (Table 6). Among 12 such cases, three cultures were not successful for three cases. Comparison of 19 cases revealed higher frequencies of aberrations (Abn) in 11 (57.9%) cases of the present investigation, whereas, 8 (42.1%) cases had higher chromosomal damage detected immediately after the disaster (Fig. 7).

Table 6. Details of the control cases with previous and present genetic information

Sl. No.	Family member	ICMR No.	Present-past =Difference	Abc %	Abn %	Abn/Abc
1.	2	0269	Difference	1.70	1.70	0.00
2.	3	0269	Difference	Inad.	Inad.	Inad.
3.		0312	Difference	Inad.	Inad.	Inad.
4.		0380	Difference	10.32	10.32	0.00
5.		0486	Difference	Inad.	Inad.	Inad.
6.		0304	Difference	NS	NS	NS
7.		0775	Difference	Inad.	Inad.	Inad.
8.		0362	Difference	Inad.	Inad.	Inad.
9.		0441	Difference	-3.55	-3.55	0.00
10.		0452	Difference	3.14	3.83	0.17
11.		0273	Difference	Inad.	Inad.	Inad.
12.	2	0274	Difference	2.30	2.30	0.00
13.	5	0274	Difference	0.50	1.50	0.33
14.		0302	Difference	-5.71	-9.05	-0.33
15.		0315	Difference	-1.55	-1.55	0.00
16.		0320	Difference	-2.82	-2.82	0.00
17.		0338	Difference	-1.84	-1.84	0.00
18.		0338	Difference	NS	NS	NS
19.	4	0346-7	Difference	1.00	11.00	1.67
20.	3	0346	Difference	13.93	13.93	0.00
21.		0354	Difference	3.93	3.93	0.00
22.	2	0382	Difference	1.77	1.77	0.00
23.	5	0382	Difference	-1.15	-1.15	0.00
24.		0493	Difference	Inad.	Inad.	Inad.
25.		0493	Difference	Inad.	Inad.	Inad.
26.		0496	Difference	1.00	2.00	0.17
27.	3	0498	Difference	-3.00	-3.00	-1.00
28.	2	0498	Difference	-0.07	2.89	0.50
29.	1	0518	Difference	Inad.	Inad.	Inad.
30.	2	0518	Difference	-4.55	-4.55	-1.00
31.		0856	Difference	NS	NS	NS

Inad. Inadequate; NS Not successful

Fig.7. Individual data on differences in genetic changes in unexposed individuals



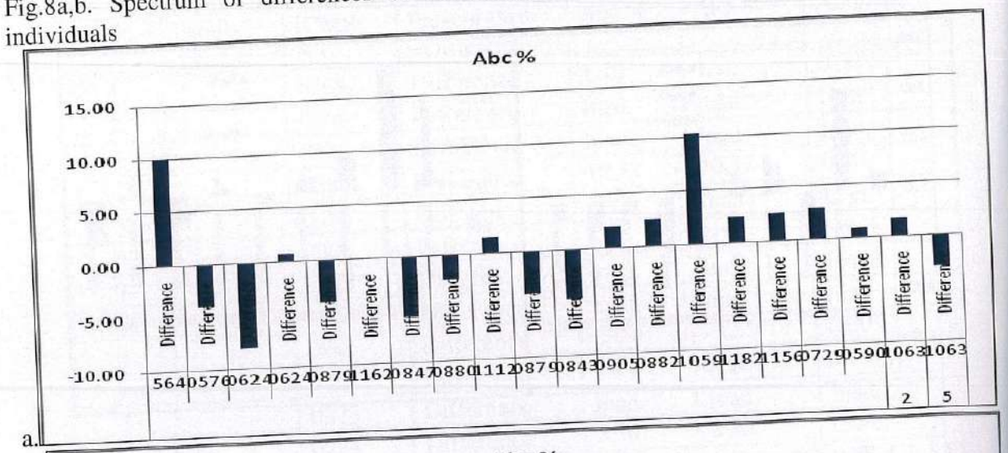
13.1.3.2. The differences of genetic condition in moderately exposed population

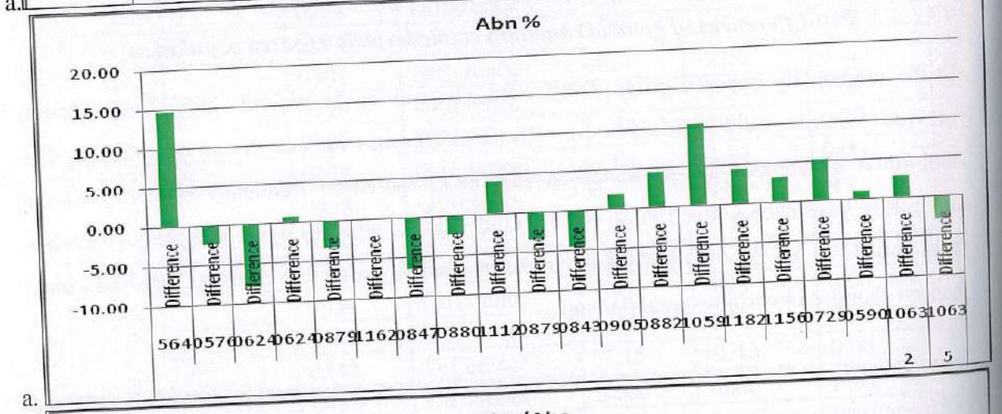
In the moderately exposed group, there were 34 cases in the present study. Among them, previous records could be identified for 29 **(85.3%)** cases for one-to-one comparison. The individual differences are presented in Fig. 8a,b. Increased frequency of chromosomal aberration have been noticed in 18 (62.1%) individuals; whereas hgier aberration frequency was noticed in 10 (34.5%) individuals measured shortly after the disaster. One individual had no changes in chromosomal damage.

The elevated levels of chromosome aberrations 30-years post disaster could be explained with confounding effects of several confounding factors.

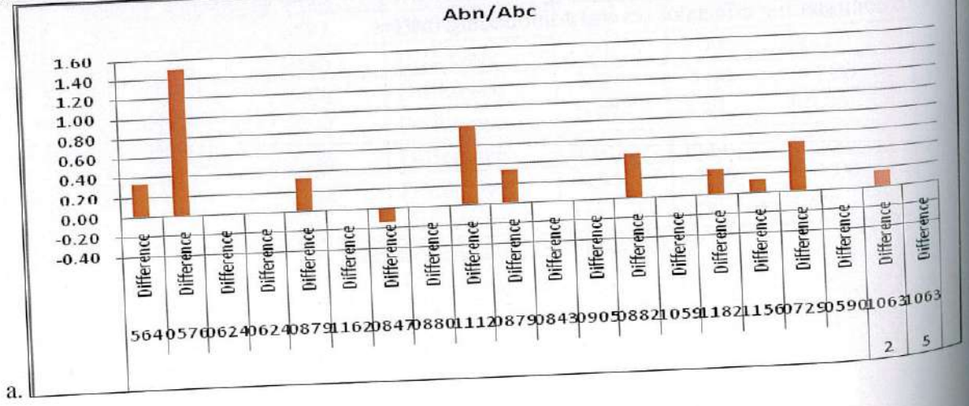
Fig.8a,b. Spectrum of differences of chromosome aberrations in the moderately exposed individuals

a.

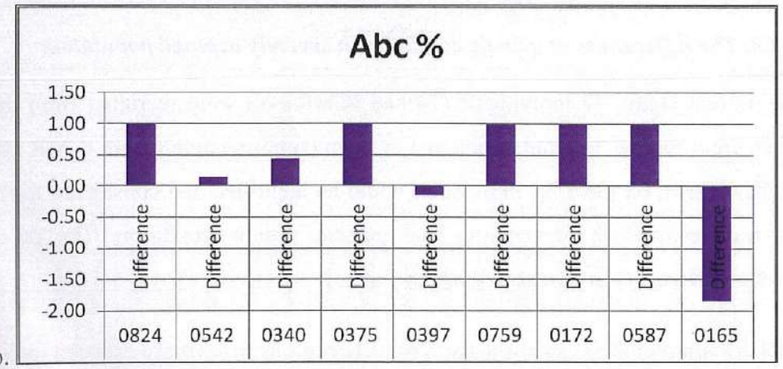




a.



b.



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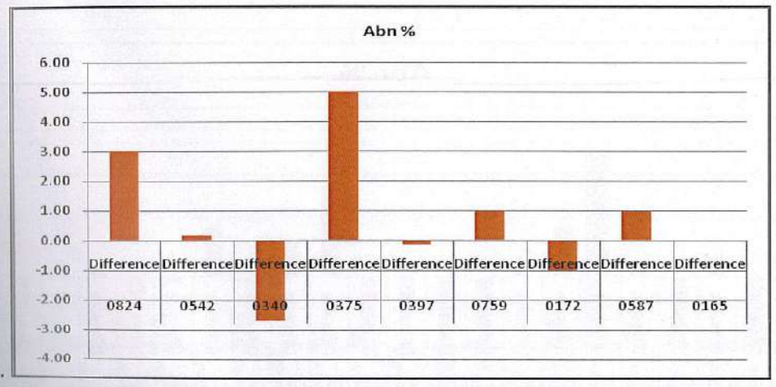

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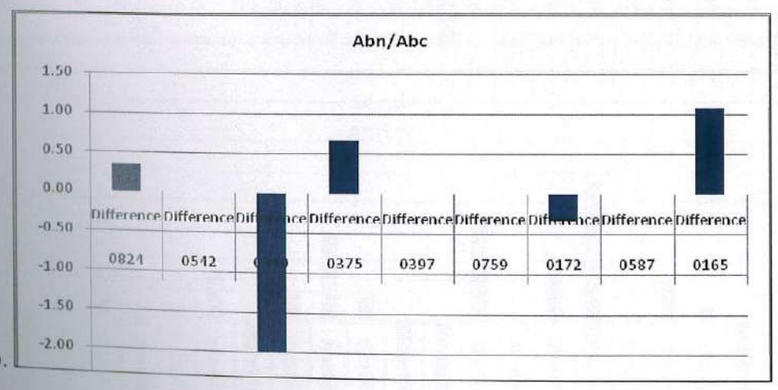


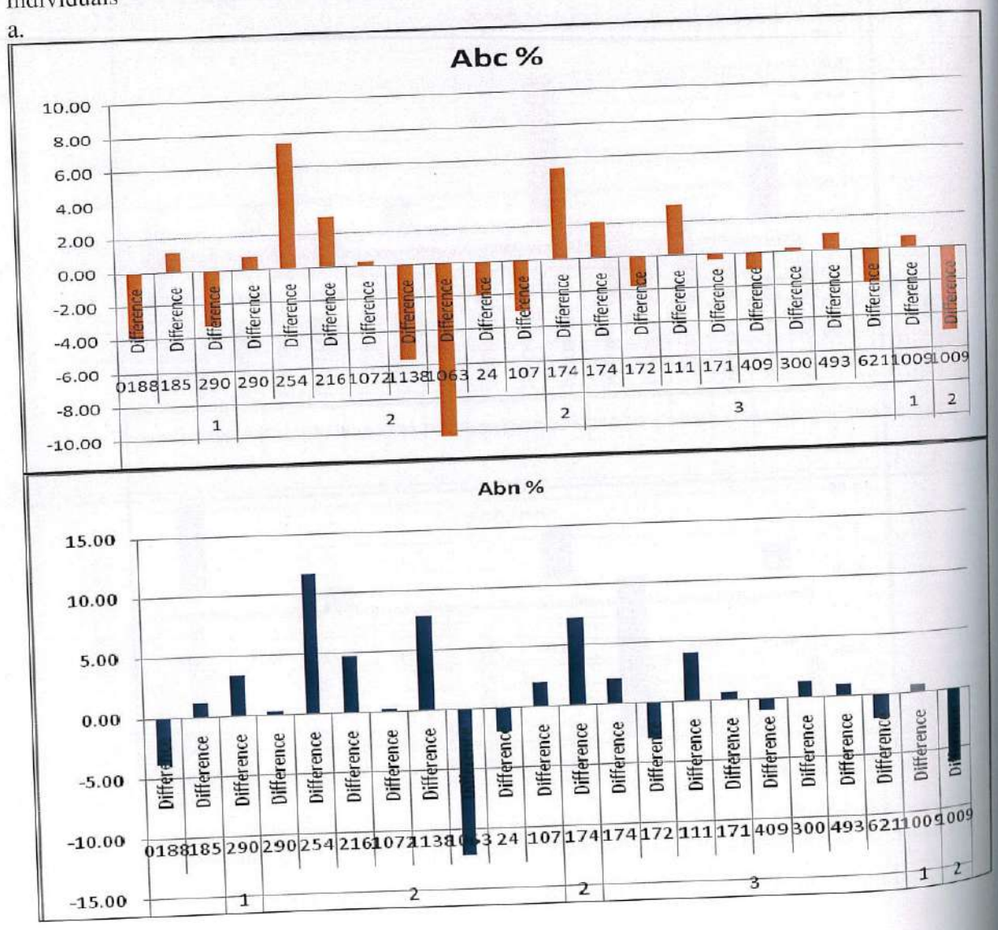
Increase in chromosome aberrations were noticed in 62.1%, 55.2% and 44.8% moderately exposed individuals for Abc, Abn and Abn/Abc parameters respectively.

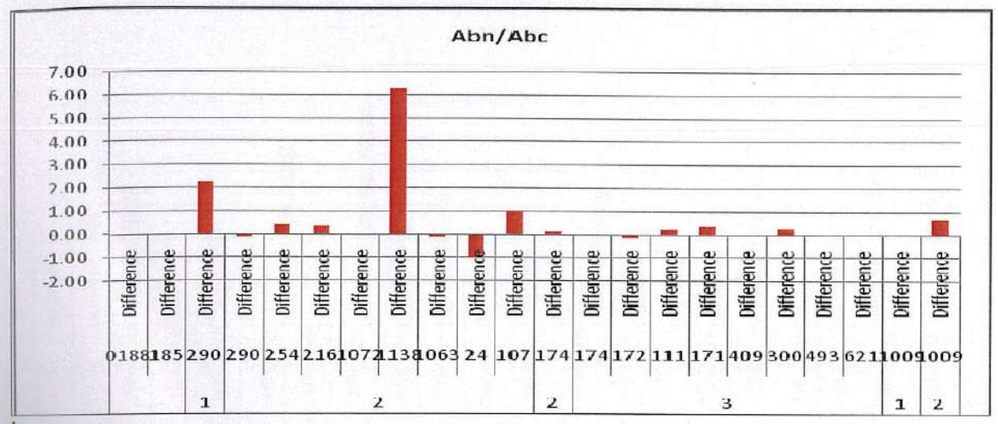
13.1.3.3. The differences of genetic condition in severely exposed population

In the present study, 77 individuals (74 had ICMR no.) were recruited from the severely exposed areas such as 1, 2 and 7 though 3 of them (spouses) didn't have ICMR no. From the previous records, 63 (**85.1%**) individuals could be identified and considered for the present one-to-one comparison of previous and present genetic condition. The details of the individual differences are presented in Fig. 9a-c.

Fig.9a-c. Distribution of changes in chromosomal aberrations in severely exposed individuals individuals

a.





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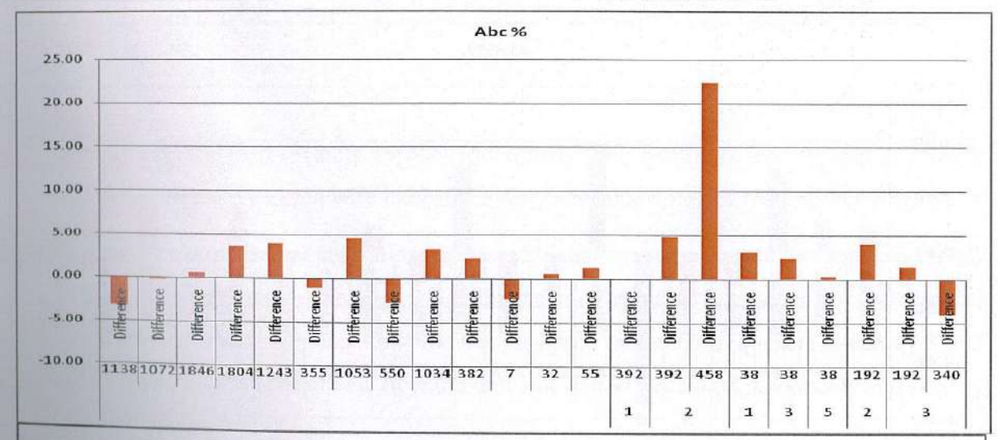

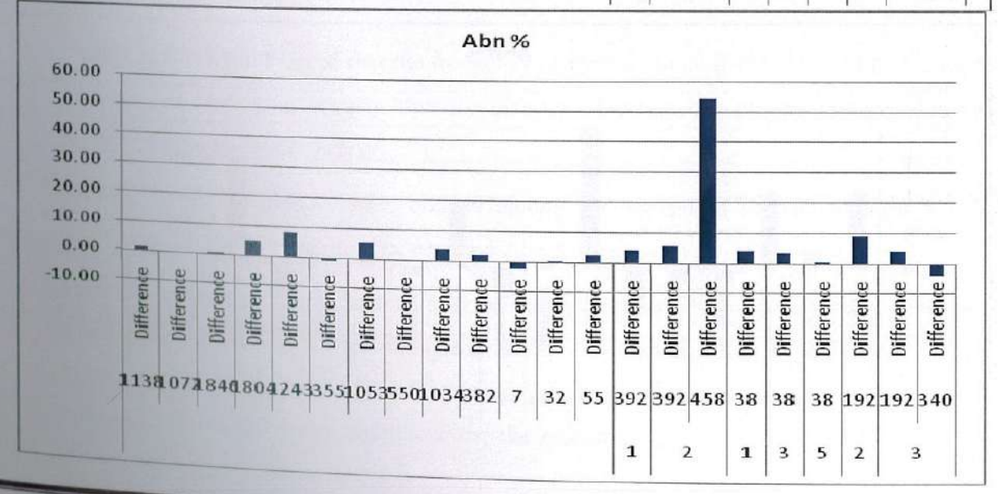

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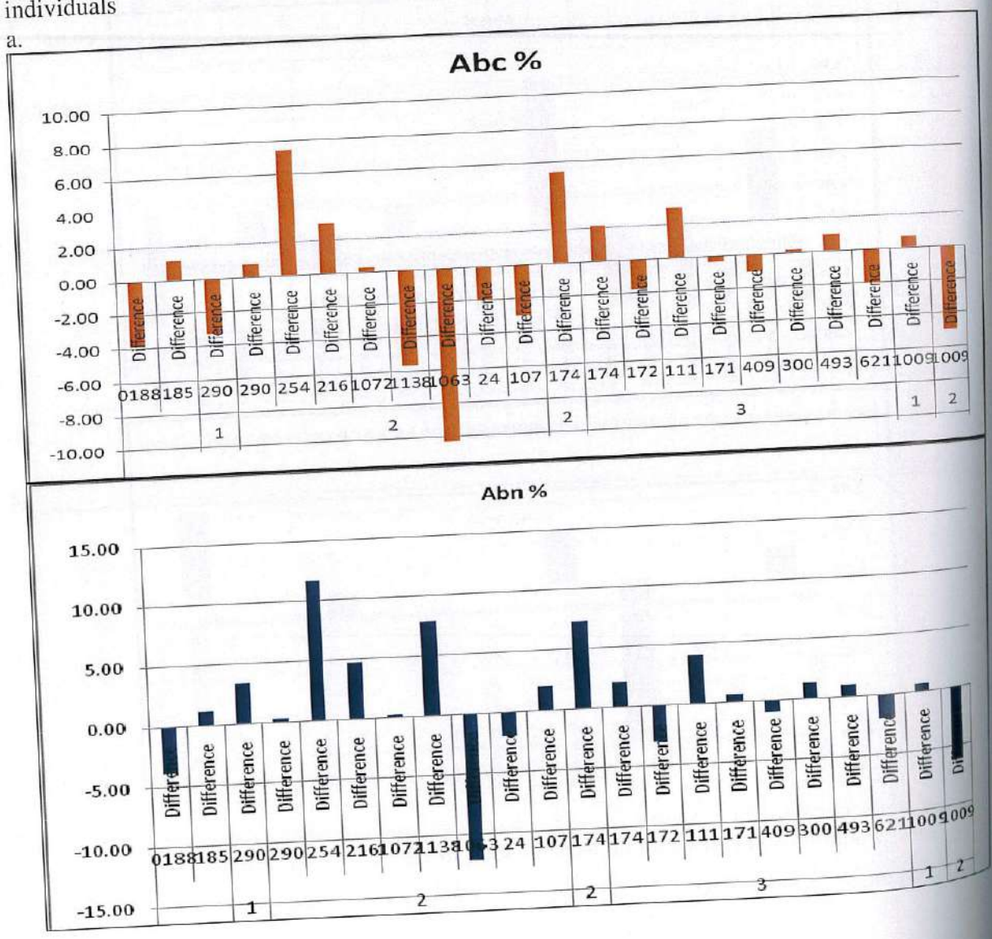
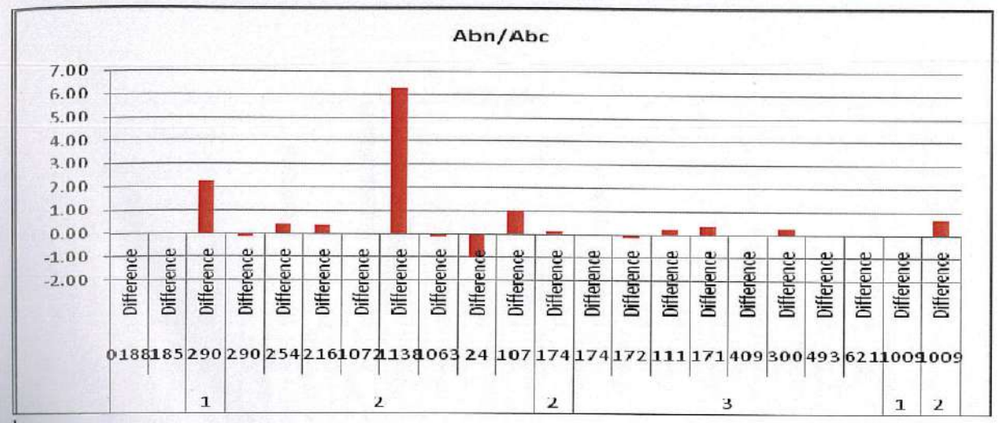
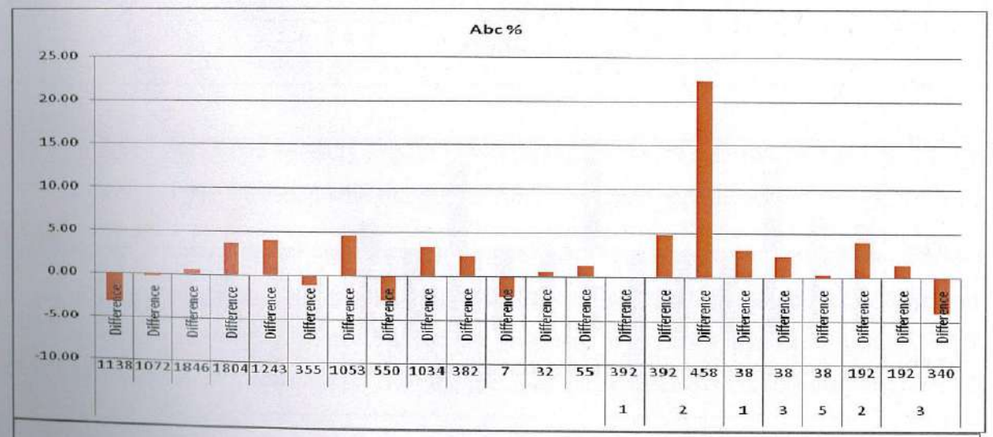
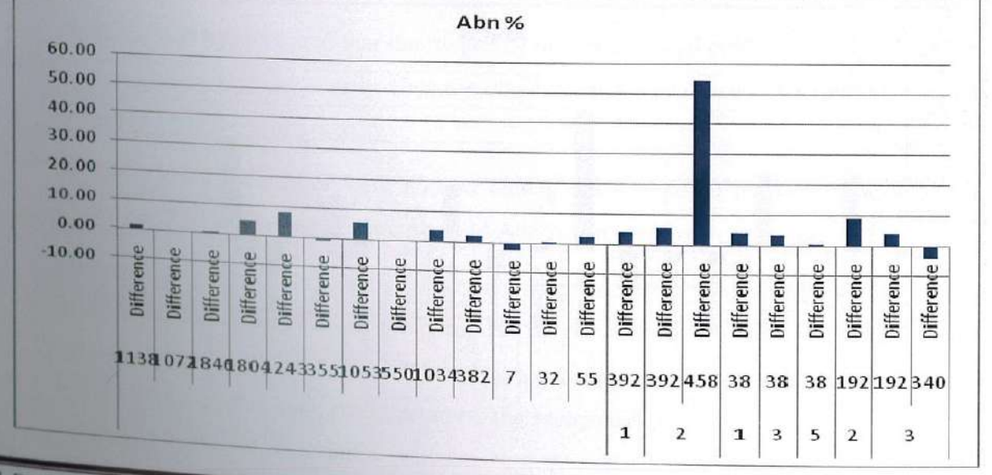
a.

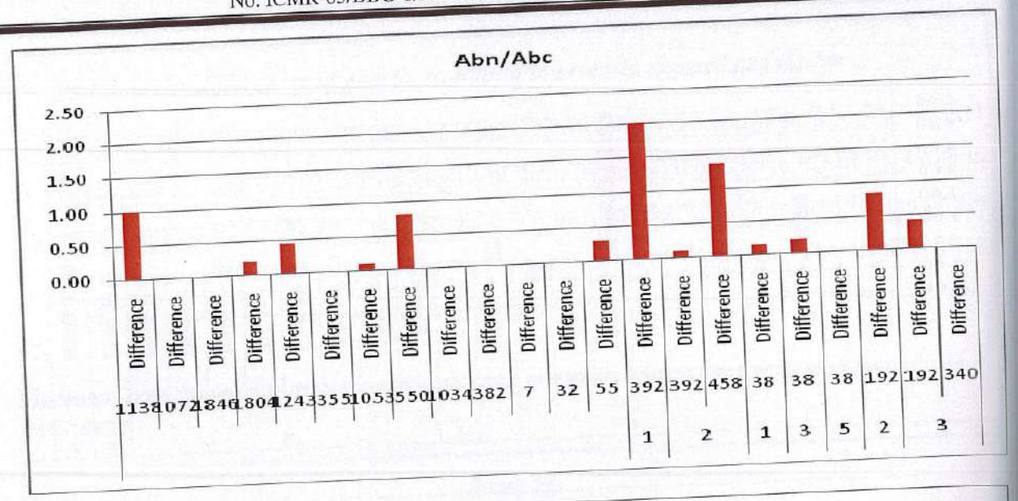




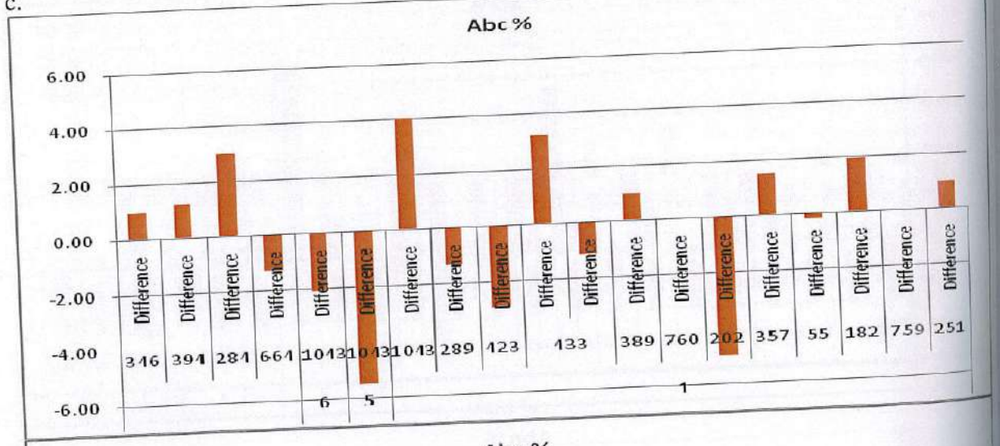
b.

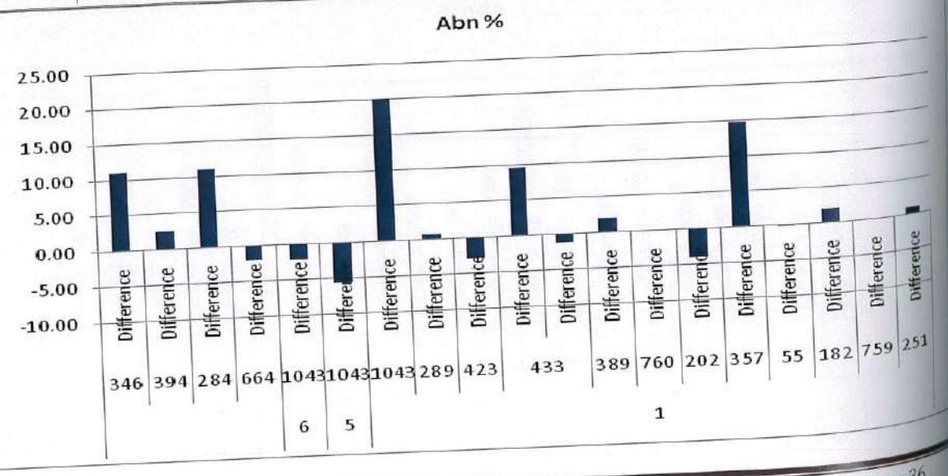


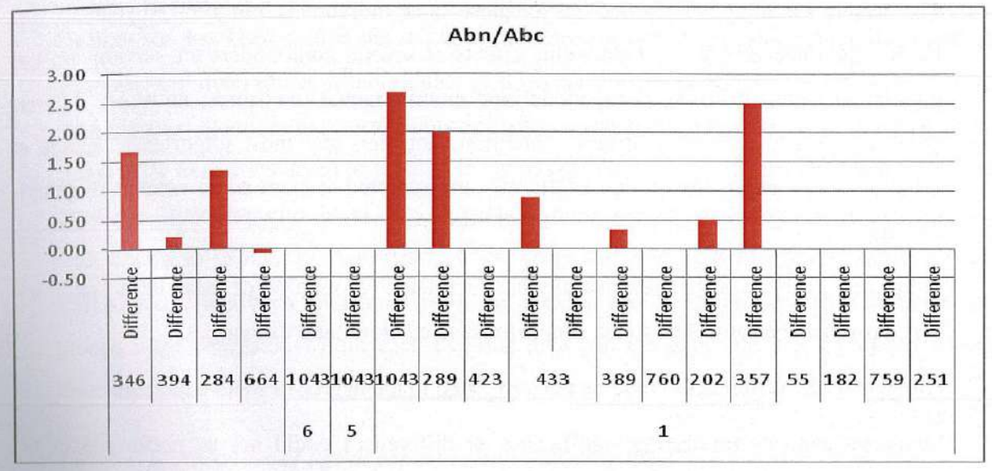




C.







Among the identified severely exposed individuals, 35 (55.6%), 42 (66.7%) and 32 (50.8%) individuals were presented with increase in Abc, Abn and Abn/Abc respectively (Fig. 9a-c).

In general, chromosomal aberrations appeared higher in the present investigation compared to that of the previous screening, which has been reflected at individual level. One-to-one comparison of Abc, Abn and Abn/Abc has presented increased frequencies, such as 57.6%, 63.04% and 48.9% respectively over the previous data collected on moderately and severely exposed population.

Ideally, it was hypothesized that the frequency of chromosomal damage would be higher in the measure carried out shortly after the disaster. However, the present investigation has presented a contradictory scenario.

Almost, similar incidences were noticed among the unexposed individuals with 52.6%, 57.9% and 21.1% increase in Abc, Abn and Abn/Abc respectively.

The possible reason could be manifold, including diversified and multiplex confounders. The previous assay was carried out during 1985-1989. Although, quality issues were strictly monitored and controlled by inter-laboratory comparison and using reagents from the same batch and lot of the same manufacturers, the recognition of aberrations were carried out from solid-stained chromosomes.

The present sampling was carried out for those same individuals, but after 30 years of gap. During the three decades, compounding effects of several confounders are obvious on any population. Age, life-style, occupational and environmental exposures, nutrition, inherent immunology and individual genetic condition, nutrition and most importantly source of drinking water, especially around UCIL, etc. are expected to exert some amount of genetic damage.

G-banding analysis in the present study has facilitated recognition of more intra- and inter-chromosomal aberrations than just simple breaks and chromatid exchanges.

Altogether, the differences could be not correlated to the degree of MIC-exposure.

Statistical analysis for testing significance of differences could not be performed in the present analysis, due to shortage of time and small sample size. However, that will be carried out after collecting similar comparative results on health parameters.

The present one-to-one comparison has depicted the picture of inter-individual variation in aberration frequency to a great detail.

Collectively, similar exercise is required on larger sample size of both previous and present investigation for comparison and grouping of increasing or decreasing trend, and statistical analysis on the pattern of changes.

13.1.4. Comparison of age at exposure and sampling time

Comparison has been carried out to check the effect of age of exposure on the incidence of chromosomal aberrations in individuals of differently exposed zones. The individuals have been grouped based on exposure status and also on the age at exposure time. Broadly, three age groups were considered to include the exposed and unexposed subjects based on age at exposure, including childhood (<1-10 years), young (11-26 years) and adult (>27 years) with a view to matching 30-40 years, 41-60 years and >60 years of age on sampling date. Exposure-age has been calculated from the present age at sampling time. The three age groups were compared within each exposed area and the exposure was compared within three age groups.

13.1.4.1. Distribution of age at exposure and sampling time

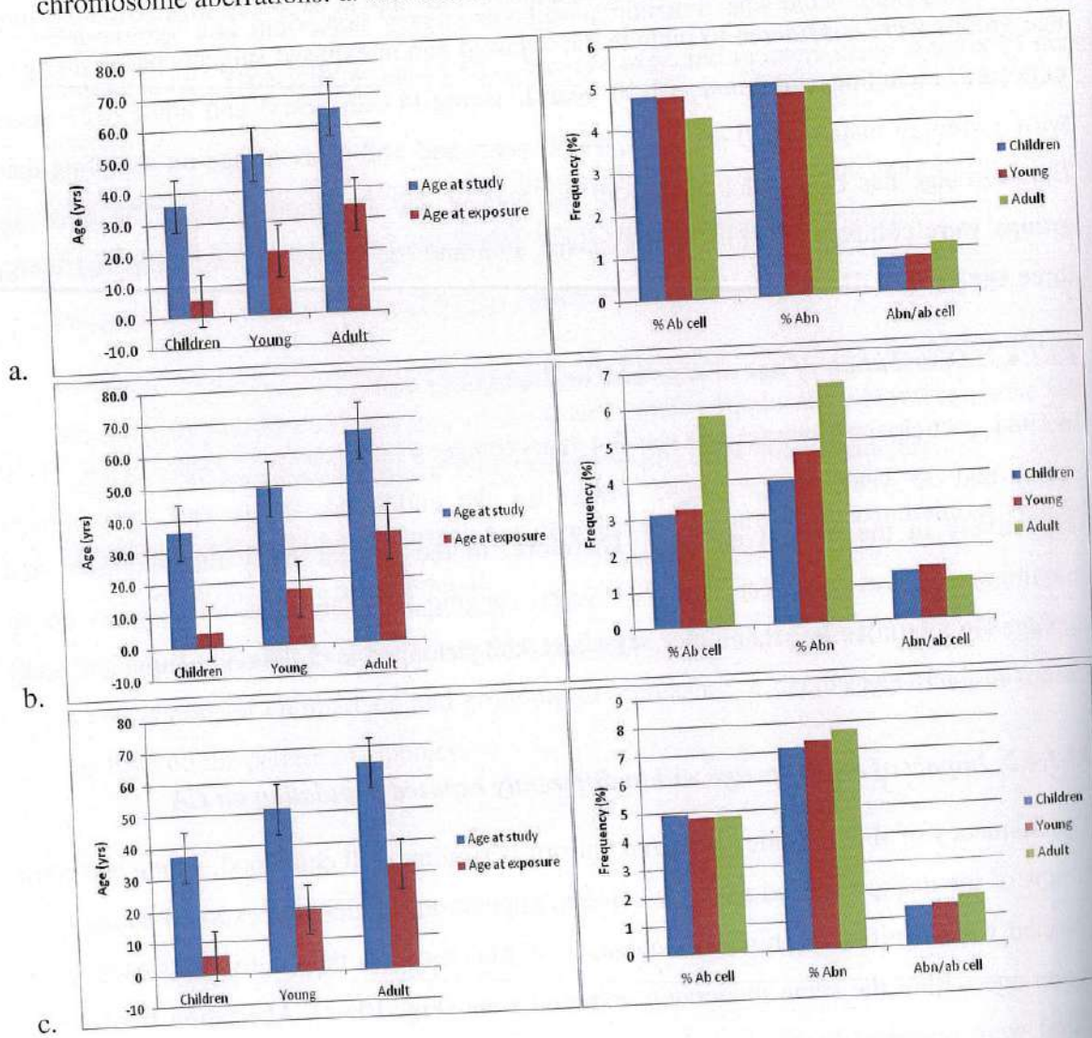
In 1984, participants' average age was 5.4 years (range: <1-10 years), 25 years (range: 11-40 years) and 35 years (range: 26-45 years) for the childhood, young and adult groups respectively in the present analysis. Therefore, in the present study the minimum and maximum age was 30+ years and 70+ years, ranging from 36 years to 66 years on an average (Fig. 10). Among them 24%, 44% and 32% belonged to childhood, young and adult groups respectively.

13.1.4.2. Impact of exposure age within differently exposed population on CA

The frequency of abnormalities appeared uniformly similar in all childhood, young and adult groups of the unexposed and severely exposed population. Moderately exposed adults were detected with significantly higher frequencies of Abc and Abn than individuals exposed at young age within the same moderately exposed zone (Fig. 10a-c). Aberration frequencies (Abn) were observed higher in individuals exposed at young and adult age compared to childhood exposure; however, the differences were not statistically significant due most likely to higher SD values, which indicate higher dispersion of aberration frequency among individuals. The incidence of Abn/Abc was higher in the adult group than exposure at childhood and young age of both control and severely exposed zones. However, Abn/Abc frequency was significantly lower in the adult group compared to childhood and young age

groups exposed moderately. In contrast, the Abc and Abn frequencies were higher in the adult group. Abn/Abc was markedly high in individuals exposed at childhood in both moderately and severely exposed areas (Fig. 10a-c).

Fig. 10. Age of MIC-exposed individuals at exposure and sampling time and frequency of chromosome aberrations: a. control; b. moderately exposed; c. severely exposed

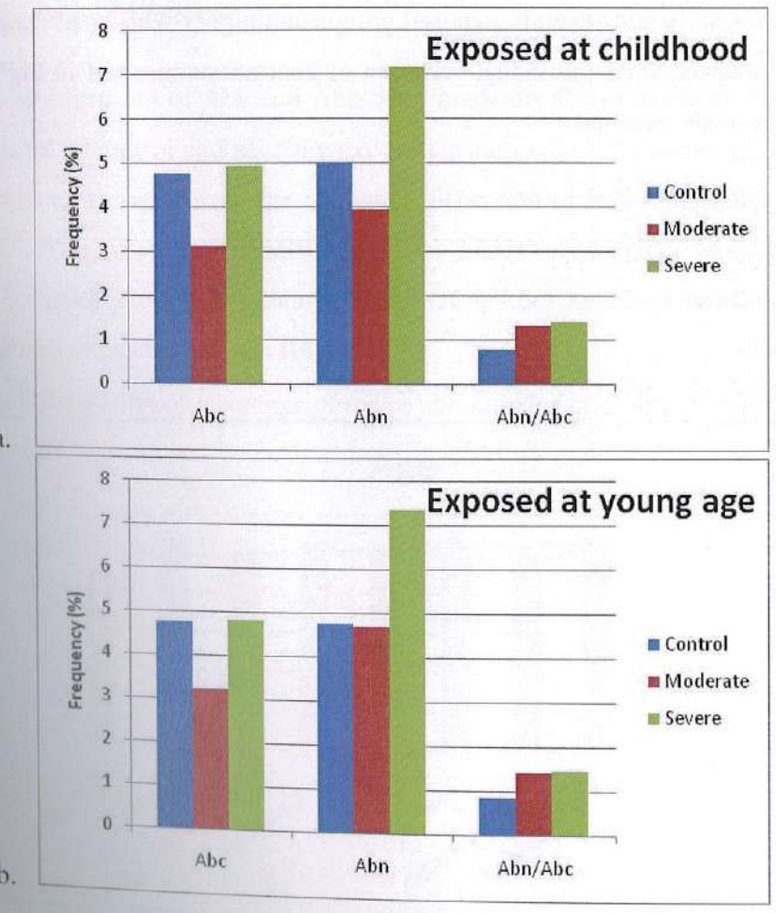


13.1.4.3. Impact of MIC-exposure on CA in different age groups

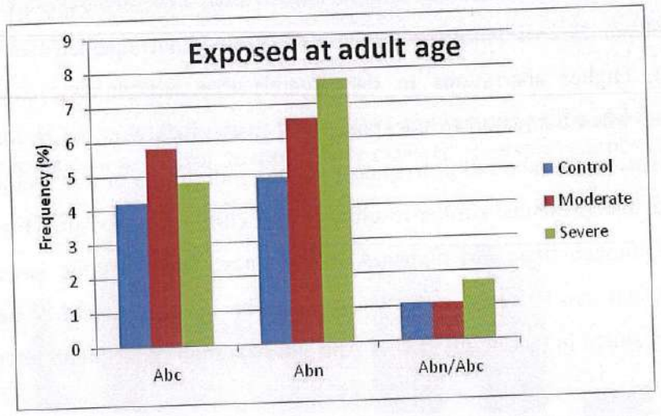
The frequency of Abn among the individuals exposed at childhood was slightly higher in severely exposed group than unexposed control, though there was no difference in Ab cell. In contrast, children exposed moderately have presented lower frequencies of Ab cell and Abn than even unexposed controls. However, frequency of Abn/Ab cell was significantly

higher in this group indicating larger Abn/Ab cell. Severely exposed children also have higher frequencies of Abn/Abc, which is revealed in frequencies of Abn and Ab cell (Fig.11a). Higher aberrations in the severely exposed children were not statistically significant when compared to unexposed and moderately exposed children, mainly due to higher dispersion of aberration frequency among individuals of this group. MIC-exposure at young age also presented similar result to that of childhood exposure (Fig.11b). Exposure at adult age, though presented higher Abn-frequency, didn't acquire larger number of Abc (Fig.11c), and that has been reflected in Abn/Abc. Adult-exposure at moderately exposed zone has resulted in marginally higher Abc and Abn than control with lower Abn/Ab cell.

Fig. 11. Impact of exposure age on chromosome aberrations in different exposed areas: a. childhood; b. young; c. adult



c.



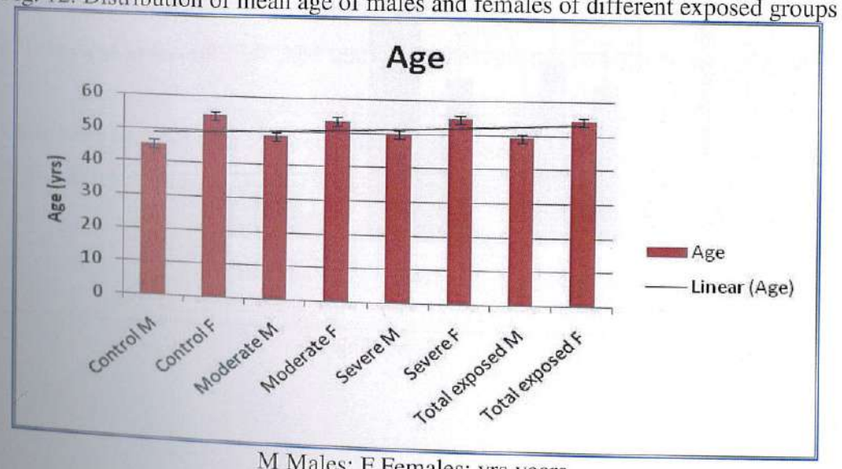
Altogether, exposure age has impacted on acquired aberrations resulting in higher Abn/Abc in both moderately and severely exposed groups and higher Abn in adults exposed severely to MIC. However, inter-individual variation of aberrations resulted in higher dispersion of frequencies of aberrations.

13.1.5. Frequency of chromosome aberrations in males and females within and between exposed groups

The frequency of chromosome aberrations was compared between the two sexes within and between differently exposed groups. The age-range of the subjects appeared uniform in both males and females, though females appeared with slightly higher age compared to that of males of different groups (Fig. 12). There was no significant difference of abnormal cells (Abc) and aberrations (Abn) in females of different exposed and control groups. However, frequency of aberrations were MIC-exposed females compared to controls, though the difference was not statistically significant due mainly to higher dispersion of aberrations frequency, which was revealed in high SD values (Fig.13a). The incidence of aberration per abnormal cells (Abn/Abc) was not different in different groups in females. Higher dispersion indicates inter-individual variation.

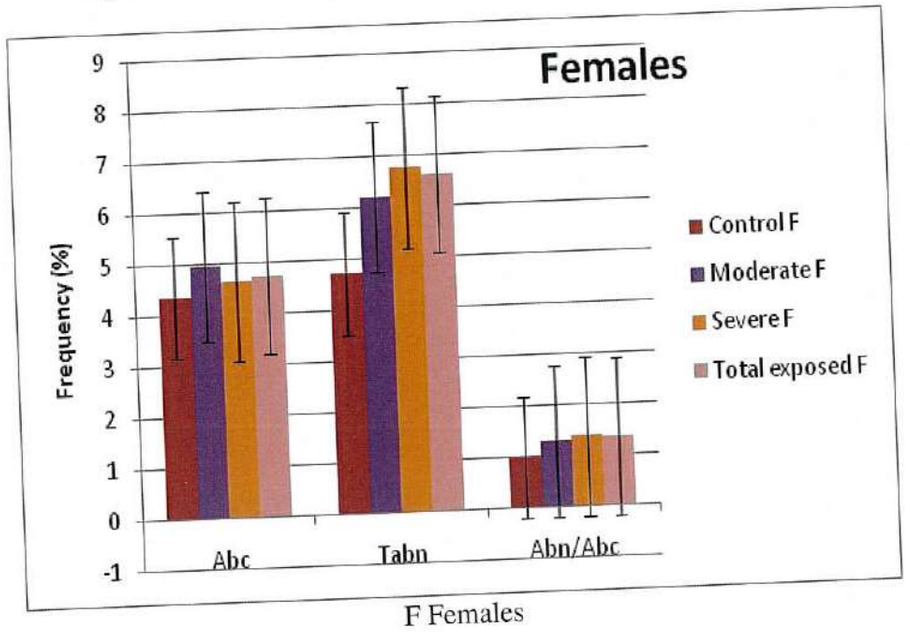
In males, the frequencies of Abc and Abn were markedly lower in the moderately exposed group compared to control and severely exposed groups (Fig.13b); however, the differences were not statistically significant due to higher dispersion or inter-individual variation. The frequency of Abn was significantly higher in males of severely exposed group than moderately exposed group. The ratio of Abn (Abn/Abc) appeared markedly high in the severely exposed males than others (Fig.13b).

Fig. 12. Distribution of mean age of males and females of different exposed groups



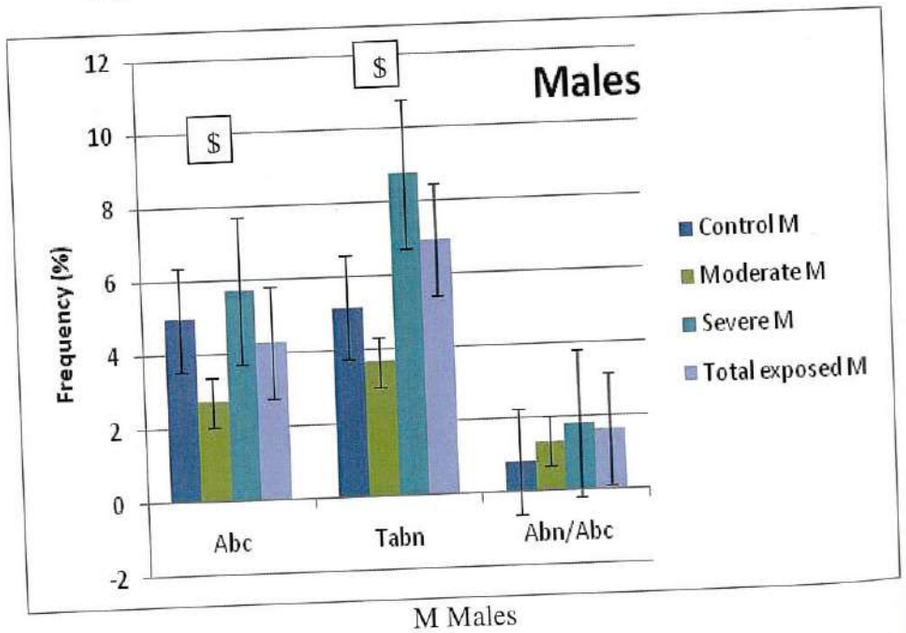
M Males; F Females; yrs years

Fig.13a. Distribution of chromosome abnormalities in females



F Females

Fig.13b. Distribution of chromosome abnormalities in males



M Males

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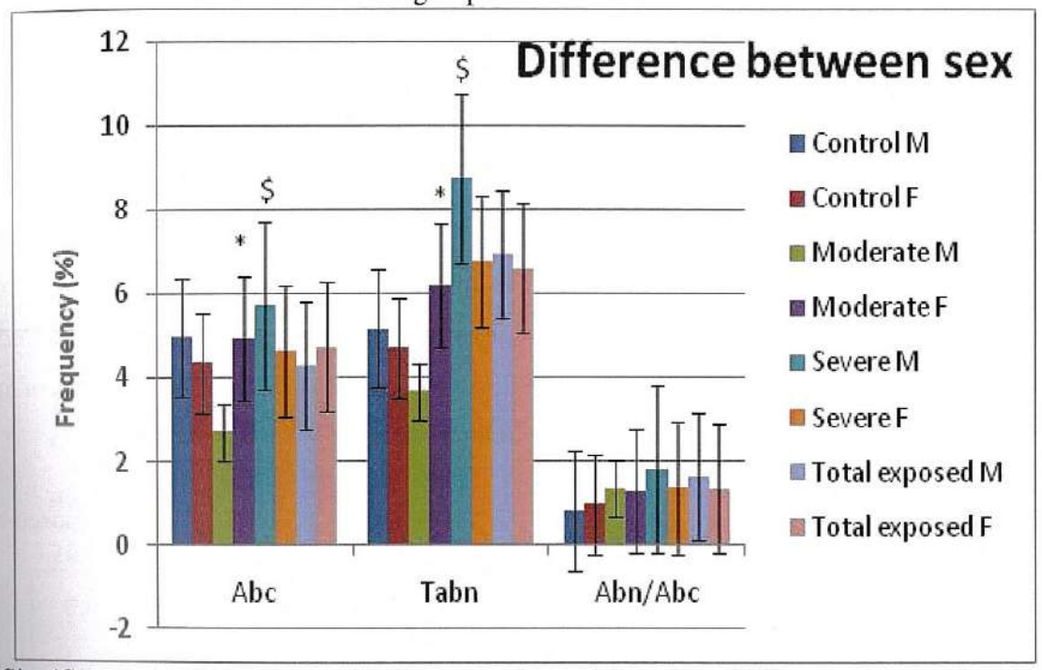
Fig. 14. Distribution of chromosome aberrations in males and females of differently exposed groups and control



*Significant at <0.05 compared between males and females within same exposed groups; $significant at <0.05 when males were compared between moderately and severely exposed groups

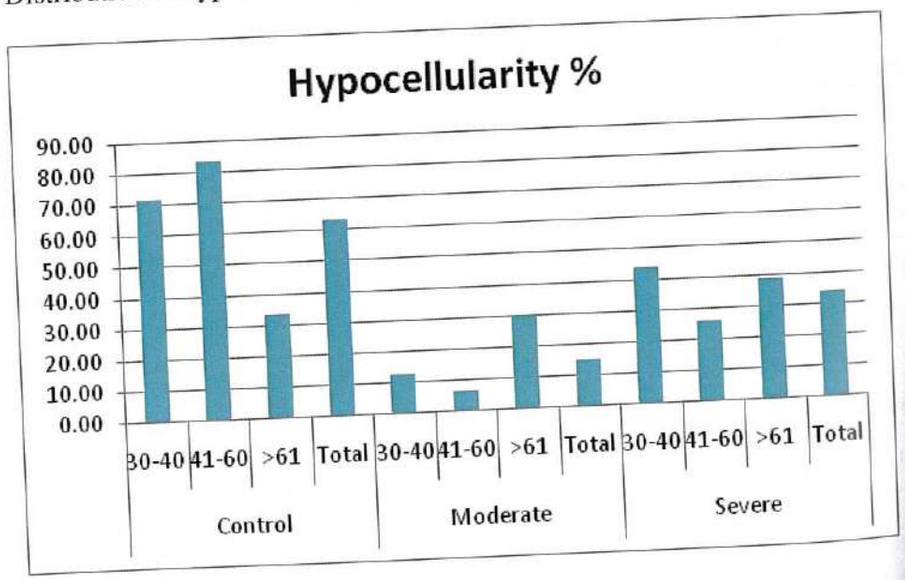
Collectively, the difference of Abc and Abn between the two sexes were higher in the moderately exposed females compared to males of this group, which was statistically significant. On an average, the frequencies were higher in exposed males than females, though the differences were not statistically significant (Fig. 14).

13.1.6. Hypocellularity in peripheral blood and acrocentric association in differently exposed population

13.1.6.1. Hypocellularity

Besides chromosome aberrations, several cases were appeared with extremely hypocellular culture of the peripheral blood, which led to exclusion of few cases from the final comparison. The cases with a minimum of 25 metaphases studied were included for final analysis. The incidence of hypocellularity is presented in Fig.15. Hypocellular condition was noticed in the sampling carried out in 2014 in JP Nagar area, and the observation was mentioned in one of NIREH's scientific meeting held in September 2014. A routine investigation on blood parameters, particularly complete blood count (CBC) needed to be carried out for adjustment of cells in culture and monitoring of general health of people; however, the proposal was not approved by that committee.

Fig.15. Distribution of hypocellularity in different age groups of differently exposed zones

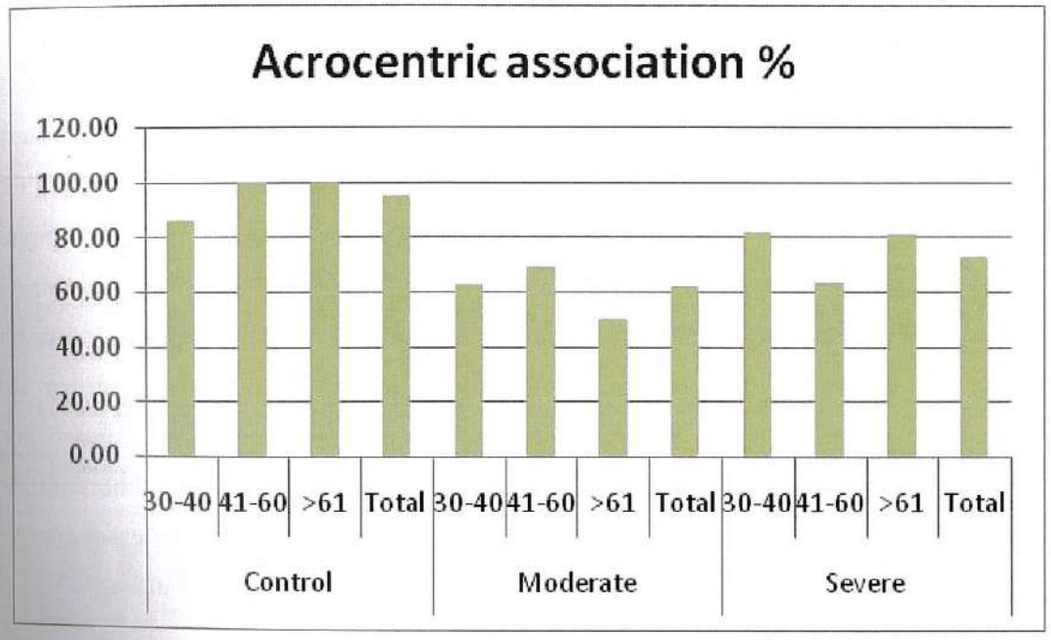


13.1.6.2. Acrocentric association

Interestingly, high frequency of acrocentric association was noted in almost all areas of Bhopal included in the present study, which was relatively lower in the moderately exposed area compared to control and severely exposed zones (Fig.16). The incidence was higher in

the control group, which might have been influenced by less number of samples studied in this group. The number of chromosomes ranged from 2-10 of 12 acrocentric chromosomes (group D: 13, 14, 15; group G: 21, 22) in association. I some assembly, the acrocentric chromosomes appeared to be jumped on other chromosomes lying in the vicinity of such association.

Fig.16. Distribution of acrocentric association in differently exposed and unexposed zones



This situation of acocentric association was highly interesting; however, it requires further investigation on the type of aggregation (groups), chromosomes involved and order/sequence of chromosomal arrangement in the assembly. Such study could open up a new area of research on acrocentric association, especially its clinical significance and the chemistry of attraction of different chromosomes in such association. Further investigation could be carried out on Bhopal population in keeping the environmental, life-style, occupational, age, and nutritional factors into account.

13.1.7. Constitutive abnormalities detected in the present study population

Constitutive abnormalities (ConA) occur congenitally and are present in every cell uniformly or in mosaic pattern. Such abnormalities appear *de novo* or inherited from one or both parents. G-banding analysis in the present study has enabled identification of constitutive or genomic abnormalities via ISCN classification along with recognition of chromosomes involved and their breakpoints. In the present study, 11 individuals (8.5%) of total 130 were detected with ConA involving chromosomes 9 (3 cases), 16 (5 cases), 6 and 11 (1 case), 14 (1 case) and 15 (1 case) in the form of deletion, inversion and balanced translocation (Table 7; Fig.17). The frequencies within differently exposed groups indicate 7 cases (9.5% within group and 63.6% among the cases with ConA) from severely exposed area; 3 cases (8.8% within group and 27.3% among the cases with ConA) from moderately exposed area; and 1 case (5.6% within group and 9.09% among the cases with ConA) from control area. However, further tests are recommended, including high resolution banding and test for fragile sites in specially formulated culture medium. Also further study is strongly recommended on larger sample size of these and more other exposed zones of different municipal wards, following constitutive G-banding. Constitute abnormalities were not considered for compilation of acquired abnormalities, and thus, these cases were treated similarly with other individuals who otherwise had normal karyotypic pattern.

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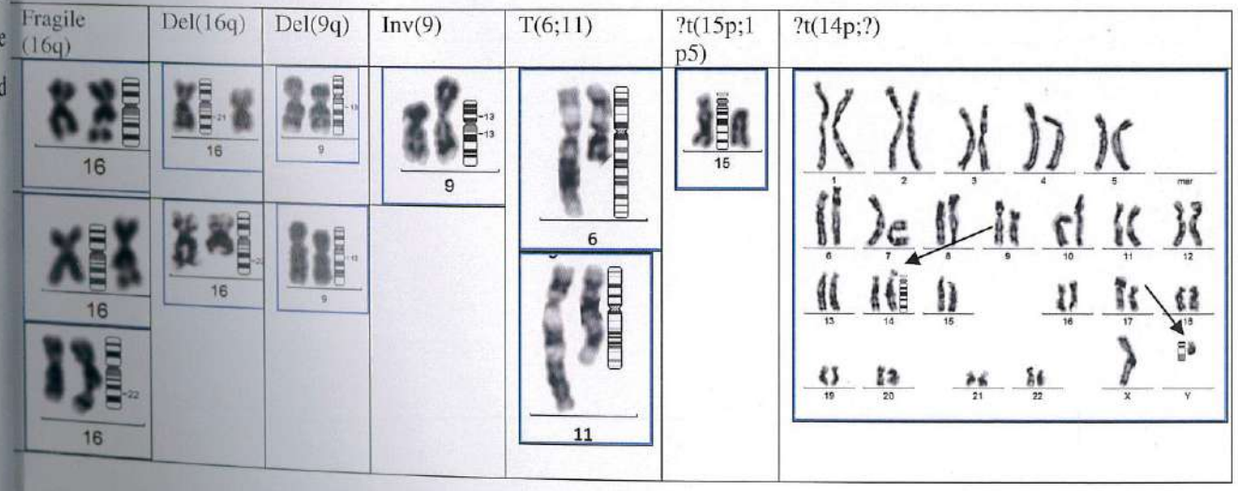
Table 7. Constitutive abnormalities detected in MIC-exposed Bhopal Population 30 years post disaster

ICMR No.	Area	Exposure status	Age/Sex	Abnormality	Frequency within group		Test recommended
1138	1	Severe	59 Y/M	46,XY,?del(9q13) [84]	3%		HRB; P, S, C
1009	1	Severe	38 Y/M	46,XY,?del(9q13) [38]*			HRB; P, S, C
1243	1	Severe	50 Y/F	46,XX,fra(16q22) [100]	4%		Fragile analysis; P, S, C
355	2	Severe	40 Y/F	46,XX,?fra(16q22) [100]			Fragile analysis; P, S, C
357	7	Severe	40 Y/M	46,XY,fra(16q22) [76]*			Fragile analysis; P, S, C
192	7	Severe	60 Y/M	46,XY,smallY,?t(14p;?)	1.4%	9.5%	HRB; P, S, C
1804	1	Severe	45 Y/F	46,XX,inv(9)(p11q13) [100]	1.4%		P, S, C
1009	3	Moderate	45 Y/F	46,XX,?del(16q22) [100]	5.9%	8.8%	P, S, C
759	5	Moderate	86 Y/M	46,XY,?del(16q22) [100]			S, C
375	5	Moderate	45 Y/M	46,XY,15p+/15s-?t(15p;15p)	2.9%		HRB; P, S, C
496	16	Control	70 Y/F	46,XX,t(6;11)(q15;q23) [100]	5.6%	5.6%	P, S, C

*Hypocellular sample; HRB High Resolution Banding; P Parents; S Siblings; C Children

Fig.17. Partial karyotypes with constitute abnormalities in different karyotypic pattern



The finding is very important to note that similar study is highly recommended for their parents, siblings and children for evaluation of the source of the aberrations; distribution among the members of blood-lineage; estimation of risk in future generations to come and future risk of the affected individuals. The study will be of highly significance for evaluation of MIC-effect meaning whether MIC exposure

resulted in origin of these inter- and intra-chromosomal rearrangements. Complete family study following genetic counseling will unravel its association with MIC-exposure. It must be noted that acquired del(16q22) is associated with hematological malignancy (FAB: AML-M4). Ideally, molecular karyotyping by array based single nucleotide polymorphism (SNP)-typing and comparative genomic hybridization (CGH) would be of immense help for recognizing small submicroscopic deletions, duplications, etc. and copy number variations (CNV), at least for these index cases and their blood-linked relatives.

13.1.8. Differences in chromosome aberrations in members of the same family

The subjects bearing the same ICMR # were assumed to belong to the same family. These cases were segregated and analyzed for evaluating the differences in chromosome aberrations. The subjects were of different age and sex, and also different patterns of relationship (Table 8). Mostly these subjects were located in the same locality or exposed areas. Altogether 29 such families were isolated for comparison of the chromosome abnormalities within the same families of similarly exposed zones. However, due to inadequate yield of metaphases, 7 families (marked in yellow) were not considered for comparison though three members were tested on repeat samples. Finally, 22 families were considered for this analysis. One family had three members, mostly like father and two sons. The purpose of this comparison was to check inter-individual variation within the same family living in a similar environmental and socio-economic condition. The relationship among the members could be siblings, couples, parent child, and so on, which was purely assumed from the age and sex.

Table 8 . Sample matrix of the number of subjects of the same families

Area #	ICMR #													
	Severe							Moderate						
	290	346	192	1043	1009	38	55	24	185	392	254	1138	111	1308	1063	172	624	1156
1	60 m 52 f								60 m 55 f		70 f 45 m	59 m 50 f	56 f	47 m 35 f 14 f		54 m		
2					38 m			70 m 61 f					58m					
7		60 f	66f 44m	58 m 38 m 33 m		71 m 40 m	60 f 37 f								60 f 39 m		68 m 63 f	52 m 32 m
3					45 f					54 m 46 f						49 f		
10									493		498	269	518	312				
ICM R #	Con trol	346	441	338		274	362	382	60 m 53 f		60 f	50 f	54 m 48 f	70 m 37 m				
16		40 m	60 f 45 m	70 m 65 f		50 f 31 m	65 f 35 m	53 f 38 m			35 m	35 m						

Required repeat test, not included due to less no. of metaphases studied

The frequencies of Abc, Abn and Abn/Abc has been presented in each individual family to capture the pattern of variation in the same family (Fig. 18). There was no pattern of variation on the chromosomal aberrations; however, mostly older members were observed with higher CA with no differences between the two sexes (Fig.18). A consolidated display of Abc, Abn and Abn/Abc together for all members of 22 families represent indicated the pattern of variation between members and within families (Fig.18). When these variables are presented separately as Abc% and Abn%, the spectrum of variation appeared very clear (Fig. 19). Statistical analysis has not been carried out to comment on significance of these variations, due mainly to small sample size.

Fig. 18. Differences in chromosome aberrations between the members within the same family

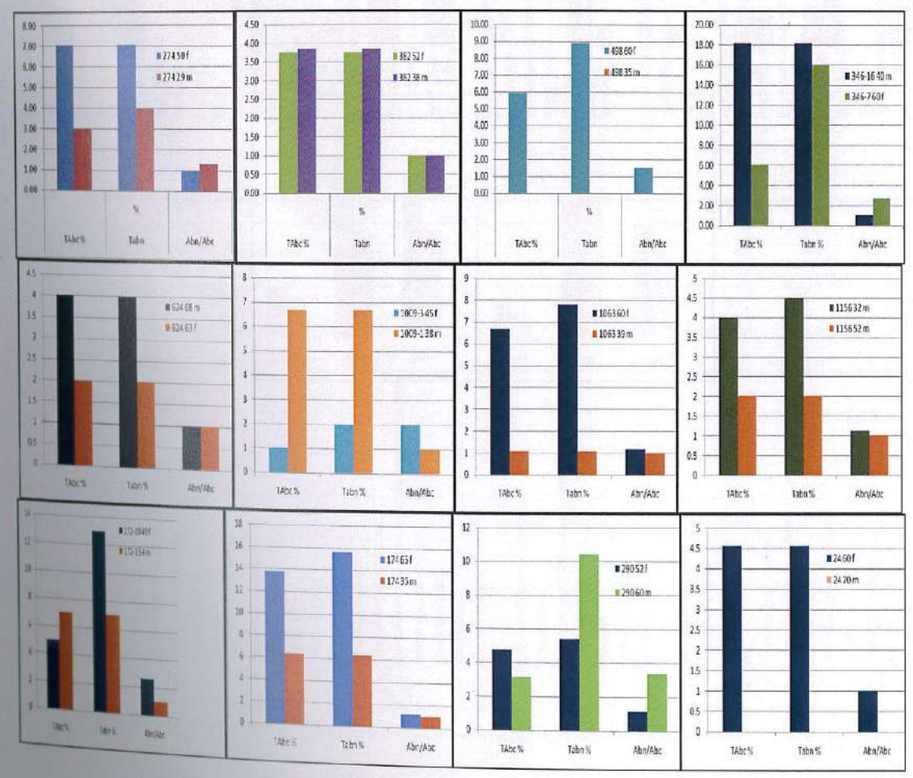
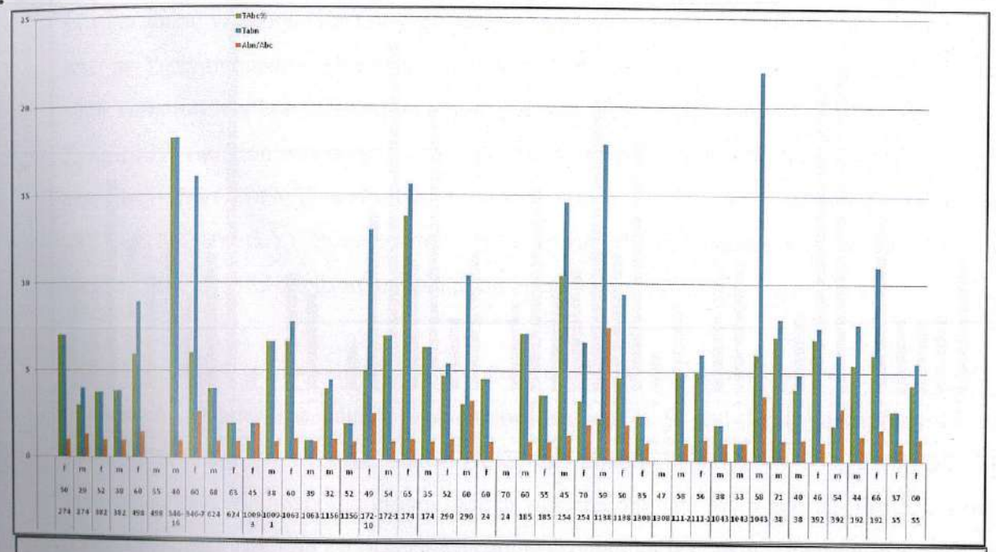
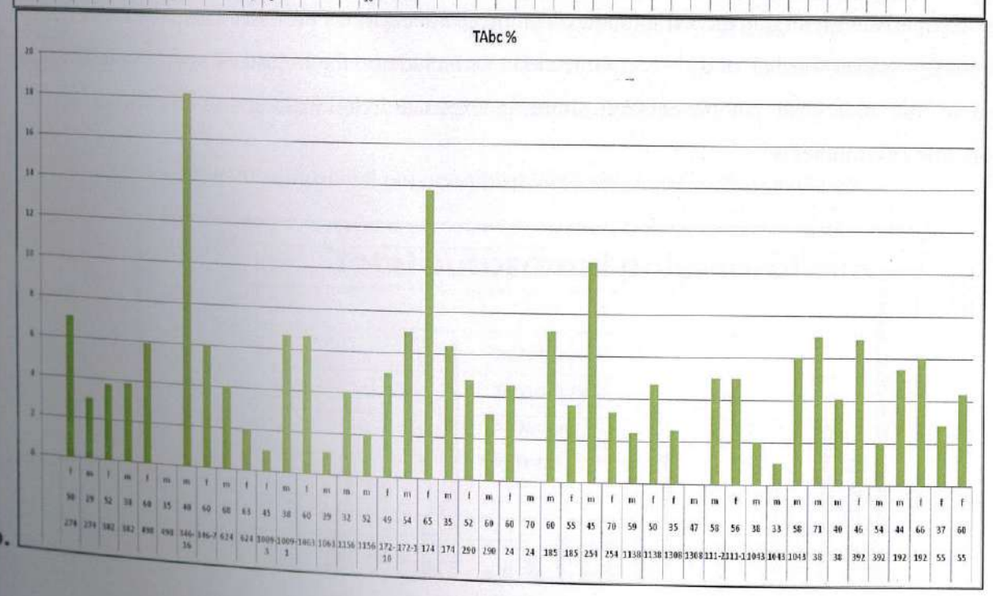


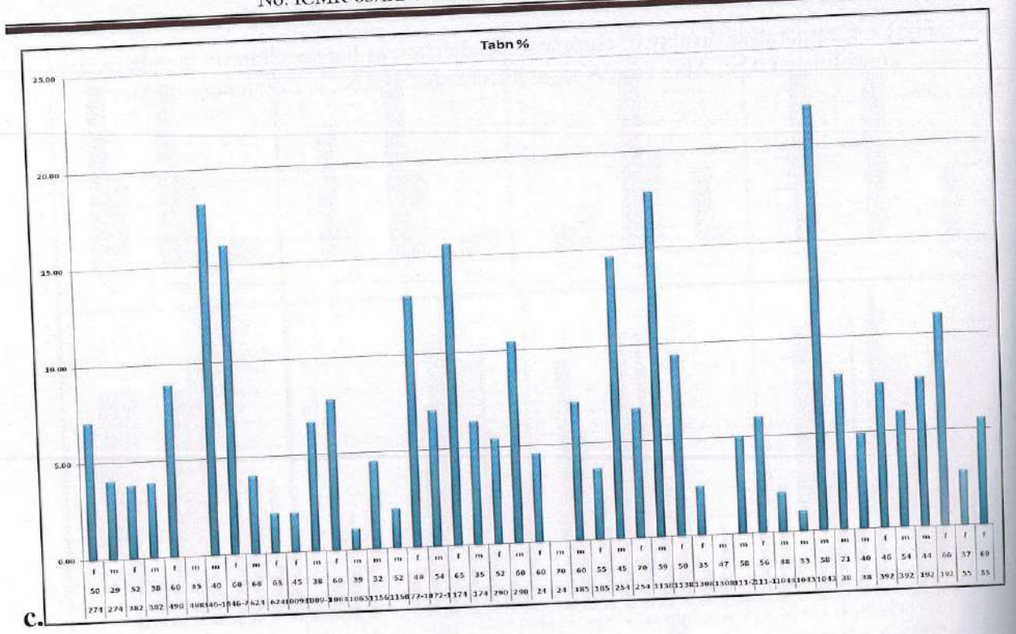
Fig.19. Comparative display of chromosome aberrations between family members: a. consolidated Abc, Abn, Abn/Abc; a. differences in Abc; c. differences in Abn

a.



b.





c.

The comparison highlights the variation in chromosome aberrations between members of the same families; however, further study is recommended to conclude on the impact of age, sex, individual genetic and biological components on similarly exposed individuals living in similar socio-economic environments.

13.1.9. Chromosomal polymorphism detected in different individuals of differently exposed zones

Chromosomal variation (var.) is often seen in apparently normal individuals in autosomes as well as Y chromosomes. However, no clinical significance has yet been established. Thus, such variation has been termed as polymorphism. Bhopal population is not an exception. Frequently, variation was noticed in pericentric heterochromatin (qh+; Het.) of 1, 9 and 16; prominent short arm in D- and G-group acrocentrics (p+) in 13, 14, 15, 21 and 22; and small (SY) or long Y (LY) chromosome. The purpose of this analysis was to correlate its incidences with MIC-exposure or habitation in exposed areas.

The frequency of chromosomal polymorphism has been demonstrated in the present study-population. Inconsistent pattern was noticed among 1, 9 and 16 in heterochromation variation in relation to exposure status. However, variation in satellite was higher in control population than MIC-exposed groups, which was predominant in 15 and 22 (Fig.20). Total Y-chromosome variation was prevalent in severely exposed group, which was mostly influenced by SY. LY-variation was inconsistent, though higher in moderately exposed group than control and severely exposed population (Fig.21).

Fig.20. Autosomal polymorphism detected in the study population

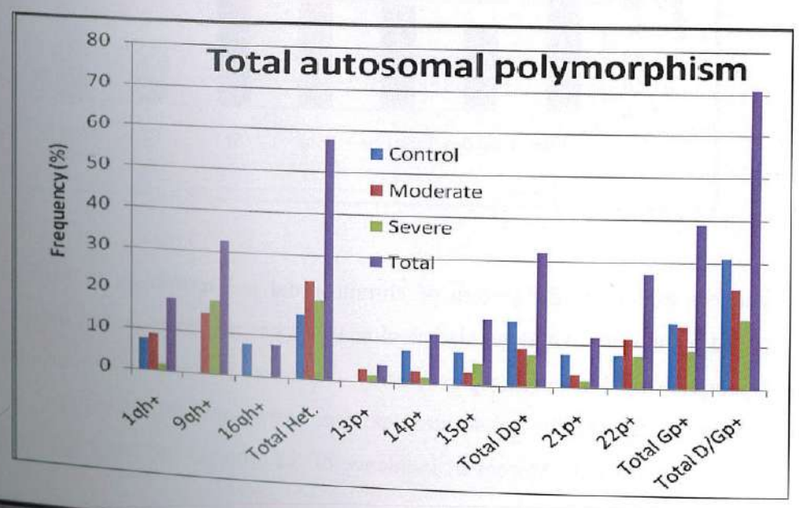
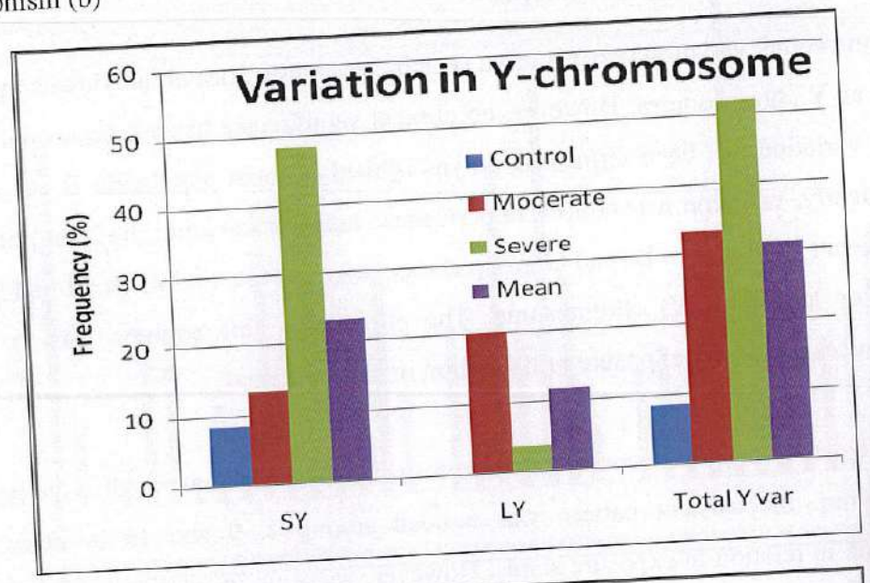
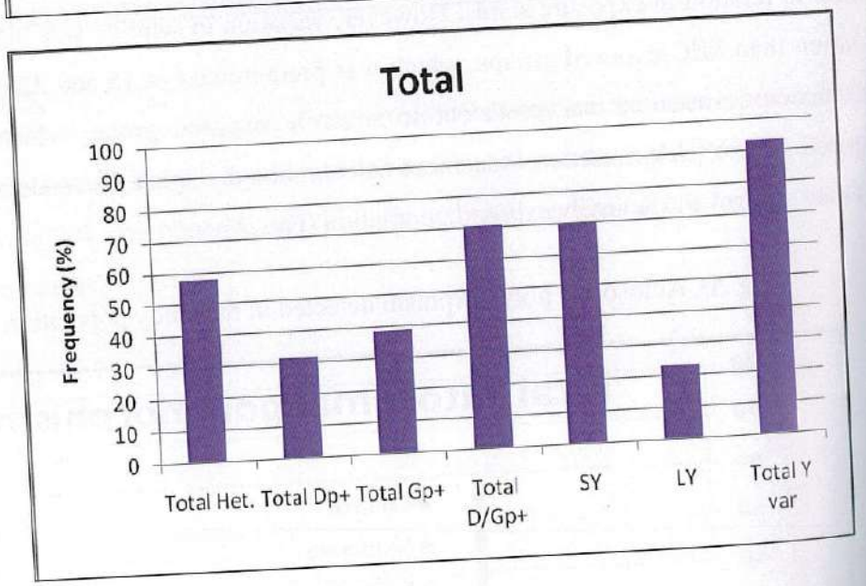


Fig.21. Variation in Y-chromosome in differently exposed groups (a) and total polymorphism (b)

a.



b.



The analysis highlights the pattern of chromosomal polymorphism in MIC-exposed and unexposed population. Although statistical analysis has not been carried out due mainly to small sample size for polymorphism-study, variation in total heterochromatin and Y-chromosome in moderately and severely exposed groups and prominent 'p+' in unexposed group was quite distinct. Moreover, incidence of SY, though has not been correlated with

age of the individuals (due to small sample size), indicates its shortening, which could be *de novo* or hereditary. One family with three male members indicated its hereditary origin. However, further study is required for attestation of association of chromosomal polymorphism with MIC-exposure, which might figure out hereditary pattern of SY in MIC-exposed population. **Nevertheless, it is noteworthy that *de novo* SY indicates its loss of heterochromatin, and could be associated with MIC-exposure because the frequency was 6 times and 1.5 times were in severely exposed group compared to control and moderately exposed group respectively. *De novo* SY could be the result of break or higher proliferation rate resulting in deletion or loss or nucleotides from its terminal region.**

13.2. Present health status of the study-population

The present study considered total 6 affected and two unaffected areas of 36 municipal wards of Bhopal, which included severely exposed (1, 2, 7) and moderately exposed (3, 5, 10), and unaffected areas (14, 16). Information on present health condition was collected on 168 individuals (41 control; 42 moderately exposed; 85 severely exposed) during 2014-2015. Three-generation pedigree also was collected for all of them. It is noteworthy that MIC-exposed population was mostly affected with respiratory system and eye-problems as major reason immediate and continuous mortality, and chronic morbidity. The purpose of the project was explained to the participants. Following counseling and after taking written consent, history-taking process was initiated. Informed consent forms were distributed in Hindi as well as in English (**ANNEXURE I & II**).

The present health problems were recorded on designed format through one-to-one interview and discussion for extracting genuine information on the present and past health during 30 years after the disaster. The data was tabulated for quantifying the different symptoms and systemic complications, and then grouped for further system-wise evaluation of exposed individuals of different areas. Briefly, reproductive health was measured as miscarriage (AEM) and infant-death (AED) after exposure due to illness; ophthalmic symptoms as decreased vision (DV), discomfort (ED), burning (BE), watering (WE) and swelling of eyes (SE); orthopedic symptoms were broadly noted into joint pain (JP), backache (BKP), body ache (BDP) and spinal pain (SPP); respiratory symptoms included chest pain (CP), cough (CG), breathlessness (BL) and breathing problem (BTR/PR); the gastric symptoms were included as stomach pain (STP), vomiting (VM), and other gastric problems (GPR); miscellaneous complaints categorized as fatigability (FT), fainting (FN), allergy (AG), headache (HD) and weakness (WK); whereas the other illnesses including diabetes (DM), hypertension (HT) and cancer (CN) were grouped as DM/HT/CN. Thus finally a quantitative data has been derived from grouping of the qualitative information for statistical analysis. Online facility of two-tailed 't' test has revealed significant differences between control and moderately and severely exposed separately, and between moderately exposed population for all health parameters such as reproductive, vision, orthopedic, respiratory, stomach, miscellaneous and DM/HT/CN, except the differences between control and moderately exposed population for orthopedic and respiratory systems, and for stomach problems between the two exposed populations (Fig.22).

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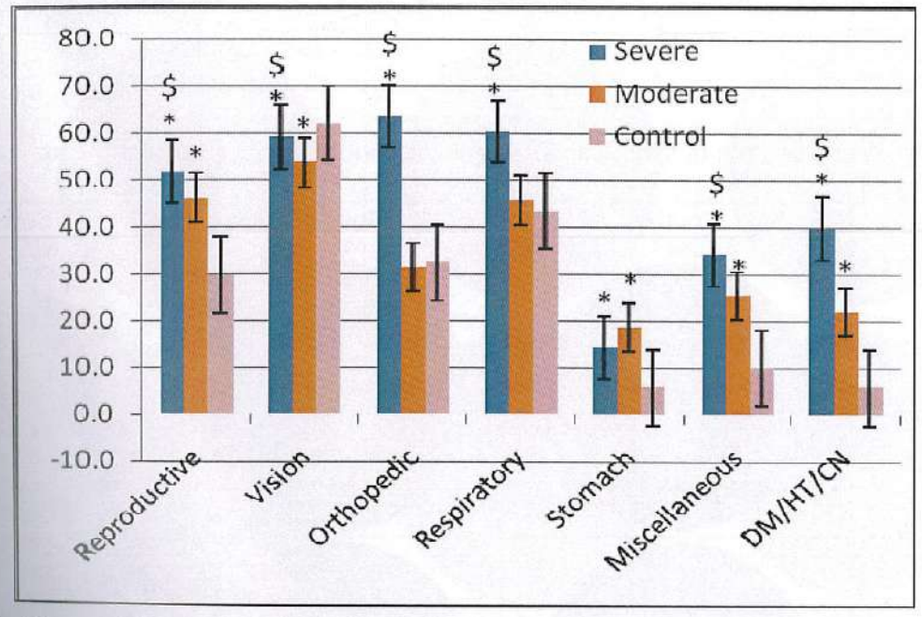
Fig.22. Health problems in control and exposed population



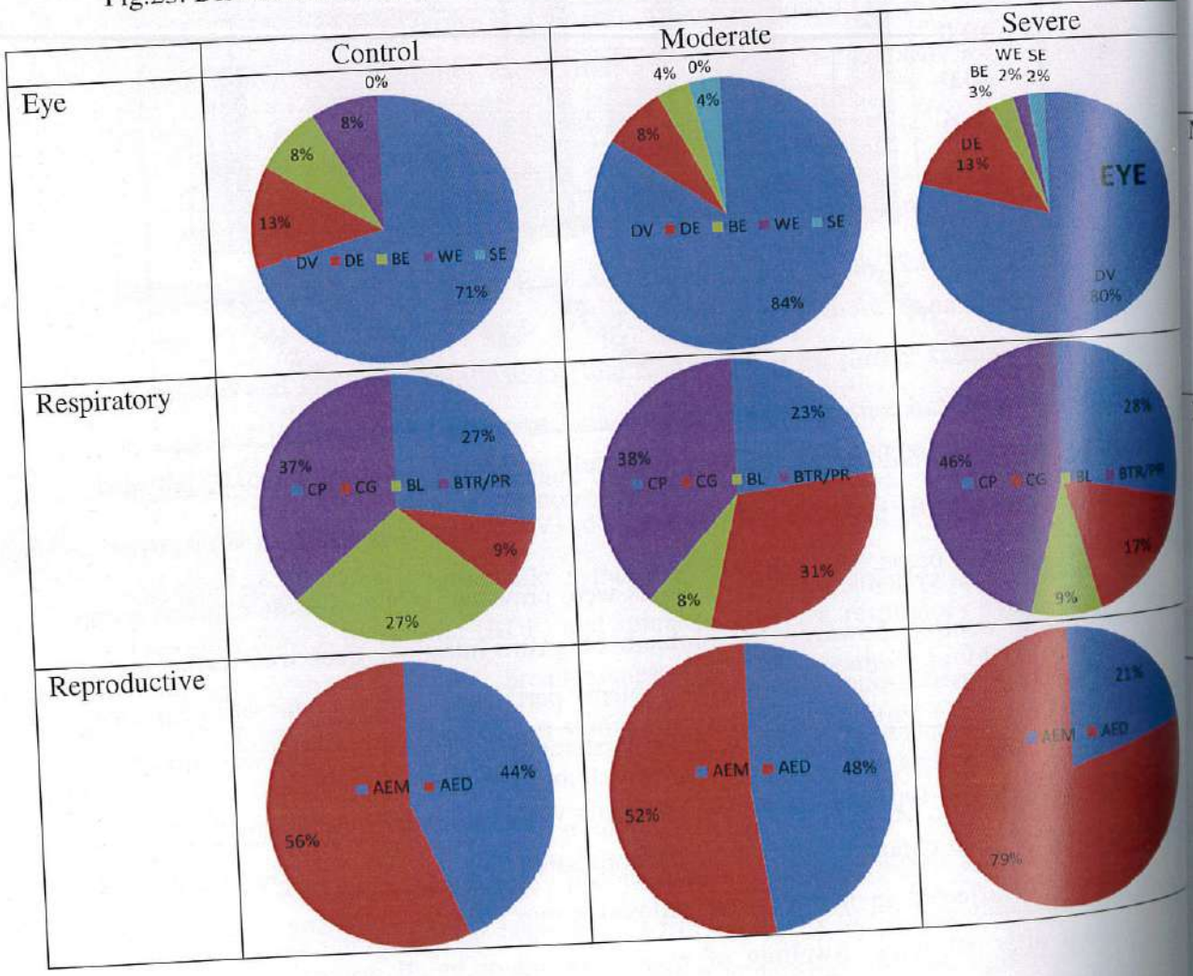
$ Significant difference moderately and severely exposed; *significant at <0.05 between compared to control

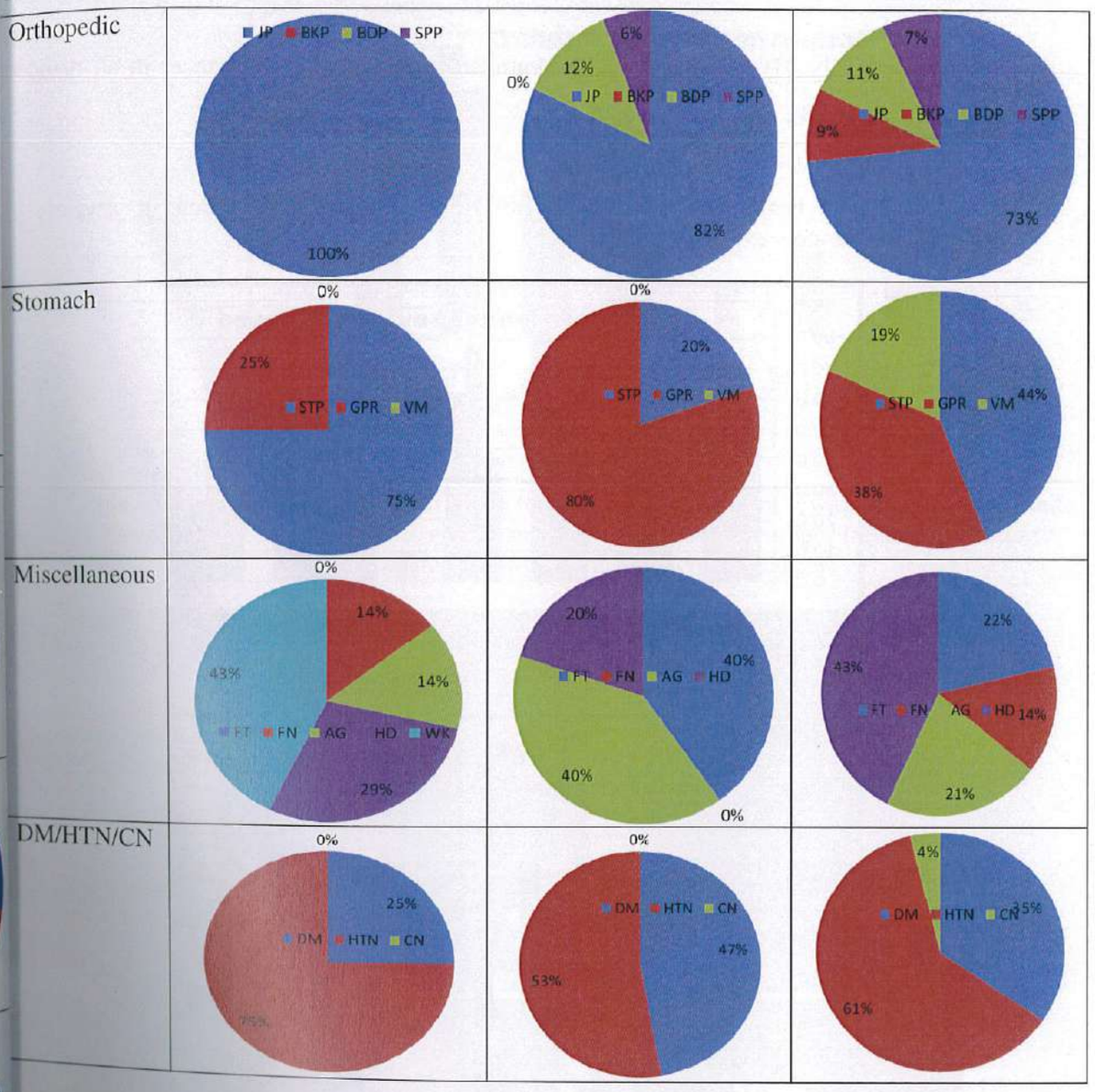
For all functional systems, the abnormalities were prevalent in the severely exposed group compared to control; however, the problem of gastro-intestinal tract was higher in the moderately exposed group. The health problems pertaining to orthopedic and respiratory systems, the differences between control and moderately exposed population were marginal.

The frequency and types of individual problems within each functional system has been described for each exposed and unexposed population (Fig.23). For eye, decreased vision was severely affected in the severely exposed group, wherein watering of eyes is still persistent after 30 years. Swelling of eyes is prevalent in all groups among different complications of eyes, which was highest in the moderately exposed group. In contrast to MIC-exposure, breathlessness is predominant in the control group. Breathing problem is

prevalent in the severely exposed group, whereas moderately exposed group was more affected with chest pain among the three groups. Severely exposed group was severely affected with miscarriage after exposure. In contrast, infant mortality was higher in the moderately exposed population in the present study. Miscarriage was slightly higher in the control group compared to moderate exposure, though the incidence of reproductive problem was significantly higher in the two exposed population collectively. Control population has described

Fig.23. Distribution of symptom-wise problems within the functional systems



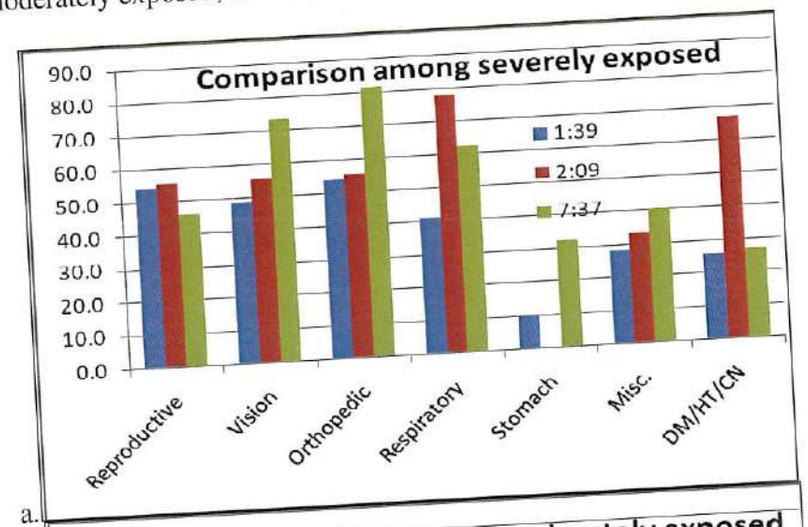


Miscarriage AEM; infant-death AED; decreased vision DV; discomfort ED; burning of eyes BE; watering WE; swelling of eyes SE; joint pain JP; backache BKP; body ache BDP; spinal pain SPP; chest pain CP; cough CG; breathlessness BL; breathing problem BTR/PR; stomach pain STP; vomiting VM; other gastric problems GPR; fatigability FT; fainting FN; allergy AG; headache HD; weakness WK; diabetes DM; hypertension HT; cancer CN.

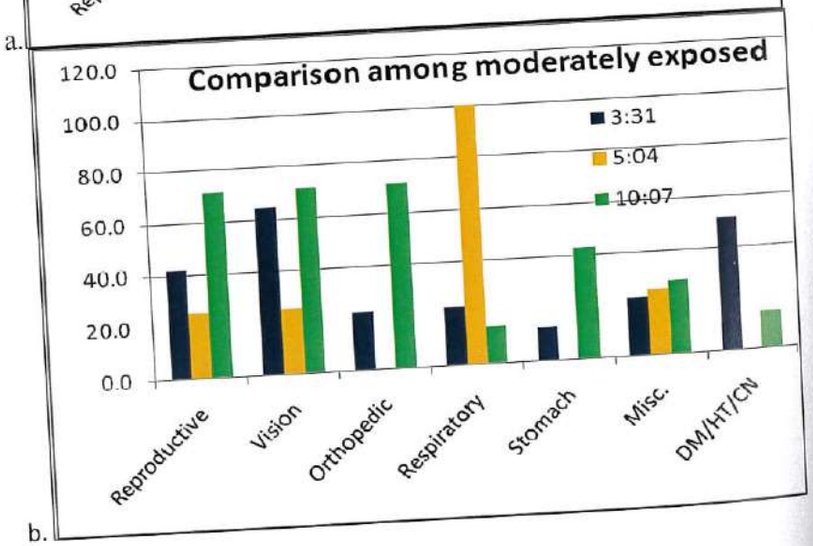
only joint pain. Severely exposed people was markedly affected with vomiting, whereas stomach pain was prevalent in the moderately exposed group. Among the mescellaneous problems, headache and allergy were prominent in the severely and moderately exposed

groups respectively. Hypertension was predominant over diabetes and cancer in all three study groups (Fig.23).

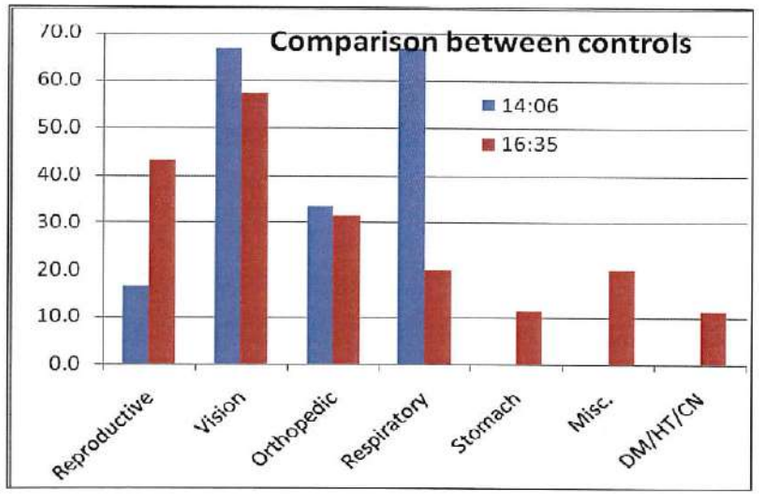
Fig.24. Area-wise health problems within differently exposed population: a. severely exposed; b. moderately exposed; c. controls



a.



b.



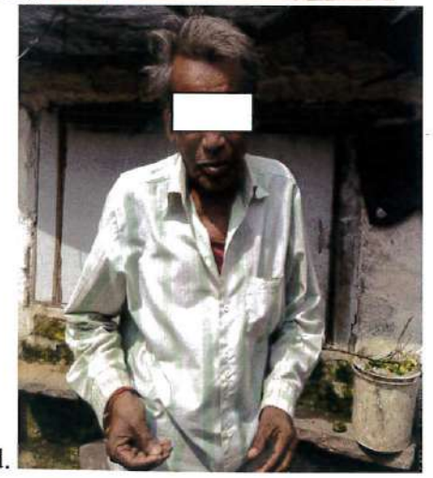
c.

Fig.25. Environmental condition of the residential areas: a. open sewage canal; b. dark and closed interior; c. life-style; d. elderly person showing excessive hair fall due to illness (? chemotherapy going on for some type of cancer)

a.

b.

c.



d.

Area-wise incidence of health problems within each exposure zone revealed that locality no. 7 was highly affected with almost all systemic complications, which was measured during 2014-2015 through interactive discussion between the affected individual and the project-staff. In contrast, the immediate vicinity to UCIL is presented with lower health problems among the localities such as 1, 2 and 7 (Fig.24a). The health problems were higher in the area no. 10 among the three moderately exposure zones; however, the sample size is too low to draw any conclusion on comparison of incidences among 3, 5 and 10 (Fig.24b). The condition was similar between 14 and 16 in the control goup (Fig.24c).

During field visit for counseling to participants, PI visited the residences and interacted with the subjects. Prevalence of weakness and cough among most of the residents in the locality no. 1 has led to suspect tuberculosis (? pulmonary). Most of the elderly of both sexes and adult females (who stayed at home during visit) appeared to be of low BMI (body mass index), which could perhaps be due to under-nutrition or unbalanced diet. Most of the elderly were observed carrying a pouch containing betel nut, lime and tobacco. However people appeared happy. The interiors of most of the today's concrete houses had a dark and close environment, which didn't have access or free flow of fresh air required for healthy breathing (Fig. 25). Collectively, their living and life-style can contrubute to a multiplex of illness, including TB or cencer. However, this study requires larger sample size for drawing a meaningful conclusion and establishing an association with exposure status 30 years post disaster.

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13.3. Discussion

It was postulated that MIC might exert genetic toxicity by binding of carbamoylating agents to nuclear proteins (Tamura et al. 1992). Genotoxic and carcinogenic potential of MIC has been reviewed by several authors (reviewed in Ganguly et al. 2017). Collectively, *in vivo* and *in vitro* geno-toxicity studies established clastogenic potential of MIC. Elevated CA was reported in exposed human and experimental animals (Deo et al. 1987; EHP 1987; Ghosh et al. 1990; Goswami 1986; Shelby et al. 1987). From these results and from considerations of the chemistry of isocyanate-DNA and isocyanate-protein reactions, it could be speculated that MIC's genotoxicity occurs through interactions with proteins affecting chromosomal structure, rather than through direct genetic mutations. Following MIC-accident, extensive investigation was carried out in different living systems on different parameters and published in Environmental Health Perspectives (EHP 1987). The reactivity of isocyanates with specific functional groups on proteins was extensively examined by Brown et al. (1987). The reactions of MIC with non-essential functional groups and the highly exothermic hydrolysis reaction would both act to compete with an interaction with specific enzymes or proteins during inhalation exposures. However, the modification of functional groups can apparently lead to antibody-formation as shown by Karol and co-workers in experimental animals and in survivors of the Bhopal accident (Karol et al. 1987). Nevertheless, the fact is that the gas released was not just MIC alone, but a number of by-products generated such as hydrogen cyanide, nitrogen oxides, carbon monoxide, phosgene, mono-methylamine and many other contaminants through exothermic reaction with water and atmospheric air and moisture. Exothermic reaction of MIC leads to its degradation and conversion to hydrogen cyanide (HCN) at ~200°C, which was evidenced in cherry red color in blood and in the viscera of some of the victims indicating acute cyanide poisoning (ICMR Technical Report 2008). However, the significance of these findings in relation to the overall health effects of MIC exposure will require further study.

The present report describes the level of genetic damage measured in 236 subjects shortly after the exposure during 1985-1989 following MIC-exposure in 1984, and in 130 subjects 30 years post-disaster. The present investigation described almost similar extent of Abc in all three groups, though Abn was higher in severely

only when compared to moderately exposed one. High SD values in all cases demonstrated higher dispersion of aberrations due to inter-individual variation. However, Abn/Abc was significantly higher in both moderately and severely exposed groups. In contrast, abnormalities measured after the accident was significantly higher in both moderately and severely exposed groups compared to the then control. Comparison of the two unexposed groups of past and present study revealed that Abc and Abn were higher in the latter group. Similar incidence was observed in the two moderately exposed groups. Also the overall frequency of abnormalities (Abn/Abc) appeared ~1.5 times higher in the present investigation compared to that of the previous study. Solid-stained metaphases could not collect detail structural information except breaks, minutes and chromatid exchanges in the previous study, whereas G-banding study has facilitated recognition of both numerical and structural rearrangements precisely in the present investigation. However, abnormal cells were significantly higher in both the exposed groups measured shortly after the disaster. Overall, the ratio of aberration (Abn/Abc) was observed higher in the present study due mainly to difference in staining-technology employed, though the culture condition followed was similar to the previous protocol.

Presence of stable rearrangements in the form of translocation, deletion, inversion, ring minutes, etc. in the present study population is a matter of serious concern. Also trisomy 8/X, monosomy 7/X, loss of Y, hypo- and hyperdiploidy, and other complex aberrations which are often reported in hematologic neoplasia, warrants follow up of cytogenetic condition in the identified individuals. In two cases, chromatid exchanges were also noticed. Persistence of minutes and chromatid exchanges were reported 1114 days post exposure (Ghosh et al. 1990). It was postulated that the chemical-induced damaged T-lymphocytes may remain circulating for long periods, which could appear as aberrations only if the cells are stimulated to divide *in vitro* (Carrano and Natarajan 1988), and thus, MIC was speculated to exert some genetic effect on T-cell precursors (Ghosh et al. 1990). In general, information on persistence of translocations in accident victims is limited, which could have been achieved if similar stable aberrations were detected shortly after the exposure as happened in accidental exposure to ionizing radiation (Lindholm et al. 1998; Pressl et al. 2000). In the present study, distribution of chromosomal rearrangements, including CK, MC

and tetraploidy was in a similar range in all exposure groups, except for t/dic and other groups of aberrations, which was slightly higher in severely exposed group. On the whole, these aberrations did not present any relationship with exposure status. However, presence of these rearrangements obviously indicated **genetic instability**.

Bhopal people have become aged by another three decades post MIC-disaster. The mean age of the present subjects was 52 years ranging from 32 to >70. Chemical-induced chromosome aberrations were demonstrated to increase with donor's age (Ghosh et al. 1991). Also age-related influence on reduced cell division and RI was reported in control individuals and in blood lymphocytes subjected to trimethyl tin and stannic chloride (Ganguly 1993a, 1993b; 1995b). Age-related cytopenia and/or aplastic anemia in peripheral blood have been described in general population. Such idiopathic cytopenia of undetermined significance (ICUS) frequently acquire aberrations/mutations, which remain silent for certain period and express as 'founder' for a number of diseases in the elderly, predominantly as myelodysplastic syndromes with a higher propensity to acute leukemia (Kwok et al. 2015; Valent and Homy 2009; Ganguly et al. unpublished). Nevertheless, toxic effects of chemical exposure may appear after variable latency period depending on the dose, mode of exposure, environmental variables and the individual genetic makeup. Also, cyanides are commonly named as 'blood agents' and get absorbed in lungs and passed to the blood to produce systemic effects (Gupta 2015). Cytopenia and nuclear changes in erythrocyte precursors reported in long-term chemical effects (Casey et al. 2015; Poynter et al. 2017; Snyder 2012), wherein ineffective and/or aplastic hematopoiesis might result in acquisition of clonal mutations, which are termed as CHIP (clonal hematopoiesis of indeterminate potential) (Steensma et al. 2015). Delayed toxic effects were reported with sulfur mustard (Gupta 2015). Mostly, chemical-induced DNA-lesions are S-dependent for expression in the subsequent divisional cycles, and thus, the circulating damaged T-lymphocytes might express aberrations when stimulated to divide (Carrano and Natarajan 1988). Also alkylating attack on DNA, which breaks DNA at nucleotides (mostly at guanine residues), might play a pivotal role in delayed expression of toxicity. Abnormalities in control population, tough could not be linked directly to that devastating MIC-exposure, its residual effect through

atmospheric dispersion of air and its interaction with aging and life-style could not be ruled out.

In the present study, the chromosomal alterations could not purely be ascribed to MIC-effect. The compounding effects of several confounding variables could multiply the genetic damage and perturb individual health to a multitude of illness. The spectrum of aberrations detected in the present investigation has to be dealt with biological and environmental consequences. A number of confounders, including life-style, living environment, nutritional factor, drinking water, occupational exposure, and inherent genetic condition are interacting (Ganguly 1994; Ganguly et al. 2017). Additionally, continuous soil contamination by chemical wastes dumped in the UCIL-site might have augmented the genetic changes through interaction with other biologic and a-biologic factors. Aging also might have contributed significantly on the extent of chromosomal rearrangements. Nevertheless, inclusion of migrated unexposed population (living in exposed areas for >10 years) would have extracted important information on long-term effects of MIC gas on the exposed population. The present pilot study has collected significant information on acquired stable rearrangements. Therefore, follow-up investigation for the indexed individuals and investigation of their siblings and progenies are strongly recommended. Further investigation at molecular level, especially in epigenetic mechanism and/or for the genes located on altered chromosomes would be important at a larger scale of sample size. Association-study on DNA-content and chromosomal involvement could provide further direction on future study on environmental and/or chemical exposure (Ganguly et al. 2000). Moreover, similar study for all family members of an exposed family, including blood-linked and unrelated spouses, would be important to measure the inter-individual variation within blood-lineage of exposed individuals and outside blood-lineage from the unrelated spouses more precisely.

The survivors suffered with eye opacity, burning and watering; bleeding through mouth, eyes and ears; chronic breathlessness and coughing; and multiple systemic and psychiatric complications (ICMR Technical Report 2008). Much of the discussion on the disaster has been reported elsewhere (Bhandari et al. 1990; Dhara 1992). Most of the information on the

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medical consequences of the Union Carbide disaster in Bhopal has been generated by the Indian Council of Medical Research (ICMR Technical Report 2008). Initial autopsy revealed cherry red discoloration of lung alongside massive pulmonary edema, emphysema, hemorrhages, visceral congestion, cerebral edema and anoxic brain damage as acute effect of gas exposure. The sub-acute phase was characterized by persistent morbidities amongst survivors of acute phase. The chronic effect was documented by a large number of study groups initiated by government and non-government organizations *in vivo* and in *vitro* in different organisms across the world (Talukder and Sharma, 1989). Survivors continue to experience high incidence of reported health problems including febrile illnesses, respiratory, neurologic, psychiatric and ophthalmic symptoms. Clinical studies have shown chronic illness including breathlessness, pulmonary fibrosis, bronchial asthma, chronic obstructive pulmonary disease, recurrent chest infections, keratopathy and corneal opacities in exposed cohorts. Loss of appetite, menstrual irregularities, recurrent fever, persistent cough, neurological disorders, fatigue, weakness, anxiety and depression persisted among the most common symptoms.

The present health survey has indicated significant illness in systemic functions such as vision, respiratory, orthopedic, and reproductive and so on in MIC-exposed population. The degree of complication is predominant in people exposed severely to MIC-gas. However, similar exercise on larger sample size, including their progenies would be meaningful. In a preliminary epidemiological survey of the effect of the Bhopal accident on pregnant women living adjacent to the facility, Varma (1991) reported a very high rate of unsuccessful pregnancies and a higher than normal infant mortality during the first 30 days of life. More than ten of these symptoms persisted among the survivors till as late as 1992 (when the last ICMR report was published) with the addition of menstrual irregularities, spontaneous abortions and neurological and mental health problems (ICMR Technical Report 2008). Six-monthly morbidity surveys from 1987 to 1991 demonstrated an increasing trend of the number of people with exposure-related symptoms. According to one study there were three times more people with respiratory symptoms in 1991 as compared to 1987. The principal findings of the ICMR was documented as the toxins of MIC-exposure crossed into the blood stream of the exposed individuals resulting in damage to the lungs, brain, kidneys, muscles

as well as gastro-intestinal, reproductive and immune systems (ICMR Technical Report 2008).

The interaction of biological and non-biological factors are of major concern for adjustment of MIC effect and tracking the cause of present health status 32 years post MIC-disaster. There are changes in the environmental admixture and population composition due to in and out migration of population. At this moment it is difficult to correlate the health effects to exposure level of MIC in different individuals. Both in the environment as well as in human body, it is apparent that MIC-effect has been diluted through natural and physiological reactions. Thus, it would be justified to consider a second control (migrated) group who were not exposed to MIC-accident in 1984 but living in the same affected areas for 20-25 years, to estimate the effects of MIC and other chemicals through soil and ground- and well-water. Collectively, the chromosomal rearrangements and clonal abnormalities recorded in Bhopal population 30 years post disaster cannot directly be correlated to MIC-exposure of 1984.

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14. Contributions made towards increasing the state of knowledge in the subject

Modernization of human living augments health-effects of environmental pollution in many ways from individual level through generations (Eisenberg 1999; Ganguly 2011; Ganguly and Kadam 2015, 2016a; Marmot and Wilkinson 2006). Socio-economically weaker population fall prey to environmental hazards to the largest extent right from their residential location to means of daily living. The dense clusters of human habitat lived around the Union Carbide India Limited (UCIL), Bhopal, were affected severely by MIC-gas leaked in 1984 from the pesticide manufacturing plant. Immediate death toll, investigation of the autopsies and monitoring of the survivors established the gravity of MIC's acute toxicity (Broughton 2005; Dhara et al. 2002; Ganguly 1993c; ICMR Technical Report 2008; Talukder and Sharma 1989).

Medical management of the gas victims was exclusively on empirical basis, since knowledge on its antidote was completely lacking. Till then, the information on the severity of MIC's toxicity was limited to a single publication (Kimmerle and Eben 1964). In fact, MIC-disaster in Bhopal had excited the scientific community of all disciplines across the globe to re-search its toxicity in different experimental systems (Bucher 1987; EHP 1987). Simultaneously, Indian scientific and medical fraternities continued research on MIC-exposed Bhopal population, which were reported by individuals and/or institutions (ICMR Technical Report 2008; Deo et al. 1987; Ghosh et al 1990; Goswami 1986; Saxena et al. 1988). Indian Council of Medical Research (ICMR), the apex body of Indian health research, continued monitoring the survivors' health through investigational schemes of different organ systems (ICMR Technical Report 2008). Till present, ICMR has been continuing medical screening of the population through epidemiological survey (Technical Report 2013). ICMR had assigned a unique identification number to each exposed family of different stratified exposed zones, which is being maintained for continual investigation.

Immediate genetic investigation on the exposed survivors was conducted by ICMR during 1985-1989 through a multi-center genetic screening program; however, outcome of that investigation has not been made available to public (the PI of this present project was

involved in that screening through Kolkata Center). *In vivo* and *in vitro* genotoxic potential of MIC was assessed by several groups in various organisms (Conner et al. 1987; Kligerman et al. 1987; Mason et al. 1987; Shelby et al. 1987; Tice et al. 1987), which has been reviewed recently by Ganguly et al. (2017). The initial genetic screening published on MIC-exposed population had shortcoming in study design, sample selection, exposure status, methodology employed, etc. However, during past three decades, there had been no further investigation to measure the long-term genetic effect of MIC, if any, on MIC-exposed Bhopal population. To quench the curiosity, ICMR, has sponsored investigation on current genetic condition of the exposed cases as a pilot study.

The present study was planned to study genetic changes in the form of chromosomal alterations in exposed and unexposed individuals. It was hypothesized that the aberration frequency would be lesser than the initial measure due to inherent detoxification system and elimination/excretion of the chemicals from the body (Baskin et al. 2008; Baud et al. 1991; Pearson et al. 1990). However, unlike radiation exposure (Awa 1997; Salassidis et al. 1995; Straume et al. 1992), chemical exposures are not followed up to exclude the persistence of stable rearrangements, which is evidenced through limitation of information in literature. DNA- alkylation, covalent binding with DNA or DNA-adduct formation, which generally happens at low concentration, may maintain the genetic damage through generations (Beland and Poirier, 1994). Additionally, environmental interaction with DNA-methylation system might aggravate the genetic changes to a complex scenario (Bowers and McCullough 2017; Pacchierotti and Spanò 2015; Ruiz-Hernandez et al. 2015). Although the present investigation cannot extract information on complex genetic-epigenetic and environmental interaction amidst several biological and a-biological confounders, this pilot survey 30-years post disaster has definitely excluded the persistence or acquisition of stable aberrations, which can cross the mechanism of cell division and remain alive, to a certain extent (Speicher et al. 2010). Furthermore, this study has tracked down the incidence of clonal changes in circulating blood cells and detected the stability of clones in exposed individuals. However, filtration of genetic changes 30-years after the disaster and correlating them solely with MIC-exposure would not be justified. It is expected that the present study would at least highlight the necessity and route of further investigation for ascertainment of genetic alterations in MIC-exposed Bhopal population and their progenies.

The present report describes the extent of chromosomal breaks and rearrangements measured on G-banded metaphases (~100 per subject) obtained from stimulated circulating lymphocytes of exposed and unexposed individuals. The spectrum of inter- and intra-chromosomal rearrangements and abnormal constitutive/genomic karyotype, including chromosomal participation and their breakpoints was attractive and drew attention for similar exercise on other family members and progenies. The more interesting part of this report is the result of comparison of past and present genetic condition of MIC-exposed victims at individual level from three exposure status. Truly, it was difficult to identify the present individuals from the previous screening records among several exposed members belonged to the same exposed family (identified by ICMR no.). Above all, analysis of genetic damage measured shortly after MIC-disaster and its comparison with the present condition is the first of its kind on MIC-victims.

The degree of chromosomal alterations (CA) has been broadly categorized as abnormal cell (Abc), aberrations (Abn) and frequency of aberrations per abnormal cell (Abn/Abc) of the previous records and the present investigation, and have been compared between different exposure status. The previous multi-center genetic investigation carried out shortly after the MIC-disaster included genetic parameters such as chromosome aberration (CA), sister chromatid exchange (SCE) and replicative index (RI). The present karyotypic classification of G-banded metaphases has further enabled identification of chromosomal involvement in two-way exchanges, complex (CK) and monosomal (MK) karyotypes, etc., which has facilitated recognition of clonal changes in the circulating blood; however, the present investigation didn't study SCE and RI. Thus, the study compares the level of chromosomal damage occurred immediately following MIC-exposure and 30-years later, in differently exposed groups. The previous data has been retrieved from ICMR's National Institute for Research in Environmental Health (NIREH)'s archive under the guidance of Scientific Advisory Committee of NIREH (ICMR) and supervision of authorized members of NIREH.

The present health status has also been collected of the participants and compared among the exposure strata.

The report is presented in two parts (13.1 and 13.2), which includes assay of past and present genetic status as chromosome aberrations in MIC-exposed and unexposed population, and present health status of the study population. Chromosome aberrations are further extensively discussed on several important topics, including chromosome aberrations detected in the present subjects 30-years post disaster; genetic changes assessed immediately after MIC-disaster; one-to-one comparison of past and present status of genetic alterations at individual level in unexposed and exposed populations; comparison of age at exposure with chromosome aberrations collected 30-years post disaster; frequency of chromosome aberrations in males and females within and between exposed groups; hypocellularity in peripheral blood and acrocentric association in differently exposed population studied after 30-years of MIC-disaster; constitutive abnormalities detected in the study population; differences in chromosome aberrations in members of the same family, and chromosomal polymorphism detected in different individuals of differently exposed zones. The health status and complications on different systems are presented in 13.2, and compared among the groups of different exposure stratum.

The present investigation has presented higher incidence of CA in respect to Abn and Abn/Abc in individuals of the two exposed groups compared to unexposed subjects. 'Other' group of abnormalities, which includes deletion, inversion, fragile sites, trisomy, endoreduplication, etc., appeared high among all structural rearrangements. Unexposed individuals also were detected with stable rearrangements. Overall, inter-individual variation was evidenced in all groups. The result of the previous genetic screening of 236 individuals described significantly higher Abc, Abn and Abn/Abc frequencies in exposed population, which was predominant in the severely exposed group. It is noteworthy that aberration frequency is higher in the present study compared to that of the previous screening; this mainly to the fact G-banding analysis has facilitated recognition of more structural rearrangements over solid-stained preparation of the previous one. Comparison of present of 111 individual has demonstrated increased aberration

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frequencies in all study groups including controls. The possible reasons are discussed in the respective section on one-to-one comparison of past present genetic condition.

Consideration of exposure age was paid importance in the present analysis to check the incidence of age-related acquisition of chromosomal alterations in MIC-exposed population. On an average, 76% of the present study population was exposed at 11-40 years of age, whose present age is ranging from 41 to >60 years. The idea was to capture age related incidence of clonal abnormalities with a view to correlating with the concept of clonal hematopoiesis of indeterminate potential (CHIP). CHIP mutations are acquired and accumulated over age, which remain silent for many individuals but express as founder mutation for elderly hematopoietic diseases such as myelodysplastic syndromes (MDS) to overt acute leukemia in 10-12% of the elderly. It is important to mention that some of the aberrations detected in the present study were specific to MDS; however, that required confirmation in bone marrow. Higher incidence of abnormalities (Abn) was noticed in the adult age group exposed to MIC, which was predominant in the moderately exposed group. On an average, aberration frequency was higher in the moderately exposed females, though overall Abn and Abn/Abc were higher in exposed males.

Approximately, 10% of the participants were detected with extremely hypocellular condition of the peripheral blood, which has been reflected further on low mitotic index, and finally they were excluded from the final data pool due to inadequate metaphase-yield. In this context, idiopathic cytopenia of indeterminate potential (ICUS) drew attraction on MIC-exposed population. However, no association of hypocellularity with MIC exposure was noticed at any age. Nevertheless, the sample size for this interpretation was small. Thus, further investigation is recommended at a larger scale. The incidence of accrocentric association is remarkable, which pulled as high as 10 0f 12 acrocentric chromosomes to form assembly among D- and G-group chromosomes of human karyogram. Further analysis on the type of aggregation, number of chromosomes involved, chromosomes participated, order/sequence of chromosomal arrangement, etc. would definitely be important, which could further be correlated to environmental and biological factors in Bhopal population. Till present, accrocentric assembly is not established with clinical expression; however, such

higher incidence in Bhopal population might establish some link to chemical exposure or some other environmental factor, and hence, that shall paid importance.

Constitutive or genomic abnormalities were observed in 8.5% of 130 subjects in the present investigation, in the form of deletion, inversion, translocation and fragile site involving chromosome 6, 9, 11 and 16. This description carries additional information on these individuals, which warrants further investigation for parents, siblings and children for understanding their clinical impact and risk on future health. A complete analysis of blood-linked family members will further establish its *de novo* origin and link to MIC exposure. It must be mentioned that carriers of del(16q22) could be at risk for acute myeloid leukemia subtype M4 of FAB/WHO classification. Therefore, monitoring of health of these individuals at shorter intervals is strongly recommended. An analysis on the distribution of CA among the members of the same family has presented wide variation; however, further study is recommended to correlate with the impact of age, sex, individual genetic and biological components on similarly exposed individuals living in similar socio-economic environments. Similarly, wide differences were observed on polymorphic variation of pericentric heterochromatin in the long arms of 1, 9 and 16; and variation in satellites of D- and G-groups chromosomes (13, 14, 15, 21, and 22). However, small Y was markedly high in the MIC-exposed population, which was prevalent among severely exposed individuals. Small Y could indicate deletion, cryptic rearrangement with other chromosomes or loss of terminal nucleotides due to higher proliferation rate.

Demonstration of systemic health problems in the present study group was important to capture the spectrum of morbidity in MIC-exposed individuals. Acute lung damage was the major cause of high mortality of gas-victims and chronic respiratory and eye problems among the survivors are well known facts. However, aftermath of MIC effects, though measured on Bhopal population continuously since disaster, can depict a wide variation when individual problems are collected and accounted for systemic disorders. In the present compilation, systemic abnormalities were prevalent in severely exposed group. However this study requires large data base. An interesting part of this analysis would be comparison and describe their immune status due to genetic instability.

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any. Nevertheless, normal karyotype in the congenitally malformed children born to MIC-exposed parents has raised a pertinent question on possibility of genetic alteration at molecular level, and thus, warrants investigation for understanding MIC-DNA interaction in the children and their parents.

Altogether, the present report is the first of its kind to describe the present status of genetic condition on MIC-exposed population. Another major discussion on the spectrum of chromosome aberrations measured shortly after disaster on gas-victims through the multi-center genetic screening has facilitated comparison of genetic condition then and now after 30 years. This picture has been precisely presented when the past and present genetic status of the same individual compared. However, the exercise needs to be practiced on larger scale of sample size on key parameters for extraction of long-term effect of MIC on Bhopal population. Nevertheless, to derive such a conclusion, at least mutations of DNA-methylation and RNA-splicing, and screening of CNVs and UPD are essential, besides conventional high resolution cytogenetics for tracking of spontaneous and acquired clones.

15. Summary of achievements and indication of scope for future work

Presence of clonal abnormality observed in few cases, showed similar balanced translocation/del/MK/trisomy in more than one cell, wherein del5q, monosomy 7, trisomy 8 and loss of Y could indicate risk of hematopoietic neoplasia (Ganguly and Kadam 2016b; Ganguly et al, 2016a, 2016b), and thus, draws serious attention for further investigation, follow-up and monitoring of health condition in shorter intervals. Some of these chromosomal rearrangements, which indicate molecular mutations, are often described in elderly individuals as clonal hematopoiesis of indeterminate potential (CHIP) (Ganguly et al. 2016b; Steensma et al; 2015). Therefore, an obvious question is whether these aberrations detected in Bhopal population are cytogenetic CHIPs, and thus, whether these rearrangements are present in circulating bone marrow cells (? malignant). Limited information on persistence of stable rearrangements and clonality in chemical or radiation exposure restricts drawing such speculation; however, such scenario definitely warrants for further studies, especially for epigenetic mutations associated with aging, xenobiotic exposure and gene-environment interaction. Precisely, screening of DNA-methylation genes (DNMT3A, ASXL1, TET2, EZH2), SRSF2 and SF3B1 for RNA splicing machinery, screening of CNVs and UPD, and high resolution conventional cytogenetics on a larger sample size would collect important information on the behavior of genetic alterations on current and future health of exposed individuals and their progenies. At least, the above-mentioned screening shall be considered mandatory for the individuals identified with persistent stable aberrations.

Few individuals, who were detected with del(5q), +8, -7, -Y, and complex karyotype, require examination by hematologist, and perhaps also bone marrow cytogenetics. The same is true for the cases appeared with hypocellular peripheral blood, for detection of bone marrow aplasia in adults and bone marrow failure syndrome in children or young adults.

Conventional karyotyping is must for the parents (if alive), siblings and children of carriers of constitutive genomic abnormalities. Health-monitoring at shorter intervals is strongly for carriers of fragile sites or deletion in 16q22, because del(16q22), which

harbors CBFB, may be associated with AML-M4 or MDS at older age. CBFB, though by itself does not contain any DNA binding motif or transcriptional activation domain, but forms a dimer in CBFB-MYH11 fusion transcripts.

Fetal poisoning was reported indicating the toxins crossing the placental barrier (Srivastava 2011). Normal karyotypic constitution in the physically and/or mentally challenged children, who were born to severely exposed parents, strongly recommends a serious health survey of these families, and genetic investigation for copy number variations (CNV), interstitial deletions and duplications, and uniparental disomy (UPD) following molecular karyotyping through array-based comparative genomic hybridization (a-CGH) or SNP-typing. Of course, high resolution conventional karyotyping shall be considered mandatory for recognition of balanced rearrangements. Targeted sequencing for epigenetic mutations, TET2, DNMT3A, EZH2, ASXL1 in particular, and also SF3B1 and SRSF2 of RNA-splicing and TP53 as cell cycle regulator could extract information on founder mutations associated with irregular hematopoiesis. The mutations of RAS-opathy, including NRAS and KRAS, would also be significant for the children born to MIC-exposed population, with physical or mental impairment. The normal karyotype does not really guarantee absence of mutations in these children. Such comprehensive screening will not only identify the at- risk children, but also direct the status in parents and minimize future risk of transmission.

Further evaluation of chromosomes participating in acrocentric association would perhaps open a new avenue of research on Bhopal population and direct biochemical investigation for identification of the proteins or enzymes pulling the chromosomes for aggregate, and find out the requirement of such gathering of acrocentric chromosomes. Screening of chromosomal involvement could be carried out on the processed samples of the present study.

The outcome of the present pilot study on 130 individuals described in the present report definitely highlights importance for similar exercise on larger sample size, in its immediate next phase, which won't be expensive, but would direct for further screening of prevalent CHIP mutations of the elderly and also in the context of ICUS. Molecular screening would be justified after screening persistent aberrations at chromosomal level.

Scope for further work

1. Continuation of this pilot study on larger sample size for completion of the targeted investigation on exclusion of long-term MIC-effect
2. Genomic karyotyping (conventional) for the parents (if alive), siblings and offspring of the cases detected with constitutive karyotypic abnormalities
3. Follow-up study for the cases appeared with higher frequency of aberrations and complex and monosomal karyotypes
4. Bone-marrow cytogenetics for cases detected with specific aberrations and hypocellular peripheral blood
5. Investigation of chromosomal involvement in acrocentric association
6. Molecular karyotyping for CNVs, UPD and screening of epigenetic and RNA-splicing mutations.

16. Science and Technology benefits accrued:

16.1. List of research publications and conference presentations with complete details:

1. Ganguly BB, Mandal S, Kadam NN. Genotoxic and Carcinogenic Effects of Methyl Isocyanate (MIC) Reviewed on Exposed Bhopal Population and Future Perspectives for Assessment of Long-Term MIC-Effect. J Environ Anal Toxicol 7:3. 2017. DOI: 10.4172/2161-0525.1000452.

2. BB Ganguly. Is there any long term genetic effect in MIC-exposed population of Bhopal, India? Abstract of the invited talk was published in the conference book of Environmental Mutagen Society of India, held in 2013 at BARC, Mumbai.

3. BB Ganguly. Impact of environment on mutations of hematologic malignancies: a close look into clonal aberrations in MIC-exposed Bhopal people. Presented as an invited talk (IT 16) in the International Conference on Advances in Cellular, Genomic and Epigenomic Insights on Environmental Mutagenesis and Health and 41st Annual Meeting of Environmental Mutagen Society of India. Held in School of Life Sciences, Manipal University, Manipal, India, during January 27-29, 2017.

4. S. Mandal et al. Genetic Sequel in Bhopal Population 32 years post MIC Exposure. Poster (P 35) presented in the International Conference on Advances in Cellular, Genomic and Epigenomic Insights on Environmental Mutagenesis and Health and 41st Annual Meeting of Environmental Mutagen Society of India. Held in School of Life Sciences, Manipal University, Manipal, India, during January 27-29, 2017.

5. Ganguly BB, Mandal S, Banerjee N. Chromosomal mutations of hematologic malignancies of the elderly correlated with chromosomal aberrations of indeterminate potential in MIC-exposed Bhopal population. Accepted for presentation in Global Toxicology 2017, to be held in Chicago, USA during July 20-22, 2017.

Other publications of the PI (2013-2017)

2017:

1. **Ganguly BB**. Small-molecule inhibitors of epigenetic mutations as compelling drug-targets for myelodysplastic syndromes. Curr Cancer Drug Targets 2017; 17 (in Press). E-pub Ahead of Print. DOI: 10.2174/1568009617033014500 2.

2016:

2. **Ganguly BB**, Kadam NN. Understanding Social Determinants for Children in Difficult Circumstances: An Indian Perspective. Int J Ped Child Health 2016; 4: 77-88. DOI http://dx.doi.org/10.12974/2311-8687.2016.04.02.3.
3. Mandal Shouvik, Kadam Nitin N, Ram Sabita M, **Ganguly Bani B**, Shenoy Vanith U. Ectodermal Dysplasia and Anodontia associated with Ring Chromosome 18. Contemporary Dentistry 2016; 6(3):220-224. 10.5005/jp-journals-00000-0000.
4. **Ganguly BB**, Mandal S, Kadam NN, Banerjee D, Chandra S, Dolai TK, Agarwa MB. Experience of Conventional Cytogenetics in Elderly Cytopenic Indian Patient Suspected with Myelodysplastic Syndromes. Blood 2016 128:5488.
5. **Ganguly BB**, Kadam NN. Down syndrome: from the age of characterization to th era of curative approach. The Nucleus. Published online: October 21, 2016. DO 10.1007/s13237-016-0187-y.
6. **Ganguly BB**, Kadam NN, Mandal PK. Complexity of chromosomal rearrangeme in a Down syndrome leukemia. Accepted by J Cancer Res Therapeutics 2016 (in pres Ahead of print online.
7. **Ganguly BB,** Dolai TK, De R, Kadam NN. Spectrum of complex chromosom aberrations in a myelodysplastic syndrome and a brief review. J Can Res Ther 2016; 1 1203-1206.
8. **Ganguly BB**, Kadam NN. Mutations of myelodysplastic syndromes (MDS): A update. Mutation Research Reviews 2016; 769: 47–62. doi:10.1016/j.mrrev.2016.04.009.

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2015:

9. **Ganguly BB**, Mandal S, Dolai TK, De R, Prasad P, Chakraborti P. All MDS-specific structural chromosomal rearrangements in a single case. Leuk Res 2015; 39 (Suppl 1):S94-S95. DOI: http://dx.doi.org/10.1016/S0145-2126(15)30190-9.
10. **Ganguly BB**, Kadam NN. Health of the underprivileged children: A close look into a subset of tribal group and street population of India. New Ind J Ped 2015; 4(3):156-166.
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12. Mandal PK, Pandey G, Mondal M, **Ganguly B**. A rare case of acute myeloid leukemia-M6 in a 2-year-old child with complex karyotype. Clin Cancer Investig J 2015; 4: 756-758.
13. **Ganguly BB**, Ramachandran K, Boricha BG, Srivastav A, Kulkarni S, Mitra BK, Kadam NN. Chromosome aberrations in fetal samples: an Indian scenario. European Society of Human Genetics, Glasgow, Scotland, UK, June 6-9, 2015. Reproductive Genetics/Prenatal Genetics, J01-58.
14. **Ganguly BB**, Sen K, Kadam NN. Geriatrics disease-burden from genetics point of view. American Society of Human Genetics, held a Baltimore, MD, USA in October 6-10, 2015.

2014:

15. **Ganguly BB,** Kadam NN. 2014. Prenatal diagnosis of fetus with short limbs caused by three abnormal chromosomes inherited from both parents. Int J Hum Genet, 14(2): 83-90.
16. **Ganguly BB**, Mandal S, Kadam NN. 2014. Prenatal detection of chromosomal aberrations and its reflection at adult age. 2451M, ASHG, San Diego, October 18-22, 2014.
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19. **Ganguly BB**. Karyotyping as a quality check for stem cell banking. J Cell Sci Ther 2013, 4:4. http://dx.doi.org/10.4172/2157-7013.S1.026.

20. **BB Ganguly**, NN Kadam, S Mandal, et al. Varieties of CML at chromosomal level. Ind J Hemat Blood Tranfus Vol 29(4), pp. 330, 2013. Doi. 10.1007/s12288-013-0302-y.

21. Complex chromosomal abnormalities in MDS. B. Ganguly, S. Mandal, M. Ghosh, T. Dolai, M. Bhattacharyya, D. Banerjee, D. Banerjee, S. Chandra. Leukemia Research Volume 37, Supplement 1 , Page S57, May 2013.

22. Clinical expression of de novo 18(q-) syndrome in a child with multiple congenital malformations. S. Mandal, B. B. Ganguly, N. N. Kadam. European Journal of Human Genetics, Vol.21, Suppl. 2, pP02.240, pp. 597, 2013.

23. Still Births and Foetal Genetic Defects causing Recurrent Miscarriages in couples. N. J. Jain, K. Gomes, N. Patil, S. Salvi, B. Ganguly. European Journal of Human Genetics Vol.21, Suppl. 2, pP02.240, pp. 599, 2013.

24. Screening of chromosomal aberrations in infertility work-up. B. B. Ganguly, G. Khastgir. European Journal of Human Genetics, Vol.21, Suppl. 2, pP02.240, pp. 599, 2013.

16.2. Manpower trained in the project:

a. Senior Research Fellow: 2 (1 trained for counseling and enrolment of participants; however, left at early phase). The present SRF is trained for cytogenetic analysis and reporting.

b. There was no provision in this pilot study

c. Other Technical Personnel trained: 2. Both were trained for cell culture and chromosome preparation; however, the first technical staff was based at Bhopal from Nov. 2013 till May 2016. Therefore, he could not develop this technique. He was trained for counseling to participants and collection of pedigree and health history.

III. Patents taken, if any: Nil

IV. Products developed, if any: Nil

17. Procurement/usage of equipment:

There was no provision of any equipment for the PI. Requirement of microscope, thermobryte and imaging software were sanctioned for NIREH. However, PI does not have any idea since the entire analytical work has been carried out at PI's host institute at Mumbai.

a.

S.No.	Name of Equipment	Make/ Model	Cost FE/₹	Date of Installation	Utilisation rate %	Remarks regarding maintenance/breakdown

b. Suggestions for disposal of equipment. Not applicable

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

18.1. *Non-availability of proposed and approved fund and staff*

18.1.a. The project was submitted to ICMR in December 2010 against ICMR's 'CALL FOR PROPOSAL' for evaluation of long-term effect of MIC on Bhopal population; however, the project was approved as NIREH's intramural project to PI (who was/is not associated with NIREH, Bhopal). PI didn't receive any intimation about this system from ICMR, Delhi where the project was actually submitted. ICMR's reference no. was 65/BBG-1/NCD-II.

18.1.b. The approved project bearing approval No. NIREH/IMP/BBG/2013/01 dated 24.10.2013 was forwarded to Director, MGM New Bombay Hospital, Vashi and PI by NIREH along with terms and conditions of the approval and forms for statement of expenditure, utilizations certificate, submission of report, etc. in October 2013. Both fund and staff were not given to the PI for initiating the project, though it was clearly stated in the approval letter to be forwarded to PI's Institute in Mumbai.

18.1.c. The issues of short comings and deviation from the letter of approval of the project were discussed in all of the NIREH's SAC meetings held in 2014 and afterwards. However the project could not be started due to some of the ICMR/NIREH's administrative reasons. The project was approved for only one year i.e. till 23.10.2014.

18.1.d. The staff projected (one SRF and one lab assistant) for the work was recruited by NIREH immediately next month (Nov. 2013) after the approval and stationed at NIREH but they left the project after two years and seven months (in place of one-year's duration of the project). SRF left in Feb. 2015 (after one year and four months) and Technician left in June 2016 (after two years and seven months). Thus, there was no staff available to PI for conducting the laboratory work till July 2016. The samples collected during this period were initiated at NIREH, but processed at PI's host institute with the help of MGM's staff and financial support.

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18.1.e. The fund was handled by NIREH and not given to PI for the work till 2016. Meanwhile, approximately 1/4th of the total sanction was forwarded to PI's Institute in September 2014, which was returned to NIREH since there was no time left for conducting any work from the approved project duration which was ending in next month i.e. October 2014. Later, NIREH forwarded Rs.5 L to PI's institute in November 2015; however, head-wise break-up was not provided by NIREH for utilizing the fund (salary head was completely utilized at NIREH and also some part of the travel and consumables). Later, Rs.1736177 was received at PI's institute **by end of July 2016** after adjusting expenses made by NIREH at Bhopal.

Staff was not transferred to PI's Institute for carrying out the project work since November 2013 till this period.

18.1.f. Later, the technician and SRF were recruited in July and August 2016 respectively after receiving the remaining of the project fund in July 2016 from NIREH. Following one month's training, they started further processing of the samples collected till May 2016.

The blood samples collected during 2014-2016 were processed by the staff of PI's Institute, and consumables provided by the PI's institute.

18.1.g. The project was extended without additional budget/fund till June 30, 2017.

18.2. Deviation of duration of the project

The project was approved for one year from 24.10.2013 to 23.10.2014. However, due to some administrative issues at NIREH, the project has actually started from August 2016 (after two years nine months). The reasons are mentioned above. The project was extended till December 2016, and later till June 2017.

Although consent and pedigree collection of 174 participants was performed by the earlier staff, analysis of pedigree was completely pending till May 2016 (till the time the staff was placed at NIREH).

After training the newly recruited staff in August 2016, microscopic and further analysis was initiated.

18.3. Deviation of sample numbers

The target was to study 100 individuals from each of exposed and unexposed groups.

Thirty year post-disaster, it was difficult to trace those individuals (presently they have families with two more generations). Since many of those cases have left Bhopal or relocated in new places of Bhopal, pedigree and consent of **174** cases could be collected. Only **44 consents of unexposed cases** could be obtained.

Blood samples of **143** cases (excluding the repeats) including **35 controls (of 44 consents)** have been collected by the project staff till May 2016 (Table 1,2).

Many consented individuals have declined to participate or unavailable at the time of blood collection.

Therefore, the projected control numbers could not be fulfilled.

The projected number could have been achieved from considering the cases studied by other centers. However, since the decision was to consider the cases studied by Calcutta (present Kolkata) center of the previous multi-center screening program, the staff didn't retrieve records from the NIREH's archive and recruit the remaining cases.

Six of 34 (6/34) unexposed cases could not be studied and reported due to inadequate morphology of metaphase chromosomes.

Ideally repeat analysis should have been carried out for another 11 cases, whose karyotype has been prepared but could not be included in the final analysis due to less number of metaphases studied. Therefore, altogether 50% of the unexposed samples (17) collected on 3-4 February, 2015 could not be considered for comparative analysis, and thus should have been repeated. The cultures were set up at NIREH.

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18.4. Deviation in culture initiation and processing

18.4.1. As per the approval, cultures were to be initiated at NIREH till harvesting and slide preparation for quality check, mainly for maintaining the integrity of the samples (**ANNEXURE III**). Remaining processing including further slide preparation, banding, microscopy, etc. were to be performed at MI's host institute at Mumbai under PI's supervision.

18.4.2. The samples collected in February, 2014 could not be cultured at NIREH due to lack of culture facilities in sterile condition. Hence, the sample were transported to Mumbai at room temperature, and cultured and processed at Mumbai. Culture reagents were brought to NIREH from Mumbai, but cultures could not be initiated at NIREH.

18.4.3. Similarly, samples collected in May 2014 also could not be processed at NIREH.

18.4.4. Samples (34 control samples) collected in February 2015 were initiated at NIREH with phytohemagglutinin (mitogen/stimulant) from NIREH's procurement. However, those cultures were further processed at Mumbai since NIREH didn't have fixative ingredients. Also, NIREH didn't validate (sterility check, etc) the culture environment. All of 34 cultures were not satisfactory due to poor metaphase-yield. Out of 34, repeat sample was collected for 17 cases and considered for final analysis; karyotype has been prepared for 11 cases, but could not be considered for data compilation due to extremely low mitotic index; and 6 could not even be karyotyped due to extremely hypocellular condition in their peripheral blood. Practically, there were 18 control samples for final analysis. One walk-in patient, who is a resident of central Bhopal has been added to control group. He was 2 years old during MIC-disaster.

18.4.5. On account of the fact that the staff was based at NIREH and the cultures initiated at NIREH were not satisfactory/acceptable, it was decided jointly by PI, CO-PI and the then Director of NIREH, that without wasting time for validation at NIREH, samples will be couriered by NIREH to Mumbai for complete processing including culture-initiation.

Another fact was that the samples collected in 2014 were transported to Mumbai for complete processing, which were fully satisfactory and considered for final compilation. The sample-integrity was not compromised.

18.4.6. A total of 113 samples, including 17 control samples, were collected by NIREH and transported to Mumbai for complete processing on six different dates (Table 3). All of these cultures were successful and considered for final compilation. Sample integrity was not compromised. Transport guidelines were followed for compliance of the logistic company.

18.4.7. Therefore, 113 samples were not cultured at NIREH as per the approval.

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19. Conclusion

Based on the outcome of the present investigation, it appears that the study was important for MIC-exposed population of Bhopal, which has estimated different degrees of acquired and constitutive chromosomal abnormalities in differently exposed and unexposed population. Since the sample size was limited to only 130 in the present pilot study, it is imperative to state that the study shall be extended on larger sample size for drawing a conclusion after 30 years of the disaster, which may at least include the following recommendations:

1. Continuation of this pilot study on larger sample size for completion of the targeted investigation on exclusion of long-term MIC-effect
2. Genomic karyotyping (conventional) for the parents (if alive), siblings and offspring of the cases detected with constitutive karyotypic abnormalities
3. Follow-up study for the cases appeared with higher frequency of aberrations and complex and monosomal karyotypes
4. Bone-marrow cytogenetics for cases detected with specific aberrations and hypocellular peripheral blood
5. Investigation of chromosomal involvement in acrocentric association
6. Molecular karyotyping for CNVs, UPD, and screening of epigenetic and RNA-splicing mutations in the elderly as well as subjects detected with stable clonal rearrangements.
7. Mutations of RAS-opathy (NRAS, KRAS, etc.) shall be included along with the items mentioned in point #6 for the children born to MIC-exposed parents, especially for physically and mentally handicapped children carrying apparently normal karyotype.

Name and signature with date

June 28, 2017
Navi Mumbai

Bani Bandana Ganguly
(Principal Investigator)

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Signatures:

Name and signature with date

NIREH, BHOPAL

1. BANI BANDANA GANGULY
(Principal Investigator)

JUNE 17, 2017

NIREH, BHOPAL

2. NALOK BANERJEE
(Co-Investigator)

JUNE 17, 2017

ANNEXURE

ANNEXURE I:

Hindi Consent Form

"भोपाल के जनसंख्या पर दिसंबर 1984 में हुए MIC गैस दुर्घटना के दीर्घ अवधि आनुवंशिक प्रभाव , यदि कोई हो "

सहभागी अनुसंधान अध्ययन की जानकारी तथा सहमति फॉर्म (form)

Title: "भोपाल के जनसंख्या पर दिसंबर 1984 में हुए MIC गैस दुर्घटना के दीर्घ अवधि आनुवंशिक प्रभाव , यदि कोई हो " .

परियोजना संख्या: 65/2 /bbg / 11-ncd-ii

प्रधान जांचकर्ता (PI)

डा. बानी bandana गांगुली, हेड, सेन्टर फॉर जेनेटिक अनुसंधान एवं स्वास्थ्य विज्ञान संस्थान कोनिदान, MGM, Kamothe, नवीमुंबई, - 410209। दूरभाष 27437895, 27430320 फेक्स : 91 22 91 22 91 , मोबाइल 9869214680, ई-मेल :mgmgeneticlab@yahoo.com

सह जांचकर्ता (Co-investigator) (सीआई)

डा. एन. बनर्जी, हेड, अनुसंधान संस्थान, राष्ट्रीय पर्यावरण और स्वास्थ्य (आई.सी.एम.आर.), कमला नेहरू अस्पताल भवन, गांधी-मेडिकल कालेज, भोपाल (मध्यप्रदेश) – 462001; टैलिफैक्स -0755- 2533976, मोबाइल 09826037631; 917552533976.

Sponsor:

भारतीय आयुर्विज्ञान अनुसंधान परिषद (आई.सी.एम.आर.) , अंसारी नगर, नई दिल्ली -110029, इंडिया.

आप को एक मानवीय आनुवंशिकी अनुसंधान अध्ययन (स्क्रीनिंग प्रोग्राम) में भाग लेने के लिए कहा जा रहा है । आप कृपया अध्ययन के कर्मचारी से कंसेट फॉर्म कि समीक्षा और किसी प्रश्न पर चर्चा कर सकती है। हो सकता है कि आप अध्ययन में भाग लेने कि अपने निर्णय लेने से पहले, आप अपने नियमित डॉक्टर से, और अपने मित्रों और परिवार से चर्चा कर सकता है। हो सकता है कि कंसेट फॉर्म कि कुछ शब्दों / सूचना आप नहीं जानते है। कृपया आप अध्ययन डाक्टर / अध्ययन स्टाफ को स्पष्ट रूप से समझाने के लिए अनुरोध करें ।

अध्ययन के डॉक्टर (या संस्था) कोई व्यावसायिक फीस और वित्तीय सहायता पूछने नहीं जाएगा।

अध्ययन के उद्देश्य

1984 के मिथाइल आइसोसाइनेट गैस दुर्घटना में जो लोग प्रभावित / संक्रमित हो चुके थे, और जो लोगों की जांच की गई (1986-1990 में), उनके, और उनके उत् पन् न संतानों के , यदि कोई हों, (जो संभव है) वर्तमान

आनुवंशिक स्थिति जांच की जाएगी और दीर्घकालिक प्रभाव (28 साल बाद) की संभावना को दूर करने के लिए पिछले आंकडे की तुलना की जाएगी. आप को इस अध्ययन में भाग लेने के लिए अनुरोध किया जा रहे हैं क्योंकि -

- दुर्घटना के बाद आपकी की जांच की गई
- आपकी वर्तमान स्थिति क्या होगी
- आपके बच्चे की जांच के तुलना किया जाएगा
- इस अध्ययन / जानकारी से आपके साथ समूचे परिवार लाभान्वित होंगे
- तीन-उत्पादन वंशावली के नैदानिक/स्वास्थ्य जानकारी दर्ज किया जाएगा
- आनुवांशिक विकारों, यदि मिला, से बचा जा सकता है
- संभावित स्वास्थ्य जोखिम की गणना की जांच होगी

प्रथम वर्ष में कुल 100 व्यक्तियों (100 परिवारों) जो लोग प्रभावित / संक्रमित हो चुके थे, और 100 व्यक्तियों, जो प्रभावित नहीं हो चुके थे(अनएक्सपोज्ड) इस अध्ययन में भाग लेंगे।

इस अध्ययन के उद्देश्य है की भोपाल में घटी मिथाइल आइसोसाइनेट गैस दुर्घटना (December 1984) की भोपाल के निवासियों के पर दीर्घ अवधि आनुवंशिक प्रभाव, यदि कोई हों, की जांच ।

चरण (Phase) 1: स्वास्थ्य अध्ययन में, जो लोग प्रभावित / संक्रमित हो चुके थे, उनकी तीन-पीढी चार्ट वंशावली में सूचना एकत्र होंगे और स्वास्थ्य स्थिति हाइलाइट होगा। पहले चरण में है स्वास्थ्य स्थिति का अध्ययन किया जाएगा। विवाह / स्वास्थ्य और शिशु जन्म / वंशावली विवरण को भी रिकार्ड किया जाएगा।

अध्ययन में भागीदार समुदायों को पंजीकृत किया जाएगा।

चरण (Phase) 2: अध्ययन यह पता लगाने के लिए और समझने के लिए किया जाएगा कि MIC के प्रभाव के परिणामस्वरूप स्वच्छंद (spontaneous) जेनेटिक आनुवंशिक प्रभाव के स्तर और क्या कोई संक्रामक (transmissible) आनुवंशिक प्रभाव हुआ है और आनुवंशिक प्रभाव कि अस्थिरता के स्तर और यह पता लगाने के लिए कि क्या कोई परिवार में हुआ है उनकी नए सिरों अथवा संक्रामक आनुवंशिक प्रभाव . Karyotype (विपथन फिंगरप्रिंट) प्रत्येक भागीदार को दिया जाएगा। महत्वपूर्ण मामलों में प्रधान जांचकर्ता परामर्श देंगे।

चरण (Phase) 3: MIC की आनुवंशिक स्तर पर दीर्घ अवधि प्रभाव गणना करने के लिए, यदि कोई हों, अध्ययन की डेटा विश्लेषण किया जाएगा । आवश्यक हो तो, एहतियाती करने के लिए उपाय की आवश्यकता को

"भोपाल के जनसंख्या पर दिसंबर 1984 में हुए MIC गैस दुर्घटना के दीर्घ अवधि आनुवंशिक प्रभाव , यदि कोई हो "

अध्ययन महत्वपूर्ण है क्योंकि वर्तमान MIC के भोपाल शहर कि नागरिक के पर आनुवंशिक प्रभाव का अनुसंधान संबंधी जानकारी उपलब्ध नहीं है।

अध्ययन प्रक्रिया

गैस दुर्घटना बाद, 1986-1990 केदौरान, एक्सपोज्ड और अनएक्सपोज्ड नागरिक के पर, एक बहु-केन्द्रित आई.सी.एम.आर. द्वारा प्रायोजित, जेनेटिक स्क्रीनिंग (screeing) के अध्ययन किए थे. अध्ययन कार्यक्रम में उन व्यक्तियों, परिवारों को सहभागिता के लिए खोजा जाएगा और प्राथमिकता दी जाएगी ।

इसमें दो अलग-अलग प्रकार के परिवारों होगा, जैसे -

1. 1984 में 25-30 वर्ष के पुरुषों और 20-25 वर्ष के महिलाओं, जो गैस दुर्घटना से प्रभावित (एक्सपोज्ड) हो चुके थे और जीवित है, की आयु आज 29 वर्ष बाद, 54-59 वर्ष तथा 49-54 वर्ष की आयु होगा. उन्हें 25-29 वर्षों के पुत्र या पुत्री (बच्चों) और 1-5 वर्ष की आयु के नाती (ग्रैंड बच्चों) (कम से कम एक) हो सकता है।
2. किशोर उम्र (10-12 वर्ष) के व्यक्तियों , जो गैस दुर्घटना से प्रभावित (एक्सपोज्ड) हो चुके थे और जीवित है, 29 वर्ष बाद, 10-14 वर्ष की आयु के बच्चों के माता-पिता हो सकता है।

1986-90 के सभी छह केन्द्रों द्वारा अध्ययन के दौरान शामिल व्यक्तियों के तीन-उत्पादन वंशावली के नैदानिक/स्वास्थ्य जानकारी दर्ज किया जाएगा और साथ-साथ नस्ल तैयार की जाएगी, जहां से नैदानिक आनुवंशिक परिणाम के बारे में सूचना उपलब्ध होगा।

गुणवत्ता आश्वासन पहले स्थान पर रखा जाएगा .

आनुवांशिक अध्ययन में शामिल होंगे -

i. 'एक्सपोज्ड' और 'अनएक्सपोज्ड' व्यक्तियों और उन व्यक्तियों के बच्चों का स्वच्छंद (spontaneous) जेनेटिक आनुवंशिक प्रभाव अध्ययन।
ii. FISH की सहायता से 'Cryptic Rearrangements' के आकलन .

इस अध्ययन में शामिल व्यक्तियों के स्वास्थ्य स्थिति का अनावरण इतिहास, वर्तमान में जांचकर्ताओ को नहीं दिया जाएगा ।

अध्ययन के पूरे समय में केवल एक बार 2-3 ml रक्त की जरूरत होगी।

संक्षेप में तकनीक

रक्त नमूनों को NIREH, भोपाल. में Mitogen-पोषक तत्व के माध्यम से, 72 घंटा, 36 डिग्री तापमान पर प्रक्रिया (culture) किया जाएगा. संवर्धित प्रक्रिया समापन होने के बाद , MGM, मुम्बई, में स्लाइड अध्ययन किया जाएगा। परिणाम PI द्वारा तैयार किया जाएगा और सीआई (CI) और आई.सी.एम.आर., दिल्ली, को परिणाम प्रस्तुत किया जाएगा।

भोपाल में अध्ययन कर्मचारियों द्वारा Karyotypes (Report) वितरित किया जाएगा; तथापि, जहां परिणाम महत्वपूर्ण है, PI परिवारोन से मामलों कि परामर्श करेंगे । वार्षिक आधार पर संयुक्त रूप से, NIREH, भोपाल में डेटा डिकोड किया जाएगा और सहभागी का स्वास्थ्य अभिलेख विश्लेषण किया जाएगा.

अध्ययन दल दौरे किए जाने के लिए सरल प्रक्रिया प्रदान करेगा और प्रक्रियाओं के अवधि प्रत्येक यात्रा के प्रारंभिक चरण (चरण-i) में एक घण्टा लग सकता है, [30 मिनट रक्त संग्रह करने के लिए और 30 मिनट रिपोर्ट (karyotype) वितरित करने के लिए]। रक्त संग्रह के बाद, 15 कार्यकारी दिनों के अंदर Karyotype तैयार किया जाएगा ।

अध्ययन में भाग लेने के लिए केवल एक ही दिन जाएगा , तथापि, आनुवंशिक आधार पर अपेक्षित परिणाम अन्तर्ग्रस्त हो, तो अनुवर्ती अध्ययन किया जाएगा ।

कोई भी सहभागी को अध्ययन से हटाया जा सकता है यदि आई.सी.एम.आर. द्वारा वित्तपोषण बंद कर दिया जाए.

आप किसी भी समय हिस्सा लेना बंद कर सकते हैं. तथापि, यदि आप अध्ययन में हिस्सा लेना बंद करना चाहते हैं, हम प्रोत्साहित करते हैं कि आप पहले अध्ययन स्टाफ से तथा अपने नियमित डाक्टर हैं बातचीत करें। या perticipation से गंभीर स्वास्थ्य प्रभाव नहीं होगा।

जोखिम और असुविधाएँ

अध्ययन के समय आपको किसी खतरे या कष्ट / असुविधाएँ नहीं होगा।

यदि आवश्यक हो तो, आपको स्वास्थ्य सूचना के लिए , परिवार के सदस्यों की वंशावली, वेतन का संग्रह, रक्त (2-3 ml) के लिए , Karyotype वितरण और परामर्श के लिए, सम्पर्क किया जाएगा। रक्त प्रशिक्षित

"भोपाल के जनसंख्या पर दिसंबर 1984 में हुए MIC गैस दुर्घटना के दीर्घ अवधि आनुवंशिक प्रभाव , यदि कोई हो "

अत:, इस अध्ययन से ऐसा कुछ नहीं होगा जैसे काम करने की असमर्थता, गर्भवती बनने की , या पिता बनने की संवेदनशील निर्णयनहीं असमर्थता नहीं होगा. इस अध्ययन से न हीं आपकी स्वास्थ्य स्थिति में सुधार होगा, न ही स्वास्थ्य खराब होगा. तथापि, यदि आवश्यक हो तो, भविष्य के स्वास्थ्य की आवश्यकता के लिए निवारक उपाय स्थापित किया जा सकता है.

लाभ

इस अध्ययन में शामिल होके, आप हमारे लिए जो जानकारी / सूचना प्रदा न करेंगे, जो MIC के दीर्घ अवधि प्रभाव , यदि कोई हो, के अध्ययन में डाक्टरों को दर्शाता प्रदान करेगा। वे अपके Karyotype प्रतिवेदन प्रस्तुत करेगा।।

हो सकता है या नहीं भी हो सकता है कि इस अध्ययन में हिस्सा लेने से आप को सीधे चिकित्सा लाभ हो. परंतु आनुवंशिकता / आनुवंशिक जानकारी / सूचना से परिवार के अन्य सदस्यों के साथ-साथ भावी पीढ़ीं / वर्तमान पीढी को लाभ होगा।

खर्चा

सभी व्यावसायिक/नैदानिक परीक्षण, विभिन्नलोगों, नैदानिक तथा प्रयोगशाला अध्ययन नि:शुल्क किया जाएगा।

भाग लेने के लिए भुगतान

आपको यह अनुसंधान अध्ययन में भागीदारी के लिए कोई वित्तीय लाभ नहीं दिया जाए गा।

फिल्लौर

इस अध्ययन से जो आनुवंशिक जानकारी होगा, यह सुनिश्चित कर वर्तमान और भविष्य परिवार के स्वास्थ्य के लिए, और अन्य खून के रिश्ते के जिए, बनुत हीमहत्वपूर्ण लाभ होगा।.

गुप्तता

यह अनुसंधान अध्ययन में एकत्रित सूचना सार्वजनिक मंच में प्रकाशित अथवा प्रस्तुत किया जा सकता है। लेकिन आपका नाम और अन्य पहचान की सूचना का उपयोग नहीं करेंगे । आपकी पहचान और शामिल चिकित्सा रिकार्ड्स नियामक मानदंडों के अनुरूप से गोपनीय माना जाएगा।

अध्ययन से संबंधित सभी दस्तावेज में केवल आपके द्वारा असाइन अपने परिवार रोगी नंबर (या कोड) और/या आद्याक्षरों आधार पर किया जाएगा. हो सकता है कि आपके सहमति से भागीदार के नाम karyotype प्रतिवेदनों में शामिल है।

सहभागी के स्वास्थ्य डेटा, परिवार आकार, अनुभव स्थिति, इत्यादि को कम्प्यूटर में दर्ज किया जाएगा, और इलेक्ट्रॉनिक मीडिया के माध्यम से प्रेषित किया जाएगा। जांचकर्ताओं और सलाहकार परिषद् और सूचना, आई.सी.एम.आर. को इस इलेक्ट्रोनिक डाटा प्राप्त होगा, तथापि सहभागी के पहचान गोपनीय रखी जाएगी ।

आई.सी.एम.आर. और MGM आचार संहिता समीक्षा बोर्ड अनुसंधान अभिलेखों की समीक्षा करेगी।

डेटा विश्लेषण करते समय, भागीदार जांचकर्ताओं को MIC के प्रभाव के संबंध में बताया जाएगा. भागीदार के अनुमति से, उनके पारिवारिक डाक्टर (जी.पी.) को उनके इस अध्ययन में भागीदारी के बारे में सूचित किया जाएगा ।

ऐच्छिक सहभागिता/ अध्ययन से वापस

इस अध्ययन में भाग लेने के लिए अपने निर्णय स्वैच्छिक है। आप किसी भी समय अध्ययन में भाग लेने से इनकार कर सकते हैं , या अध्ययन से आपको वापस ले सकते हैं और अध्ययन से हट सकते हैं। भाग नहीं लेने का फैसला से आप या आपके अन्य चिकित्सा परिचर्या अध्ययन पर कोई असर नहीं पडेगा।

यदि आपका डॉक्टर यह महसूस करती है कि आपको यह अध्ययन से वापस लेना आपके सर्वोत्तम हित में है, अध्ययन डाक्टर आपके सर्वानुमति बिना आपको अध्ययन से हटा सकते हैं.

इस अध् ययन में बने रहने के लिए हम आप को नई जानकारी देंगे यदि आपके स्वास्थ्य / कल्याण,पर असर तो।

अध्ययन संबंधी चोट के लिए चिकित्सा देख-रेख

इस अध् ययन के फलस्वरूप कोई बीमारी या चोट लगने का खतरा नहीं है ।

प्रश्न

भागीदारी के बारे में या आपके अधिकार के बारे में आप किसी प्रश्न पूछने के लिए स्वतंत्र हैं। अध्ययन के दौरान या बाद में यदि कोई प्रश्न उठाया गया, अध्ययन कर्मचारियों और अध्ययन डाक्टर, NIREH, भोपाल को संपर्क

"भोपाल के जनसंख्या पर दिसंबर 1984 में हुए MIC गैस दुर्घटना के दीर्घ अवधि आनुवंशिक प्रभाव , यदि कोई हो "

जब तक कि आपके पास सभी प्रश्नों का संतोषजनक उत्तर नहीं है, यह सहमति फॉर्म पर हस्ताक्षर नहीं करना ।

सहमति के विवरण

मैं इस कंसेट फॉर्म पढा है । मैं , इस अवसर पर डॉ. बानी bandana गांगुली, प्रधान जांचकर्ता और डा.एन. बनर्जी, सह जांचकर्ता या उनके / उसके अध्ययन स्टाफ से अनुसंधान और विचार-विमर्श किया, और अपने प्रश्न का उत्तर मुझे बताया गया है। अध्ययन के लाभ-जोखिम समझाया गया है। मेरा यह मानना है कि अध्ययन दल में शामिल किसी भी सदस्य अनुचित रूप से प्रभावित नहीं किया। मैं समझता हूं कि इस कंसेट फॉर्म में हस्ताक्षर करने के बाद इस के प्रति मेरे लिए दी जाएगी। इस अध्ययन में भाग लेने के लिए अपने निर्णय स्वैच्छिक है। मैं समझता हूं कि किसी भी समय मैं अध्ययन में भाग लेने से इनकार कर सकता हूं , या अध्ययन से आपने आपको वापस ले सकते हूं । मैं इस अनुसंधान में भाग लेने के लिए सहमत हूं ।

मैं समझता हूं कि निजी आनुवंशिक जानकारी पहचान गोपनीय रखी जाएगी । मैं जांचकर्ताओं द्वारा और अनुसंधान परिषद् और आचार बोर्ड के कर्मचारियों द्वारा अपने चिकित्सा रिकाईस का निरीक्षण करने के लिए अधिकृत करता हूं।

कंसेट फॉर्म पर हस्ताक्षर करने से में किसी भी रूप से एक अनुसंधान अध्ययन में भागीदार की कानूनी अधिकार नहीं छोड रहा हूं ।

मैं इस बात से सहमत हूं कि इस अध्ययन से संबंध में संपर्क किया जा रहा है। हां □ नहीं □

मैं इस बात से सहमत हूं कि मेरे परिवार चिकित्सक को अधिसूचित किया जा रहा है की मैं इस अध्ययन में भागीदार हूं । हां □ नहीं □

दिनांक_____

सहभागी हस् ताक्षर_____

(दिन/माह/वर्ष)

सहभागी प्रकाशित नाम _____

[यदि बच्चों के साथ अध्ययन होगा, निम्नानुसार माता-पिता या बच्चे की कानूनी अभिभावक के और बच्चों की सहमति प्राप्त किया जाएगा]

माता-पिता/ कानूनी अभिभावक के हस् ताक्षर _____ दिनांक_____

(दिन/माह/वर्ष)

माता-पिता/ कानूनी अभिभावक के नाम _____

बच्चे के हस् ताक्षर_____ दिनांक_____

(दिन/माह/वर्ष)

बच्चे का प्रकाशित नाम _____

[अध्ययन के लिए सहमति देने के लिए अक्षम लोगों के लिए , निम्नानुसार कानूनी अभिभावक के सहमति प्राप्त किया जाएगा]

_____ अभिभावक के हस् ताक्षर _____ दिनांक_____

(दिन/माह/वर्ष)

"भोपाल के जनसंख्या पर दिसंबर 1984 में हुए MIC गैस दुर्घटना के दीर्घ अवधि आनुवंशिक प्रभाव , यदि कोई हो "

कानूनी अभिभावक के प्रकाशित नाम_____

[यदि तृतीय पक्ष हस्ताक्षर हैं, निम्नलिखित जानकारी शामिल होगा, यदि लागू हैं,]

मैं, पुष्टि कर रहा हूं कि सहभागी और सहभागी के प्रतिनिधि तथा कानूनी रूप से स्वीकार्य प्रतिनिधि को जानकारी और सूचना सही रूप से समझाया गया , और सहभागी इस अध्ययन में भाग लेने के लिए सहमति दी.

साक्षी हस् ताक्षर_____ दिनांक_____

(दिन/माह/वर्ष)

विटनेस प्रकाशित नाम _____

सहभागी को जानकारी और सूचना सही रूप से समझाया गया , और सहभागी जानबूझकर इस अध्ययन में भाग लेने के लिए सहमति दी.

प्रकाशित नाम _____ दिनांक_____

(दिन/माह/वर्ष)

हस् ताक्षर_____

इस अध्ययन में भूमिका_____[अधिकृत/योग्यता के सदस्य अनुसंधान अध्ययन दल अर्थात् जांचकर्ता, नर्स, आदि]

[संभावना है कि अध्ययन दल के सदस्यों के परिवार के सदस्य अध्ययन में भाग ले - सूचना निम्नानुसार प्राप्त किया जाना चाहिए]

अध्ययन दल के सदस्यों से सम्बन्ध _____[उदाहरणार्थ, अध्यापक/प्रोफेसर सदस्य या परिवार]

ANNEXURE II:

English Consent Form

'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'

RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

Title of Study

"Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984".

Project number: 65/2/BBG/11-NCD-II

Principal Investigator (PI)

Dr. Bani Bandana Ganguly, Head, MGM Center for Genetic Research & Diagnosis, MGM Institute of Health Sciences, Kamothe, Navi Mumbai 410209. Tel. 91 22 27437895, Fax: 91 22 27430320, Mobile 91 9869214680, e-mail – mgmgeneticlab@yahoo.com.

Co-Investigator (CI)

Dr. N. Banerjee, Head, National Institute of Research on Environmental Health (ICMR), Kamla Nehru Hospital Building, Gandhi Medical College, Bhopal-462001 (Madhya Pradesh-India) Telefax-0755-2533976, Mobile-09826037631, 91 7552533976.

Sponsor

Indian Council of Medical Research (ICMR), Ansari Nagar, New Delhi 110029, India.

You are being asked to participate in a genetic screening programme (a human research study). Please take your time to review this consent form and discuss any questions, you may have, with the study staff. You may take your time to make your decision about participating in this study and you may discuss it with your regular doctor, friends and family before you make your decision. This consent form may contain words that you do not understand. Please ask the study doctor or study staff to explain any words or information that you do not clearly understand.

The study doctor (and or/ institution) is (are) not receiving professional fees and financial support from the participant to conduct this study.

Purpose of Study

This genetic screening will be conducted to study current status of genetic condition of those exposed to methyl isocyanate in 1984 and examined during 1986-1990, and to compare with the previous genetic data to exclude long term effect of MIC 28 years after exposure, if any, and their progenies who were born after the disaster to those exposed parents (as many as can be traced). **You are being asked to take part in this study because**

- **You were screened after the disaster**
- **Your current status will be compared with the previous condition**
- **Screening of your children will be done for comparison**
- **Your entire family will be benefitted with these information**
- **Clinical/health information will be recorded in three-generation pedigree**

- **Genetic aberrations, if detected, can be prevented from transmission**
- **Possible future health risk will be calculated from the screening**

A total of 100 individuals (families) from exposed group and 100 from unexposed group will participate in the first year of this study.

The purpose of this study is to investigate long term genetic effects of MIC, if any, on Bhopal population exposed to MIC-gas leak in 1984.

Phase 1 studies: Health information will be collected in three-generation pedigree chart which will highlight the health condition since 1984 of the exposed person and their children. Health condition will be studied in the first phase. The pedigree will also record the details of marriage and child birth and their health.
Participants will be registered in exposed and control groups.

Phase 2 studies: *Genetic study will be carried out to find out* the level of spontaneous genetic effect to understand the level of instability and also to find out whether there is any transmissible genetic effect in any family occurred de novo or as a result of MIC-effect.
Karyotype (chromosomal fingerprint) will be given to each participant. Cases with significant result will be counseled by PI at one-to-one basis.

Phase 3 studies: Data will be analysed to calculate long term of effects of MIC, if any, at genetic level. The study will further highlight the necessity for introducing precautionary measures, if required.

This research is important because currently there is no information available on genetic effect of MIC on Bhopal population.

Study procedures

Genetic screening was carried out after MIC-disaster on exposed and unexposed population in a multi-centric screening program sponsored by ICMR during 1986-1990. Those individuals, today families, will be traced and given priority for participation in this study.

There will be two different types of families, such as

1. In 1984, if men of 25-30 years and women of 20-25 years (child bearing age) got exposed and survived, they will be 51-56 years and 46-51 years old after 26 years, and they can have male and female children of 20-25 years (appx) and grandchildren of 1-5 years old, at least one.
2. Individuals with exposure at pre-adolescent age (10-12 years) will become parents of 1-16 years old children after 26 years.

3-generation pedigree will be prepared along with clinical examination from all those included for cytogenetic studies during 1986-90 by all six centers. This would provide information about clinical genetic effects.

Quality assurance will be put in place first before further cytogenetic studies.

Cytogenetic study will include

i. Study of spontaneous and constitutive chromosome aberrations in those individuals of exposed and unexposed group and the children born to those individuals.
ii. Estimation of cryptic rearrangements by FISH for above mentioned cases.

In this present study, investigators will be kept blinded about participant's exposure status and health history.

2-3 ml peripheral blood will be required only once during the entire study period.
Technique in brief: The blood samples will be cultured in mitogen-supplement nutrient medium for 72 h at 37^0C at NIREH, Bhopal. After termination of cultures and completion of processing, slides will be studied at MGM Institute, Mumbai. Result will be prepared by PI and submitted to CI at Bhopal and ICMR, Delhi. Karyotypes will be distributed by study staff at Bhopal; however, PI will do counseling for the cases/families with significant results. Annually the data will be decoded jointly by PI and CI at NIREH, Bhopal and analysed with participant's health records.

The study team will provide simplified schema and/or calendar of visits and procedures to be carried out for each visit which may take an hour or so at the initial phase (phase I), 30 mins during blood collection and 30 mins for delivering the karyotype report. The karyotype result will be ready within 15 working days after blood collection.

Participation in the study will be for a single event; however, if required depending on genetic result, the study will involve follow-up.

The researcher may decide to take you off this study if funding is stopped by ICMR.

You can stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the study staff and your regular doctor first. There will not be any serious consequences or health effect from the perticipation.

Risks and Discomforts

While on the study, you are at no risk for any side effects or discomforts.
You will be contacted for health information, drawing pedigree of your family members, collection of peripheral venous blood (2-3ml) through single vein puncture, distribution of your karyotype and counseling, if required. Blood will be collected by trained phlebotomist.
Hence there are no *such things as the inability to work, potential anxiety related to the sensitive nature of the questions asked,* becoming pregnant or father a baby while on this study *etc.*

Your health condition will not improve or will not worsen while participating in this study. However, preventive measures can be installed, if required for future health.

Benefits

By participating in this study, you will be providing information to the study doctors that will show the effects of long term effect of MIC, if any, on your health. They will deliver your karyotype report. They will also take necessary measure in case of genetic instability or high rates of spontaneous chromosomal damage.

There may or may not be any direct medical benefit to you from participating in this study. We hope the information learned from this study will benefit other family members in present as well as future generation, since genetic information is passed on through heredity.

Costs

All professional / clinical advices, diagnostic and laboratory tests which will be performed as part of this study, etc. are provided at no cost to you. There will be no cost for the study treatment that you will receive.

Payment for participation

You will not be given any financial benefit for your participation in this research study.

Alternatives

Genetic information that would come from this study of yourself and other members of blood relation would be of tremendous importance for present and future health of your family.

Confidentiality

Information gathered in this research study may be published or presented in public forums. However your name and other identifying information will not be used or revealed. Medical records that contain your identity will be treated as confidential in accordance with the regulatory norms.
All study documents related to you / your family will bear only your assigned patient number (or code) and /or initials.

Depending on your consent, participant's name may be revealed in karyotype reports.

Participant's health data, exposure status, family size, etc. will be entered into the computer and transmitted electronically. Investigators, Advisors and ICMR will receive this electronic data; however, identifying information will be kept confidential.

ICMR and project advisors may have access to records containing personal health information and genetic records for monitoring quality assurance. ICMR Ethics Board

and MGM Ethics Board may review research-related records for quality assurance purposes.

While analysing the data, participant's exposure status will be disclosed to investigators for understanding the effect of exposure to MIC.
With participant's permission, their Family Physician (GP) will be notified about your participation in this study.

Voluntary Participation/Withdrawal From the Study

Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time. Your decision not to participate or to withdraw from the study will not affect your other medical care.

If your study doctor feels that it is in your best interest to withdraw you from the study, your study doctor will remove you without your consent.

We will tell you about any new information that may affect your health, welfare, or willingness to stay in this study.

Medical Care for Injury Related to the Study

There is no risk of injury or illness resulting from this study.

Questions

You are free to ask any questions that you may have about your participation and your rights as a research participant. If any questions come up during or after the study, contact the study doctor and the study staff at NIREH, Bhopal.

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

Statement of Consent

I have read this consent form. I have had the opportunity to discuss this research study with Dr._Bani Bandana Ganguly, the Principal Investigator and Dr. N. Banerjee, the co-Investigator_and or her/his study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I believe that I have not been unduly influenced by any study team member to participate in the research study by any statement or implied statements. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this genetic screening is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that information regarding my personal genetic identity will be kept confidential. I authorize the inspection of my medical records by investigators and their staff, ICMR and The Research Ethics Board.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

I agree to being contacted in relation to this study. Yes No

I agree to my family physician being notified of my participation in this study. Yes No

Participant signature_____ **Date** _____ **(day/month/year)**

Participant printed name: _____

For studies with children, consent would be obtained from the parent or legal guardian and assent would be obtained from the child as follows:

Parent/legal guardian's signature_____ **Date** _____ **(day/month/year)**

Parent/legal guardian's printed name: _____

Child's signature _____ **Date** _____ **(day/month/year)**

Child's printed name: _____

For studies involving persons who are incapable of giving consent, consent would be obtained from their legal guardian as follows:

Legal guardian's signature_____ **Date** _____ **(day/month/year)**

Legal guardian's printed name: _____

If applicable, when third party signatures are required, the following would be added:

I, the undersigned, attest that the information in the Participant Information and Consent Form was accurately explained to and apparently understood by the participant or the participant's legally acceptable representative and that consent to participate in this study was freely given by the participant or the participant's legally acceptable representative.

Witness signature_____ **Date** _____ **(day/month/year)**

Witness printed name: _____

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent

Printed Name: _____ **Date** _____ **(day/month/year)**

Signature: _____

Role in the study: _____/authorized/qualified member of the research team i.e. investigator, study nurse, etc.]

For studies that may have potential enrollment of family members of the research team the potential conflicting relationship should be disclosed as follows:]

Relationship to study team members:_____[eg. supervisor, teacher/professor or family member.]

ANNEXURE III:

Mode of Operation

ANNEXURE III

'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984' submitted by Bani B. Ganguly

(Project No. 65/2/BBG/11-NCD-II)

1. **STANDARD OPERATING PROCEDURE**

Topic: Sample collection and culture at NIREH Bhopal and analysis at Navi Mumbai

At NIREH, Bhopal

Tracing prescreened cases by Research Officers of NIREH

Sampling: Blinded sampling.

Sample: 2-3 ml Peripheral blood for each subject in sterile sodium heparin vacutainer

Cell culture: At NIREH following standard protocol by project staff and maintained at 37°C for 72 hours. Harvesting will be done following standard colchicine-hypotonic-fixative schedule.

Transportation of processed sample: At room temperature by project staff from NIREH to Mumbai

At MGM Institute of Health Sciences (MGMIHS), Mumbai

Registration of processed samples with lab code: The processed samples will be unpacked at MGMIHS and registered with lab code and no.

Slide preparation and banding: Slides will be prepared and stained following FPG staining for M1 metaphases and GTG-banding for karyotyping. FISH will be considered after analysis of M1 cells for spontaneous aberrations and karyotyping.

Analysis: Analysis of spontaneous analysis will be carried out by JRF on 100 M1 cells. JRF will be responsible for technical operation as well. The result will be reviewed by SRF and PI. Karyotyping and FISH will be done by SRF and reviewed by PI.

Reporting: After every cycle, summary of the result will be submitted to PI at NIREH along with two slides randomly selected from the batch. The result will also be submitted to ICMR, N. Delhi. PI at NIREH may send the slides to any other lab for cross check or the PI of Mumbai may send to BARC or ACTREC for comparison.

Reporting of karyotypes: Karyotype will be given to all participants. Abnormal karyotypes will be distributed by PI of Mumbai lab for genetic counseling and necessary guidance to individual and family members.

Comparative analysis: The current result will be compared with earlier result jointly by PI-MGMIHS and PI-NIREH. The current result will also be analysed jointly correlating with demographic and health status.

Final report: Final report will be prepared by PI-MGMIHS, and discussed with PI-NIREH and Project Advisor. The report will finally be submitted to ICMR, N. Delhi.

SOP was prepared by Prof. Bani B. Ganguly, PI and checked by the CI, Dr. N. Banerjee

FLOW CHART OF THE OPERATION OF THE PROJECT

1. Activities at NIREH, Bhopal under the guidance of Co-PI:

Two Research Officers (RO) from NIREH (Bhopal)

⇩

Tracing pre-Screened Families

Collection of pedigree and current history

Label the vacutainers with code
Collection of Blood (2-3 ml in sodium heparin vacutainer)
Do not open tube. Inject the sample through cap.
Do not place sample on ice.

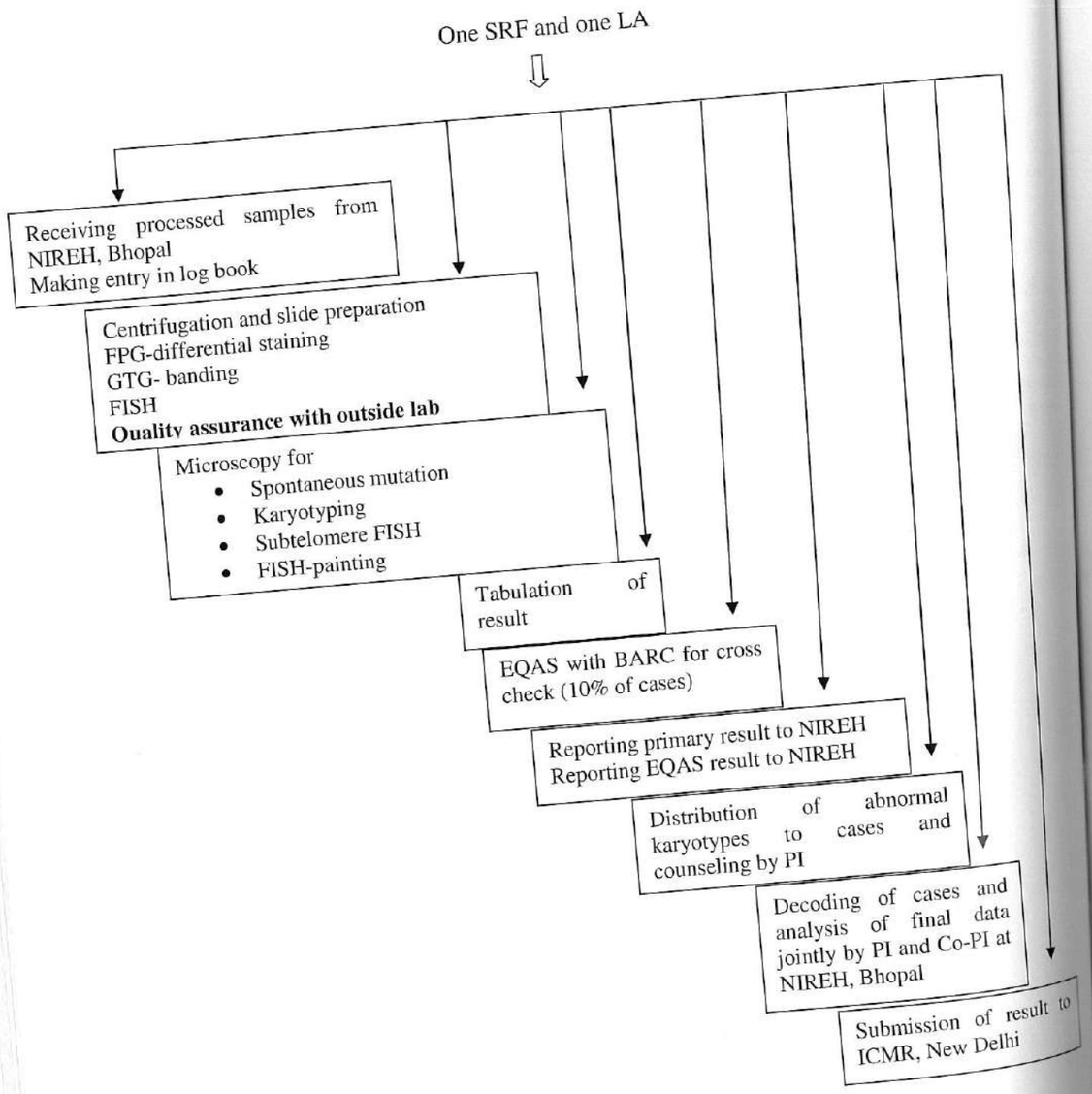
Setting up of cell culture by project staff (4/sample: 2+BrdU, 2-BrdU)
Label culture tubes and pour complete medium
Inoculate sample and incubate at 37°C for 72h

Harvesting of cultures after 72h following standard technique by project staff
Preparation of one slide for quality checking

Feeding history in computer system
Receiving result from Mumbai and feeding in system

Statistical analysis of genetic data with qualitative and quantitative attributes

2. Activities at MGMIHS, Mumbai under the guidance of PI:



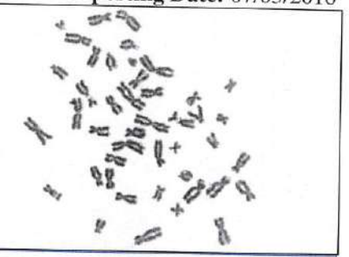
ANNEXURE IV:

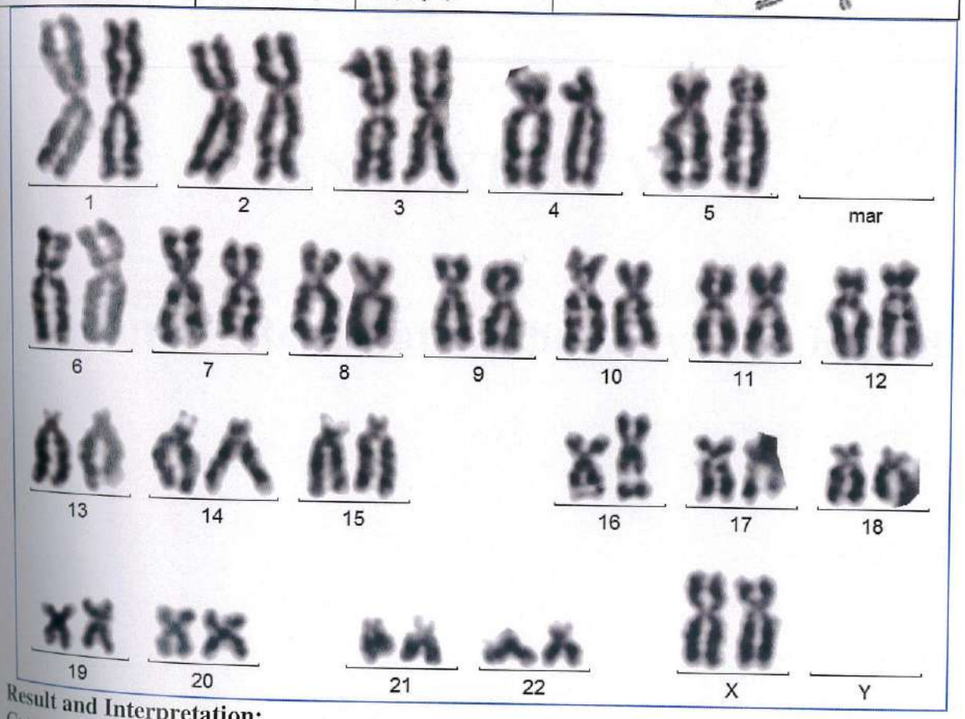
Sample Karyotype Report

CHROMOSOME ANALYSIS IN WHOLE BLOOD

Name: XXXXXX **Age/Sex:** 50 Y/F **Area/ICMR No.:** 01/1243 **Exposure:** Severe
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied**: 100
Collection Date: 25/04/2016 **Culture Date**: 27/04/2016 **Reporting Date:** 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Fra(16q22)
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX,fra(16q22)	Fra(16q22)





Result and Interpretation:
Cytogenetic testing following PHA-stimulated whole blood culture and GTG-banding revealed 46,XX,fra(16q22) chromosomal pattern in all cells examined.
Karyotyping is mandatory for siblings and offspring.
Genetic counseling is strongly recommended.

Dr. Bani B. Ganguly Ph.D, FICMCH
Consultant Geneticist
E-mail: mgmgeneticlab@yahoo.com

ANNEXURE V:

Signature of Recipients of the Karyotype Reports

Area 1

1 Govind Prajapati — 1308
Kalpna — 1308
Durga - daughter

(10) Nafees Ali — 1138
Shafika Ali — 1138

3. Mrs. Punabai — 185
Mr. Bundel Singh — 185

4- Mr. Kailash — 857 Rajak

5. Tulsa Bai — 254
Jamuna Prasad — 254 Reddy

6. Kashi Bai → 188

7. MD. Sahid → 1009

8. Miss Shabnam → Shiddhika Bee. (Mother) 1072

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(1) Area 1. BABU LAL — 024 → 9/9/2014

SUSHILA BAI — 024

LOCALITY NO. 1

SR. NO.	ICMR NO.	PATENTS NAME	KARYOTYPE	RICEIVED DATE	NAME & SIGNATURE	
1	✓90	Kamala Bai	[illegible]	15/6/17	केशरबाई	[illegible]
2	✓111	Kashi Bai			Tarachand	[illegible]
3	✓107	Umedi Bai			Vinod	
4	✓171	Bhagwati Bai			Kammobai — Soniya	
5	✓171	Veni prasad				Kamalabai
6	✓174	Kamala Bai			Kamlabai	
7	✓174	Santosh			Chhagan Ahirwar	chhagan
8	✓188	Kashi Bai			Usha	[illegible]
9	✓216	Guddi Rasene			Manish	मनीष
10	✓290	Nirmala				[illegible]
11	✓290	Ramesh Srivastav		15/6/2017		
12	✓300	Sushila Bai				[illegible]
13	✓404	Raisa Bee				
14	✓409	Sanjoy Sharma				[illegible]
15	✓474	Bilkis Bee				
16	✓493	Kaniza Bee				
17	✓523	Sahida Bee				
18	✓621	Zulekha Bee				
19	1243	Malti Bai				
20	1804	Shadma Anjum				
21	1846	Leela Bai				

Kareem bax / Risaldar / J.P. Nagar — 15/6/17 Bhopal

Received — हस्ताक्षर

01/1128 / Nabees ali 01 → नफीसअली
01/1138 / Shafika bee 02 →

01/1243 / 02 Malti bai मालतीबाई — मालती

01/1308 / 01 Govind prajapati
02 Kalpana — कल्पना

01/1009/01 Sahid Noor Ashma

01/1804 Anjum Fairpran. इसरार खान / Son

01/1846 · Leelabai – 02 — [illegible] Son

LOCALITY NO. 2

SR. NO.	ICMR NO.	PATENTS NAME	KARYOTYPE	RICEIVED DA	NAME & SIGNATURE	
22	111	Rashid Khan				
23	355	Sitara Bee				
24	550	Zubeda Bee				
25	740	Md. Saheed				
26	1034	Shazadi Bee				
27	1053	Irfan				

LOCALITY NO.7

SR. NO.	ICMR NO.	PATENTS NAME	KARYOTYPE	RICEIVED DA	NAME & SIGNATURE	
28	7	Mithilesh Tiwari			Manorma	मनोरमा
29	32	Brijkishor			Brij kishor	B. Kishor
30	38	Rajesh/ Raju			Swapna Dixit	
31	38	Kanta Prasad			Sadhna Dixit	
32	38	Udaynarayan	Expired			
33	55	Shabeena Bee			Deepika	[illegible]
34	55	Haseena Bee				
35	182	Rajkumari			Ravi Rao	[illegible]
36	192	Rakesh Malviya				
37	192	Kamala Malaviya			Kamla	कमला
38	202	Mullo Bai			Rakesh	[illegible]
39	251	Kala Bai			Bismarts	[illegible]
40	284	Ramcharan			Rupesh	Rupesh
41	289	Radha Bai			Radha bai	[illegible]
42	340	Laxminarayan			Krishna	[illegible]
43	346	Kanta bai			Shailendra	Shailendra
44	346	Raju				
45	357	Jagdish	Mohit			Mohit
46	389	Khema Bai	Khema			[illegible]
47	392	Draupati	Pooja			[illegible]
48	392	Lala Ram				
49	394	Kanti Bai	Hemant			Hemant
50	399	Asha Bai	Deepak			[illegible]
51	423	Parwati Bai	Narendra			Narendra
52	433	Kapuri Bai	Laxmi.			[illegible]
53	458	Ramrati Bai	Neeraj			[illegible]
54	663	Shushila Bai	Mulak			
55	656	Vashanti Borashi	Sashi		सुधा	[illegible]
56	664	Sarju Bai	Arti			[illegible]
57	759	Yashoda Bai	Shobha			[illegible]
58	760	Hari Prasad	Pramila			[illegible]
59	767	Kailash Bai	Pritipal Singh			[illegible]
60	1043	Bishan Lat				
61	1043	Neeraj	Saroj bai			सरोज
62	1043	Vinod				

LOCALITY NO. 3

SR. NO.	ICMR NO.	PATENTS NAME	KARYOTYPE	RICEIVED DA	NAME & SIGNATURE
63	542	Shahna			
64	564	Amisha Begam			
65	576	Peepu			
66	590	Md. Mushtaq			
67	594	Aneesha khatoon			
68	624	Nosha Khan			
69	624	Naseem Akhtar			
70	721	Saida Bee			
71	729	Rami Bee			
72	824	Humera			
73	843	SM. Furkan			
74	847	Israil			
75	879	Md. Sabir			
76	880	Naseem Bano			
77	882	Md. Salim			
78	905	Munni Bai			
79	1009	Bebi			
80	1059	Aliya Bee			
81	1063	Mubeen Khan			
82	1063	Saida Bee			
83	1103	Femlada Bee			
84	1112	Md.Rais			
85	1156	Firoz			
86	1156	Naim			
87	1162	Musharat Jaha			
88	1182	Aisha Bee			

LOCALITY NO. 5

SR. NO.	ICMR NO.	PATENTS NAME	KARYOTYPE	RICEIVED DA	NAME & SIGNATURE
89	375	Nandkishor			Smt Seema Khare
90	397	Ushadevi Vishwakarma			

91 759 Kok Singh

Loacality No.10

SR. NO.	ICMR NO.	PATENTS NAME	KARYOTYPE	RICEIVED DA	NAME & SIGNATURE
92	165	Murlidhar			
93	172	Kamlesh			
94	263	Geeta			
95	587	Mahesh			
96	771	Laxmi Tiwari			

LOCALITY NO.16

SR. NO.	ICMR NO.	PATENTS NAME	KARYOTYPE	RICEIVED DA	NAME & SIGNATURE
100	269	Gulab Bai			
101	274	Sk haneef			Shabnam 21-6-17.
102	274	Zubeda Bee			Zubeda 21-6-17
103	302	[illegible]			Ratna
104	305	Urmila Bai			
105	312	Sharad Kanade			
106	312	Vijaya Kanade			
107	315	Rukaiyya			Aarti, Rukaiya, Suman
108	320	Suman Bai			
109	330	Komal Singh			
110	336	Tara Bai			
111	340	Raju Mandle			Tota Ram
112	354	Ramaji			GEET
113	362	Gora Bai			
114	362	Surendra			
115	380	Kamla Malvi			Kamla Vindviya
116	382	Suman rajput			
117	382	Mahipal Singh			
118	441	Ganga Bai			
119	441	Basant Solanki			
120	452	Hemraj			
121	486	Ashok Singh			
122	493	Sakum Bai			
123	493	Jagat Singh			
124	496	Choti Bai			
125	498	Mathura Devi			Shivam 16/6/17
126	498	Surendra			
127	518	Govind Singh			
128	518	Vimla			
129	771	Sabara Bee			
130	273	Geeta Bai			GEETA
131	269	Kamlesh			

SR. NO.	ICMR NO.	PATENTS NAME	KARYOTYPE	RICEIVED DA	NAME & SIGNATURE
100	269	Gulab Bai			Anita
101	274	Sk haneef			
116	382	Suman rajput			Kumud Rajput
117	382	Mahipal Singh			Kumud Rajput

4/9/17

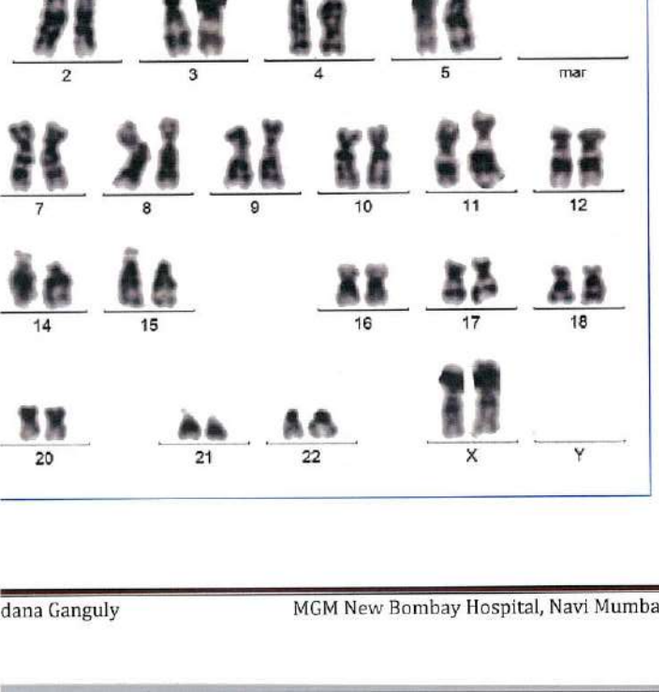
ANNEXURE VI:

Karyotypes of all Participants

CHROMOSOME ANALYSIS IN WHOLE BLOOD

/07 **Age/Sex:** 55 Y/F **Exposure:** Severe
od **Method:** Cell culture and G-banding **Cells studied**: 125
3/02/2016 **Culture Date**: 25/02/2016 **Reporting Date:** 29/02/2016

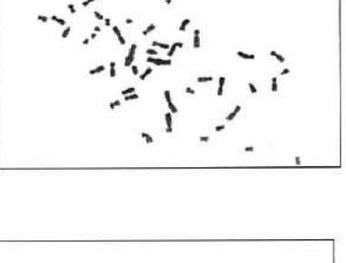
	Number	Normal/Abnormal
	6	Normal
	4	Normal
	14	9qh+
	6	Normal
	6	Normal
	4	Normal
	4	Normal
	2	Normal
	46,XX,9qh+	9qh+

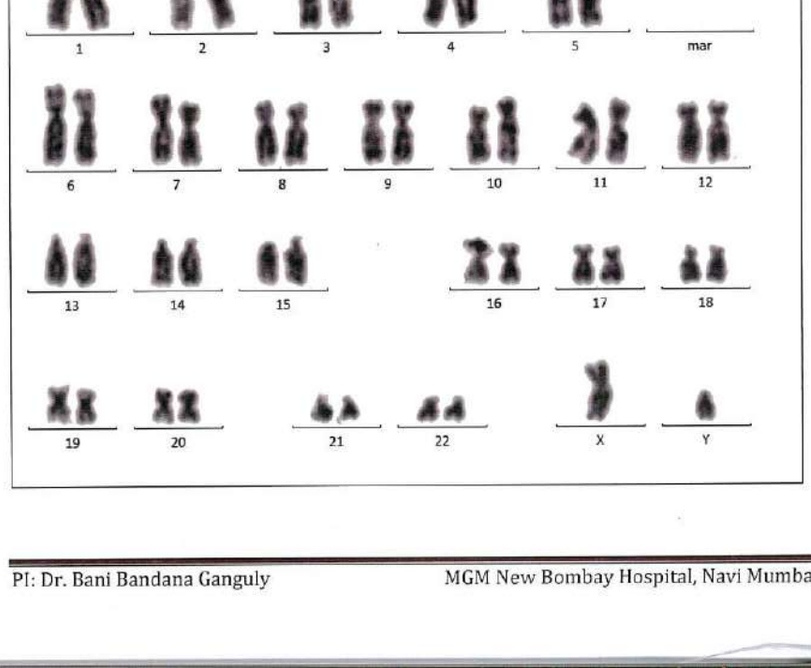


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ ICMR No: 01/ 24 **Age/Sex:** 70 Y/M **Exposure:** Severe
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied**: 07
Collection Date: 8/05/2014 **Culture Date**: 9/05/2014 **Reporting Date:** 16/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal





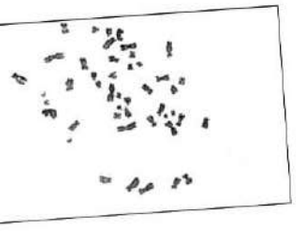
OMOSOME ANALYSIS IN WHOLE BLOOD

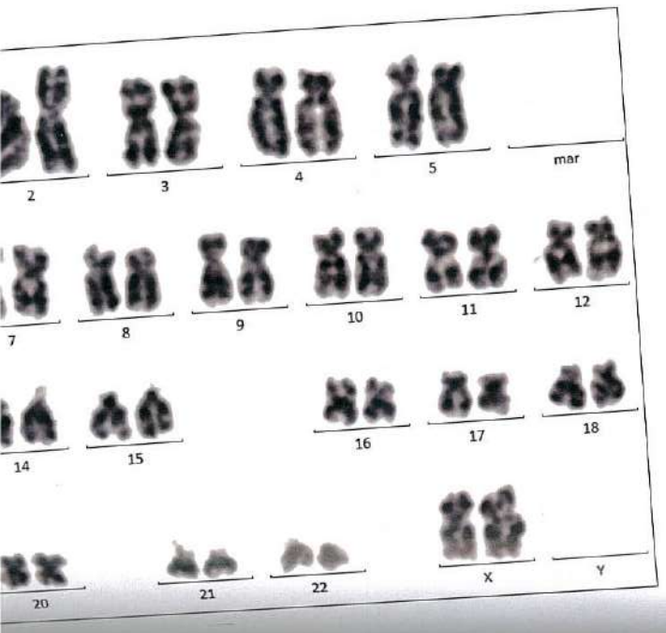
4

Age/Sex: 60 Y/F
Method: Cell culture and G-banding
Culture Date: 9/05/2014

Exposure: Severe
Cells studied: 22
Reporting Date: 19/05/2014

mber	Normal/Abnormal
	Normal
	Normal
	9qh+
	Normal
	Normal
	Normal
	Normal
	Normal
5,XX,9qh+	9qh+





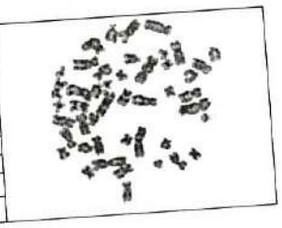
CHROMOSOME ANALYSIS IN WHOLE BLOOD

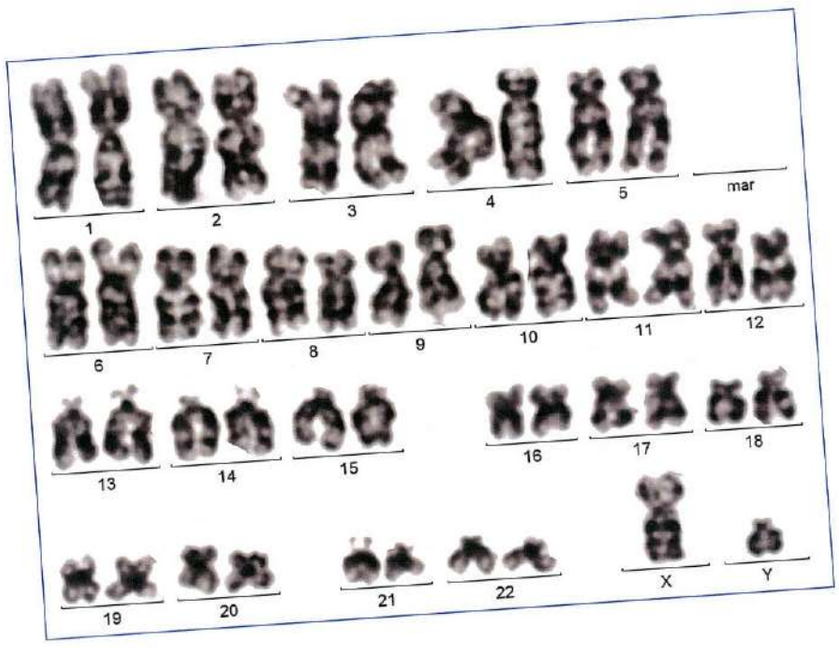
Area/ICMR No: 7/32
Sample: Whole Blood
Collection Date:

Age/Sex: 58 Y/M
Method: Cell culture and G-banding
Culture Date: 10/05/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 17/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	13p+
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY,13p+	13p+





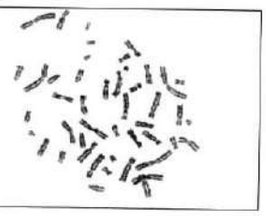
CHROMOSOME ANALYSIS IN WHOLE BLOOD

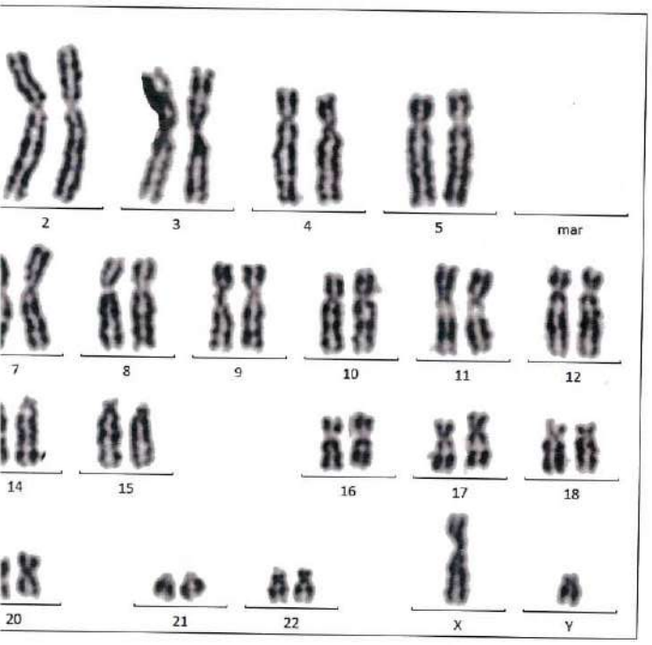
7/38
lood
23/02/2016

Age/Sex: 40 Y/M
Method: Cell culture and G-banding
Culture Date: 25/02/2016

Exposure: Severe
Cells studied: 110
Reporting Date: 02/03/2016

	Number	Normal/Abnormal
	6	Normal
	4	Normal
	14	Normal
	6	Normal
	6	Normal
	4	Normal
	4	Normal
	2	Small Y
	46,XY,small Y	Small Y





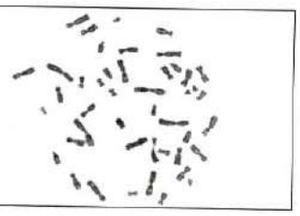
CHROMOSOME ANALYSIS IN WHOLE BLOOD

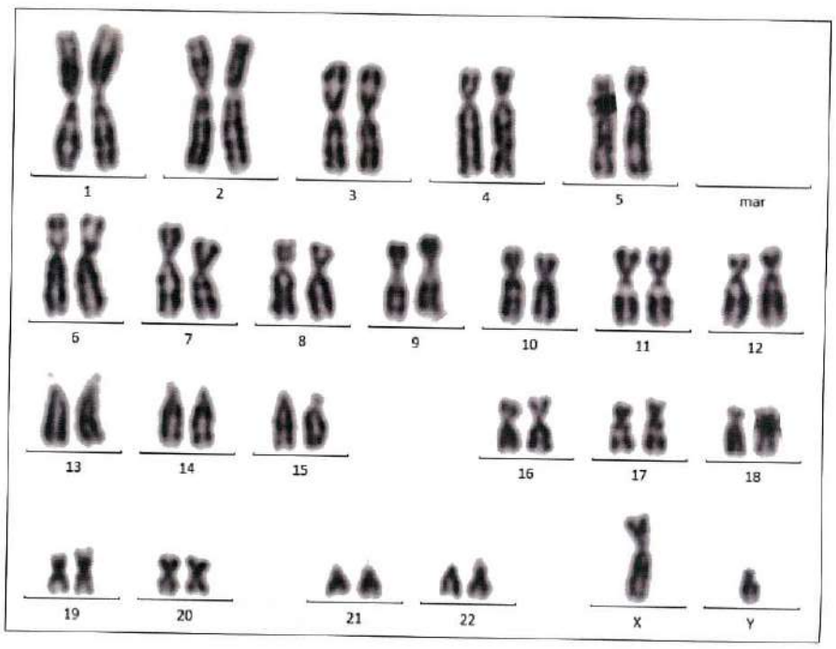
Area/ICMR No.: 7/38
Sample: Whole Blood
Collection Date: 23/02/2016

Age/Sex: 34 Y/M
Method: Cell culture and G-banding
Culture Date: 25/02/2016

Exposure: Severe
Cells studied: 128
Reporting Date: 03/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Small Y
Karyotype	46,XY,small Y	Small Y





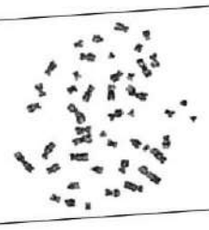
OMOSOME ANALYSIS IN WHOLE BLOOD

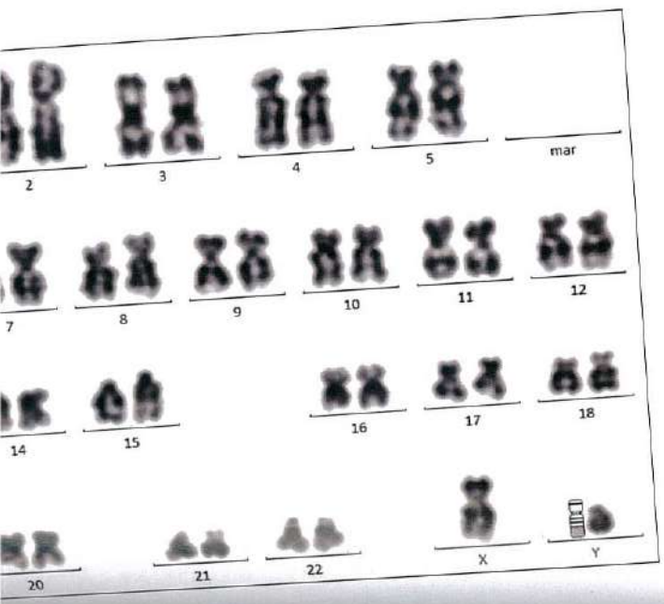
16

Age/Sex: 50 Y/M
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 27/02/2016

mber	Normal/Abnormal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Small Y
,XY,small Y	Small Y





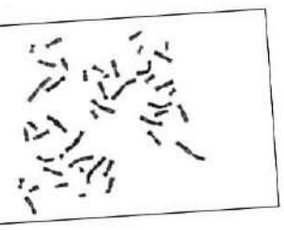
CHROMOSOME ANALYSIS IN WHOLE BLOOD

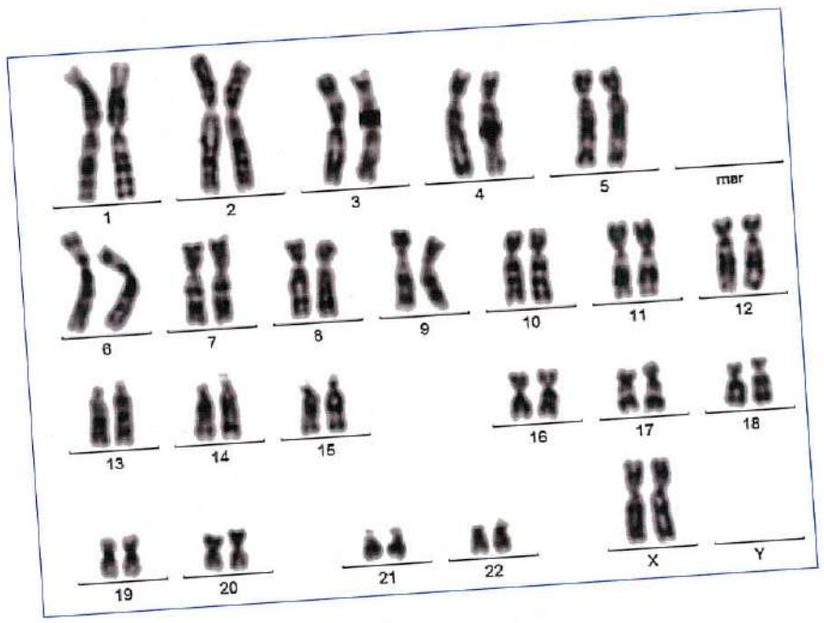
Area/ICMR No.: 7/55
Sample: Whole Blood
Collection Date: 17/02/2016

Age/Sex: 60 Y/F
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 132
Reporting Date: 27/02/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal





enetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December
1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

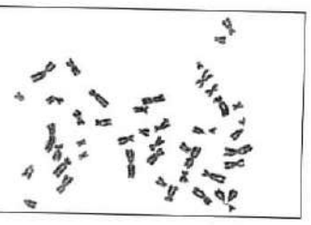
CHROMOSOME ANALYSIS IN WHOLE BLOOD

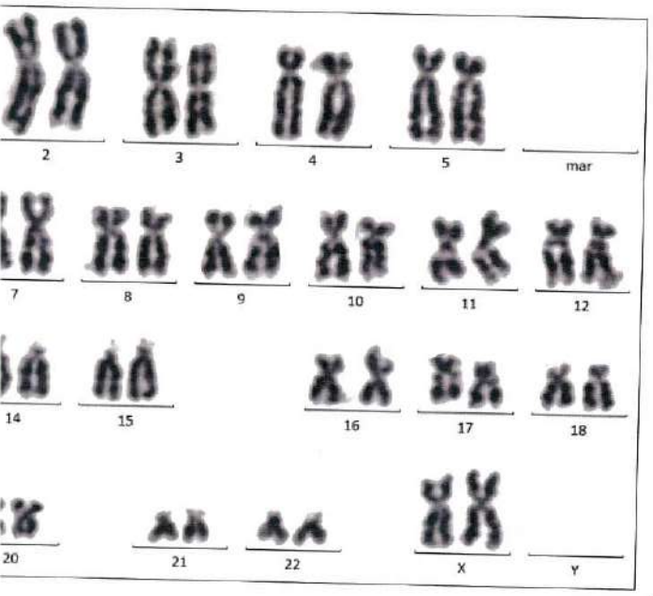
7/55
ood
3/02/2016

Age/Sex: 37 Y/F
Method: Cell culture and G-banding
Culture Date: 25/02/2016

Exposure: Severe
Cells studied: 142
Reporting Date: 12/03/2016

	Number	Normal/Abnormal
	6	Normal
	4	Normal
	14	Normal
	6	Normal
	6	Normal
	4	Normal
	4	Normal
	2	Normal
	46,XX	Normal





'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December
1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

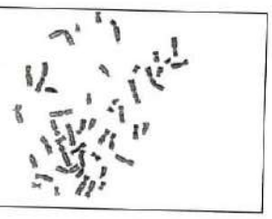
CHROMOSOME ANALYSIS IN WHOLE BLOOD

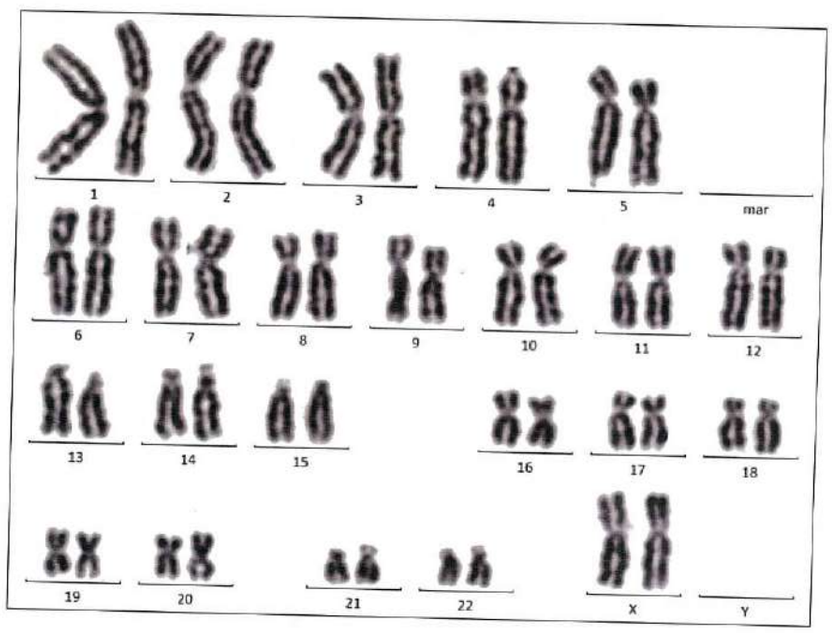
Area/ICMR No.: 1/89
Sample: Whole Blood
Collection Date: 16/02/2016

Age/Sex: 72 Y/F
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 27/02/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal



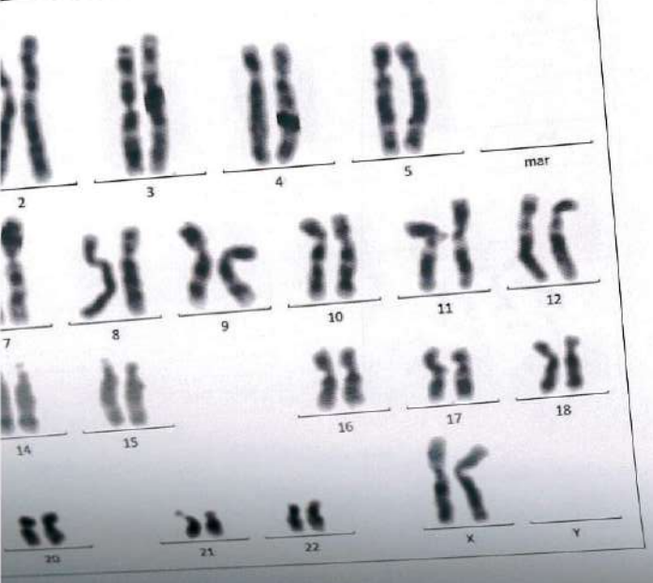


MOSOME ANALYSIS IN WHOLE BLOOD

Age/Sex: 50 Y/F
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 27/02/2016

er	Normal/Abnormal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
:X	Normal



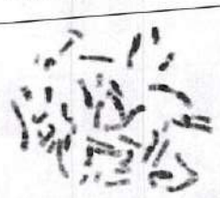
CHROMOSOME ANALYSIS IN WHOLE BLOOD

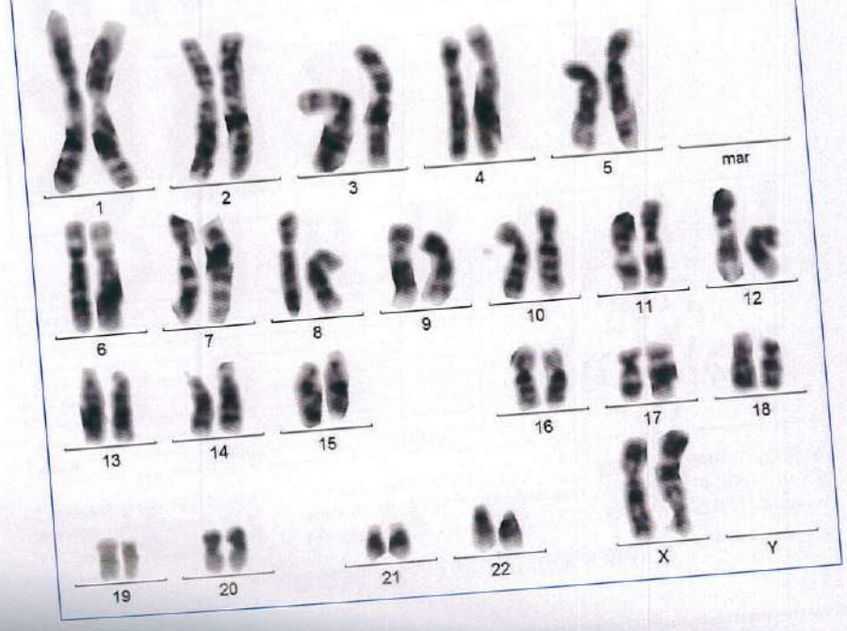
Area/ICMR No.: 1/107
Sample: Whole Blood
Collection Date: 26/04/2016

Age/Sex: 45 Y/F
Method: Cell culture and G-banding
Culture Date: 27/04/2016

Exposure: Severe
Cells studied: 60
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal





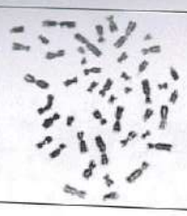
CHROMOSOME ANALYSIS IN WHOLE BLOOD

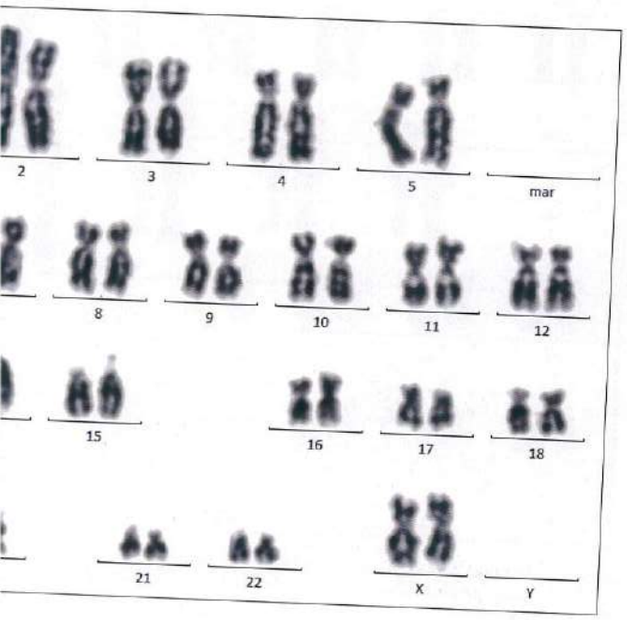
: 01/111
Blood
16/02/2016

Age/Sex: 56 Y/F
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 27/02/2016

	Number	Normal/Abnormal
	6	Normal
	4	Normal
	14	Normal
	6	Normal
	6	Normal
	4	Normal
	4	Normal
	2	Normal
	46,XX	Normal





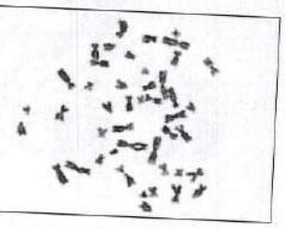
CHROMOSOME ANALYSIS IN WHOLE BLOOD

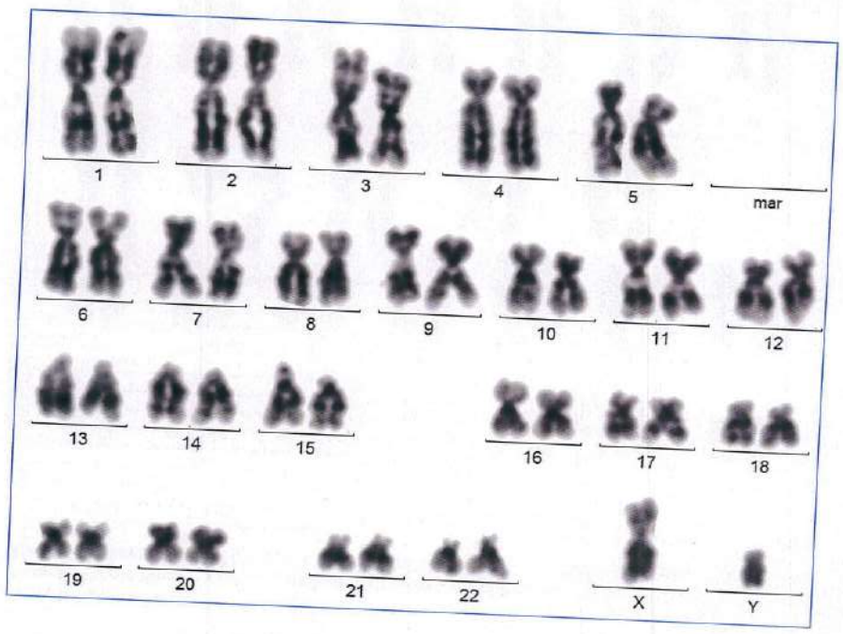
Area/ICMR No.: 02/111
Sample: Whole Blood
Collection Date: 10/03/2016

Age/Sex: 58Y/M
Method: Cell culture and G-banding
Culture Date: 12/03/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 22/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Small Y
Karyotype	46,XY,small Y	Small Y



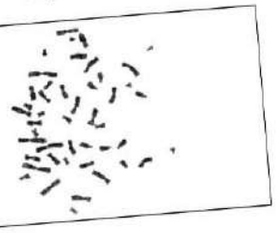


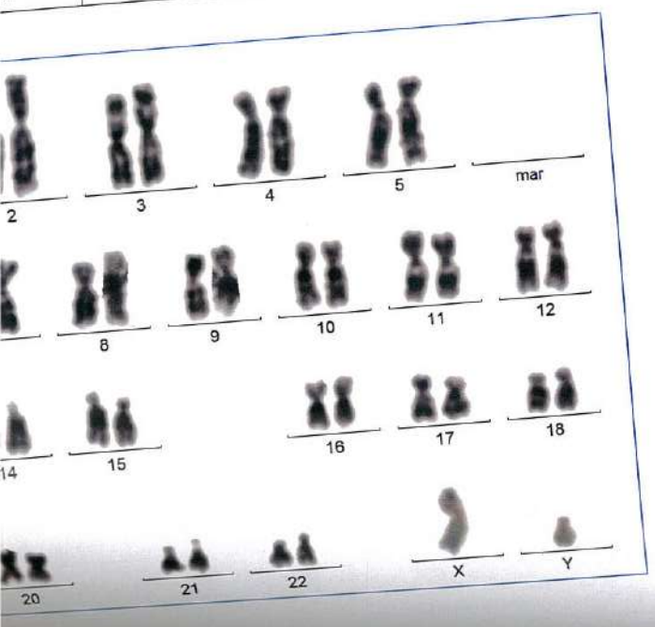
MOSOME ANALYSIS IN WHOLE BLOOD

Age/Sex: 47Y/M
Method: Cell culture and G-banding
Culture Date: 12/03/2016

Exposure: Moderate
Cells studied: 100
Reporting Date: 22/03/2016

er	Normal/Abnormal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
Y	Normal





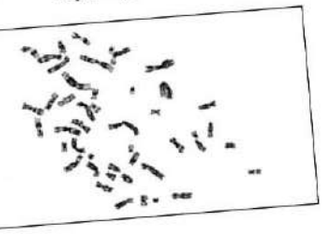
CHROMOSOME ANALYSIS IN WHOLE BLOOD

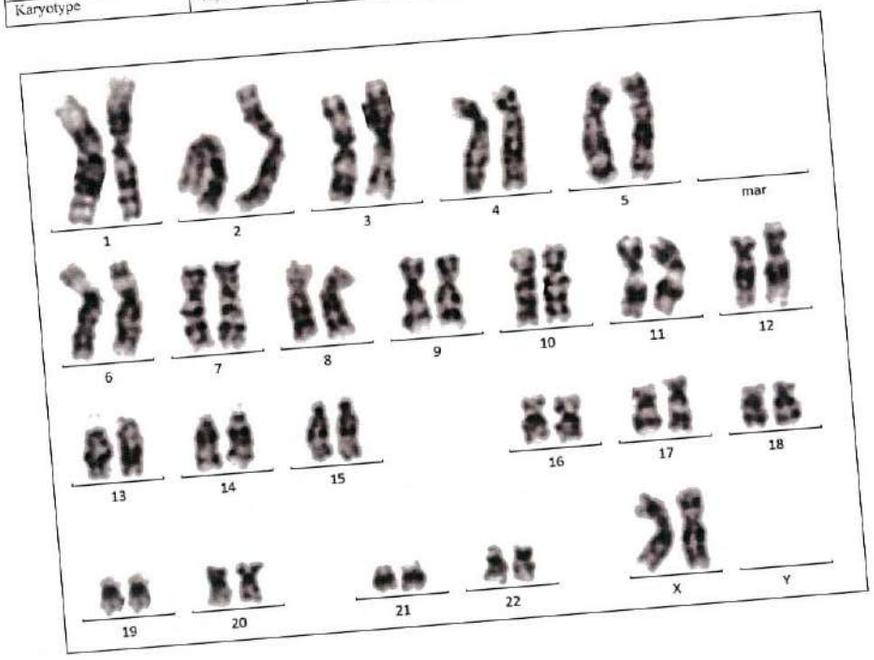
Area/ICMR No.: 1/171
Sample: Whole Blood
Collection Date: 26/04/2016

Age/Sex: 70 Y/F
Method: Cell culture and G-banding
Culture Date: 27/04/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal





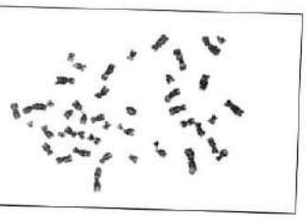
CHROMOSOME ANALYSIS IN WHOLE BLOOD

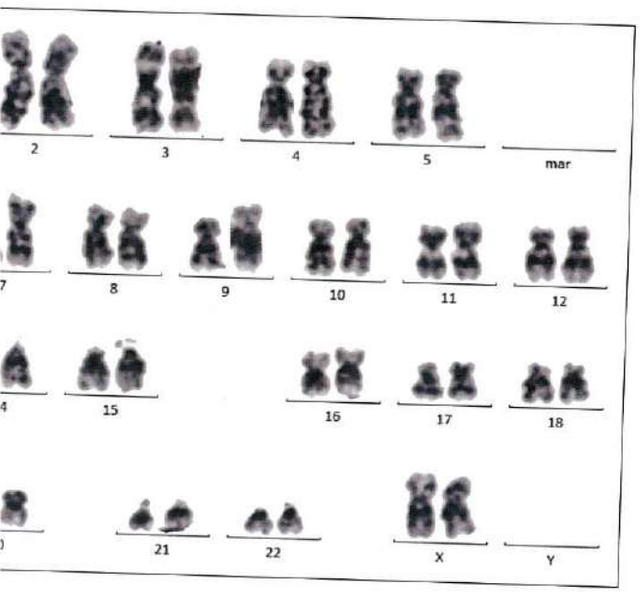
10/172
lood
1/03/2016

Age/Sex: 49Y/F
Method: Cell culture and G-banding
Culture Date: 12/03/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 22/03/2016

	Number	Normal/Abnormal
	6	Normal
	4	Normal
	14	Normal
	6	Normal
	6	Normal
	4	Normal
	4	Normal
	2	Normal
	46,XX	Normal





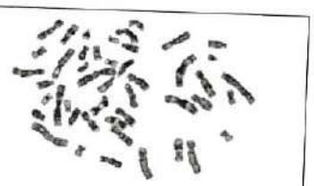
CHROMOSOME ANALYSIS IN WHOLE BLOOD

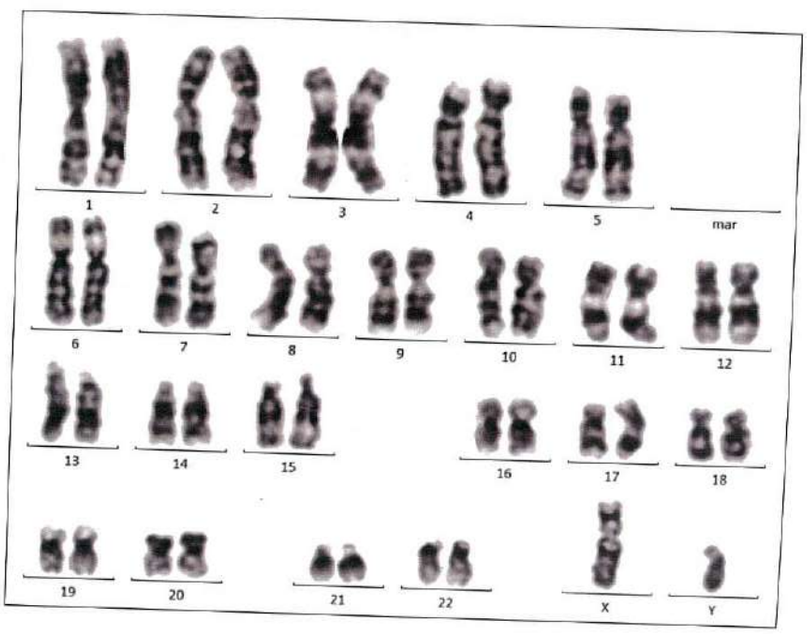
Area/ICMR No.: 1/172
Sample: Whole Blood
Collection Date: 16/02/2016

Age/Sex: 54Y/M
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 27/02/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal



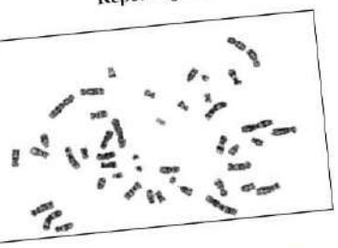


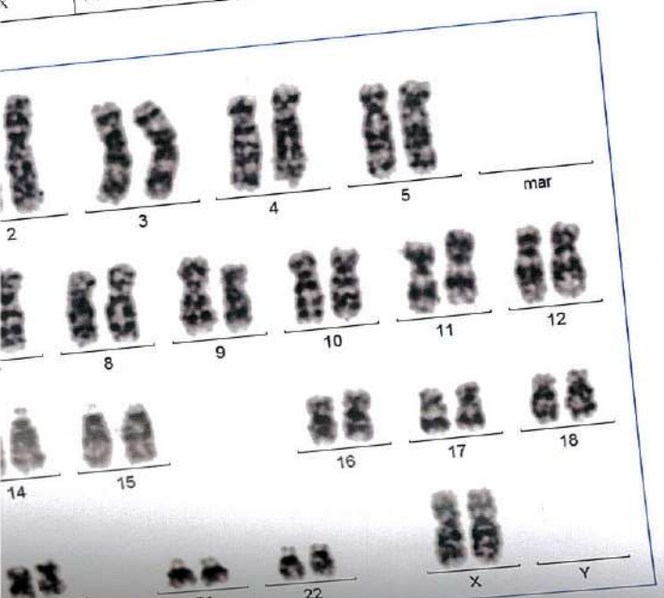
IOSOME ANALYSIS IN WHOLE BLOOD

Age/Sex: 65 Y/F
Method: Cell culture and G-banding
Culture Date: 25/04/2016

Exposure: Severe
Cells studied: 70
Reporting Date: 05/05/2016

	Normal/Abnormal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
X	Normal





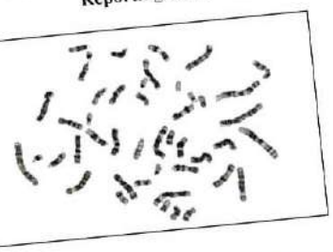
CHROMOSOME ANALYSIS IN WHOLE BLOOD

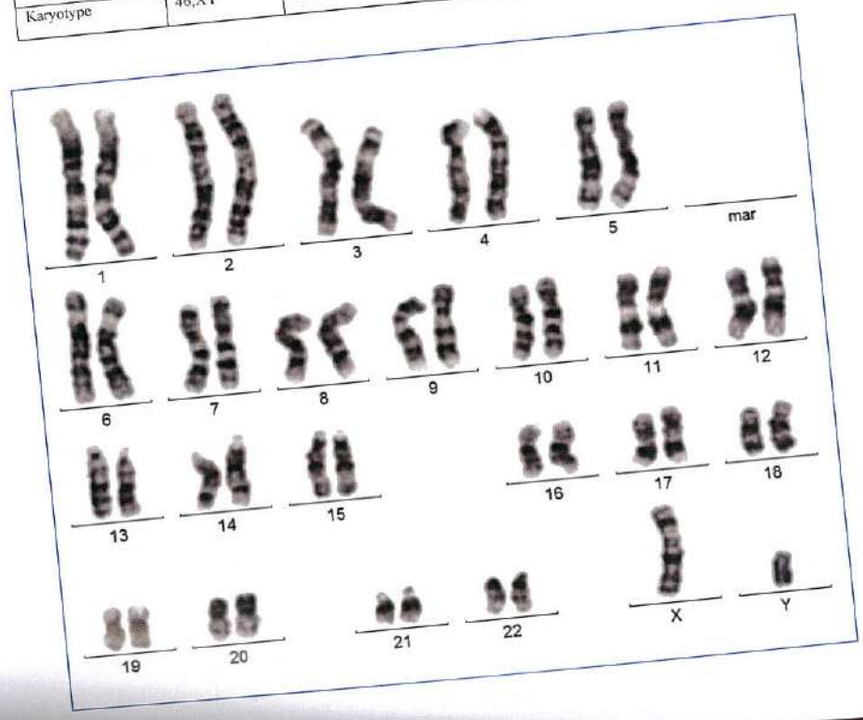
Area/ICMR No.: 1/174
Sample: Whole Blood
Collection Date: 25/04/2016

Age/Sex: 35 Y/M
Method: Cell culture and G-banding
Culture Date: 26/04/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal





MGM New Bombay Hospital, Navi Mumbai
f genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December
1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

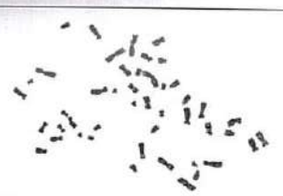
CHROMOSOME ANALYSIS IN WHOLE BLOOD

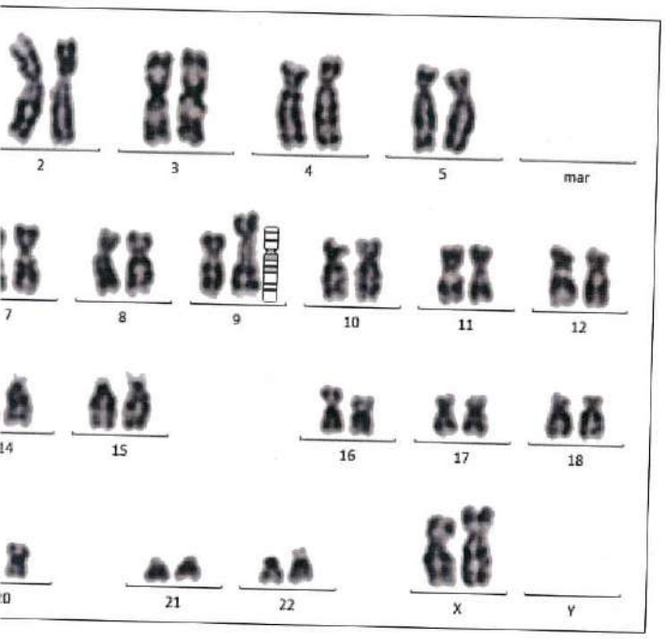
o.: 7/182
Blood
25/04/2016

Age/Sex: 52 Y/F
Method: Cell culture and G-banding
Culture Date: 26/04/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 07/05/2016

	Number	Normal/Abnormal
	6	Normal
	4	Normal
	14	9qh+
	6	Normal
	6	Normal
	4	Normal
	4	Normal
	2	Normal
	46,XX,9qh+	9qh+





'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December
1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

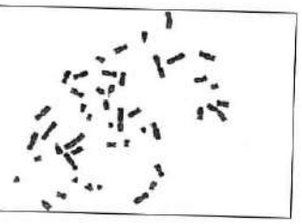
CHROMOSOME ANALYSIS IN WHOLE BLOOD

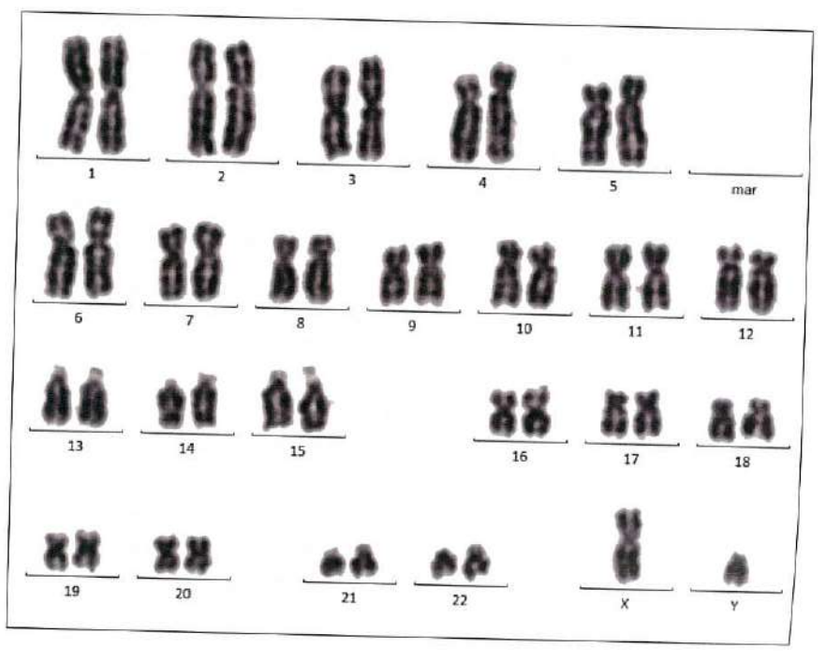
Area/ICMR No.: 1/185
Sample: Whole Blood
Collection Date: 8/05/2014

Age/Sex: 60 Y/M
Method: Cell culture and G-banding
Culture Date: 9/05/2014

Exposure: Severe
Cells studied: 28
Reporting Date: 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Small Y
Karyotype	46,XY,small Y	Small Y

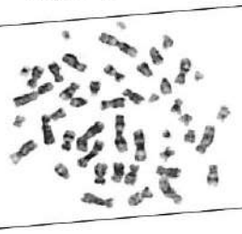




IOSOME ANALYSIS IN WHOLE BLOOD

Age/Sex: 55 Y/F **Exposure:** Severe
Method: Cell culture and G-banding **Cells studied**: 23
Culture Date: 09/05/2014 **Reporting Date:** 19/05/2014

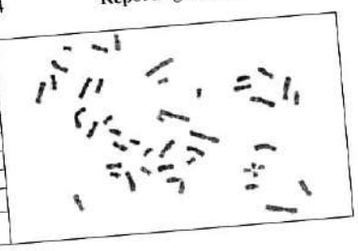
r	Normal/Abnormal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
X	Normal

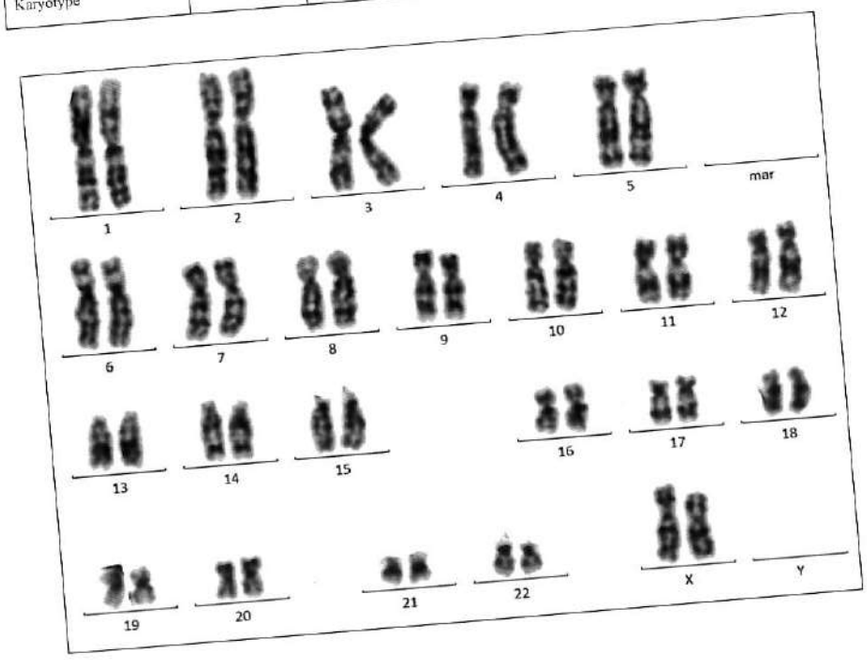


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 1/188 **Age/Sex:** 70 Y/F **Exposure:** Severe
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied**: 24
Collection Date: 16/02/2014 **Culture Date**: 9/05/2014 **Reporting Date:** 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal

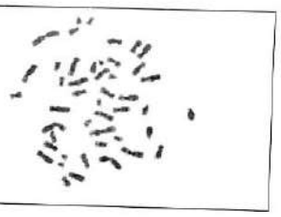


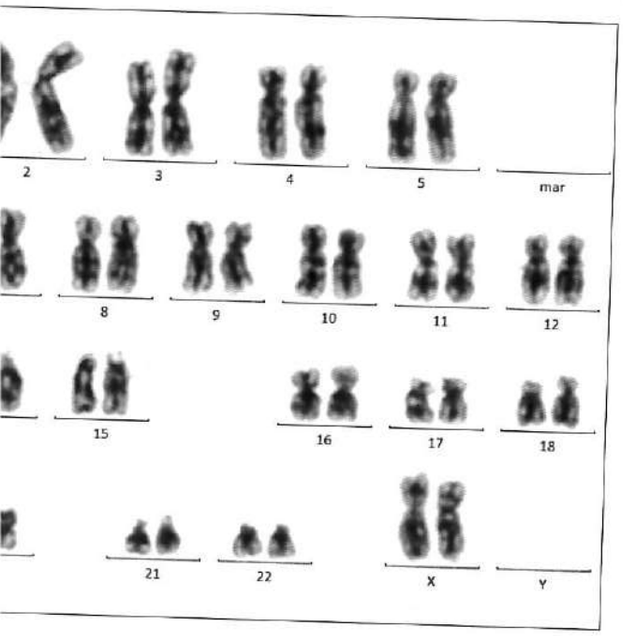


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 7/192 **Age/Sex:** 66 Y/F **Exposure:** Severe
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied**: 100
Collection Date: 17/02/2016 **Culture Date**: 17/02/2016 **Reporting Date:** 27/02/2016

	Number	Normal/Abnormal
	6	Normal
	4	Normal
	14	Normal
	6	Normal
	6	Normal
	4	Normal
	4	Normal
	2	Normal
	46,XX	Normal

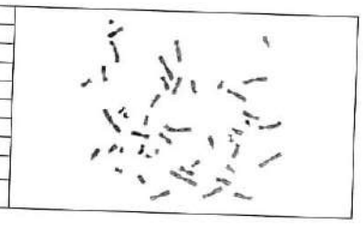


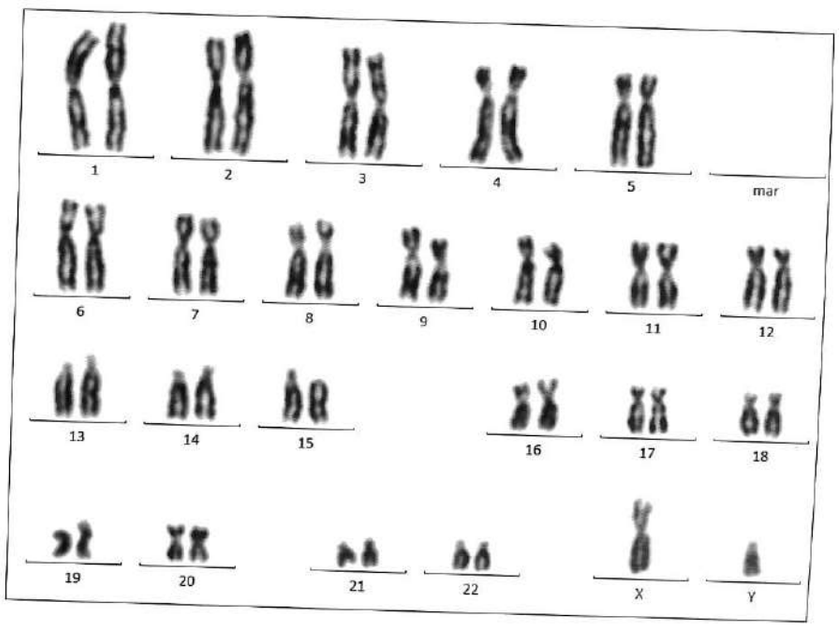


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 7/192 **Age/Sex:** 44 Y/M **Exposure:** Severe
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied**: 105
Collection Date: 23/02/2016 **Culture Date**: 25/02/2016 **Reporting Date:** 05/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Long Y
Karyotype	46,XY,long Y	Long Y



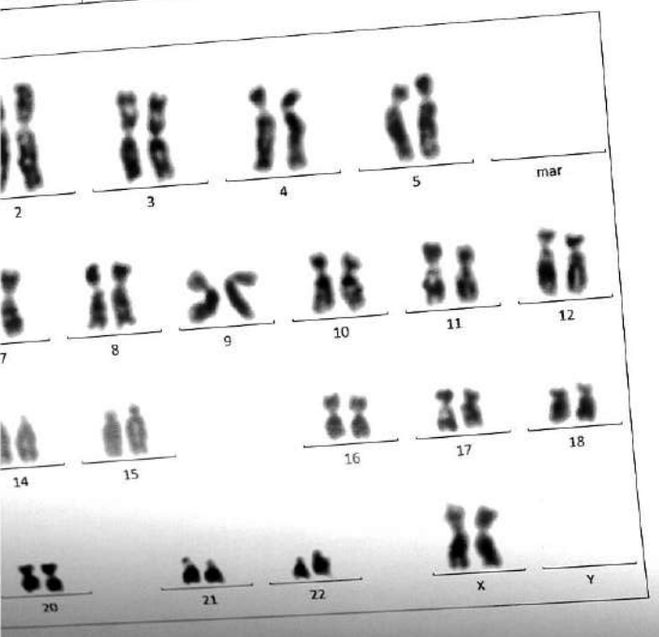


MOSOME ANALYSIS IN WHOLE BLOOD

Age/Sex: 54 Y/F
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 27/02/2016

er	Normal/Abnormal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
X	Normal



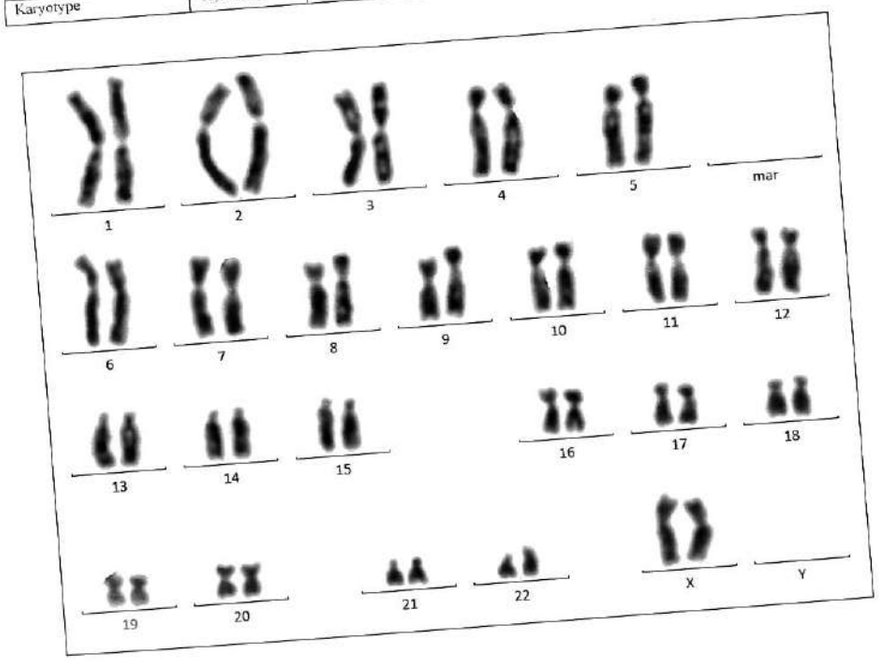
CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 1/216
Sample: Whole Blood
Collection Date: 16/02/2016

Age/Sex: 50 Y/F
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 61
Reporting Date: 26/02/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	9qh+
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX,9qh+	9qh+



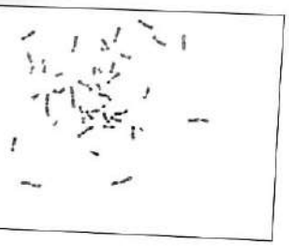
CHROMOSOME ANALYSIS IN WHOLE BLOOD

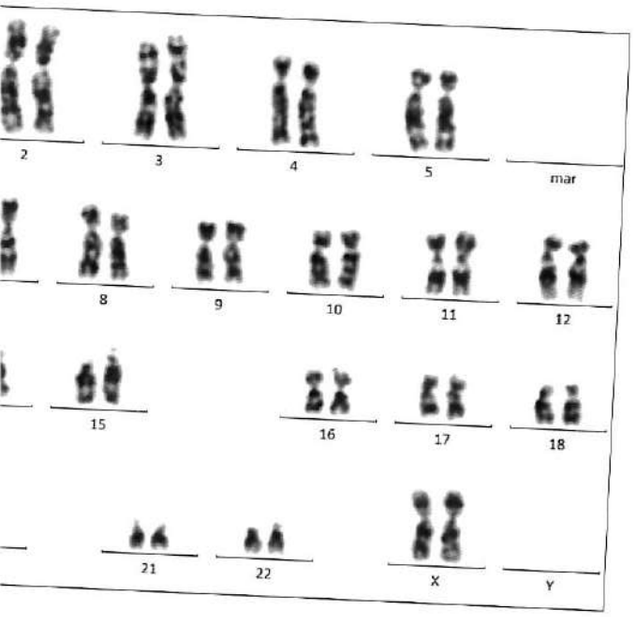
: 7/251
Blood
17/02/2016

Age/Sex: 52 Y/F
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 26/02/2016

Number	Normal/Abnormal
6	Normal
4	Normal
14	9qh+
6	Normal
6	Normal
4	Normal
4	Normal
2	Normal
46,XX,9qh+	9qh+





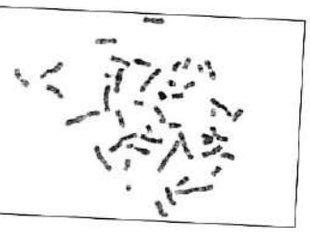
CHROMOSOME ANALYSIS IN WHOLE BLOOD

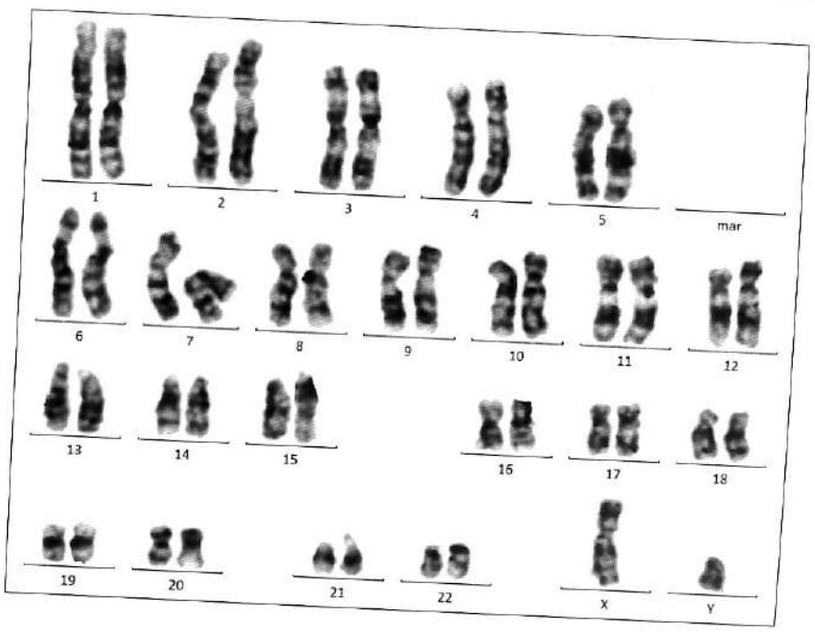
Area/ICMR No.: 1/254
Sample: Whole Blood
Collection Date: 9/05/2014

Age/Sex: 45 Y/M
Method: Cell culture and G-banding
Culture Date: 9/05/2014

Exposure: Severe
Cells studied: 38
Reporting Date: 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal



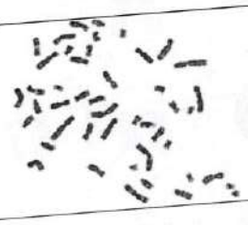


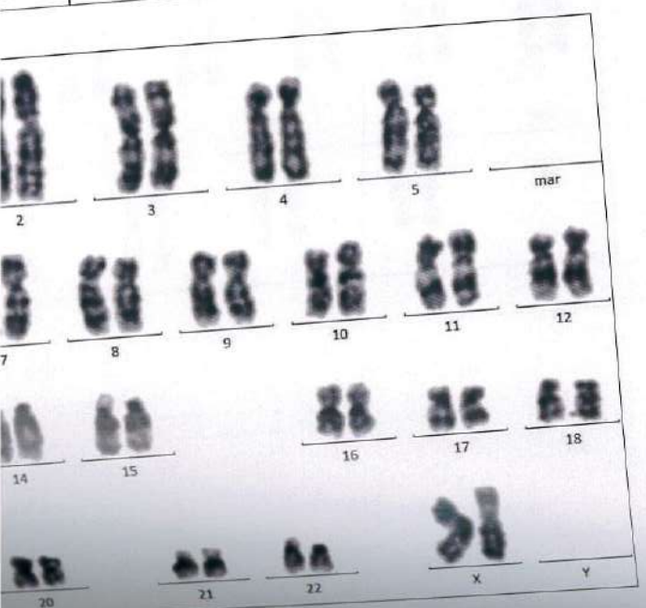
MOSOME ANALYSIS IN WHOLE BLOOD

Age/Sex: 65 Y/F
Method: Cell culture and G-banding
Culture Date: 9/05/2014

Exposure: Severe
Cells studied: 30
Reporting Date: 19/05/2014

r	Normal/Abnormal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
K	Normal





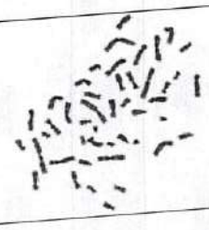
CHROMOSOME ANALYSIS IN WHOLE BLOOD

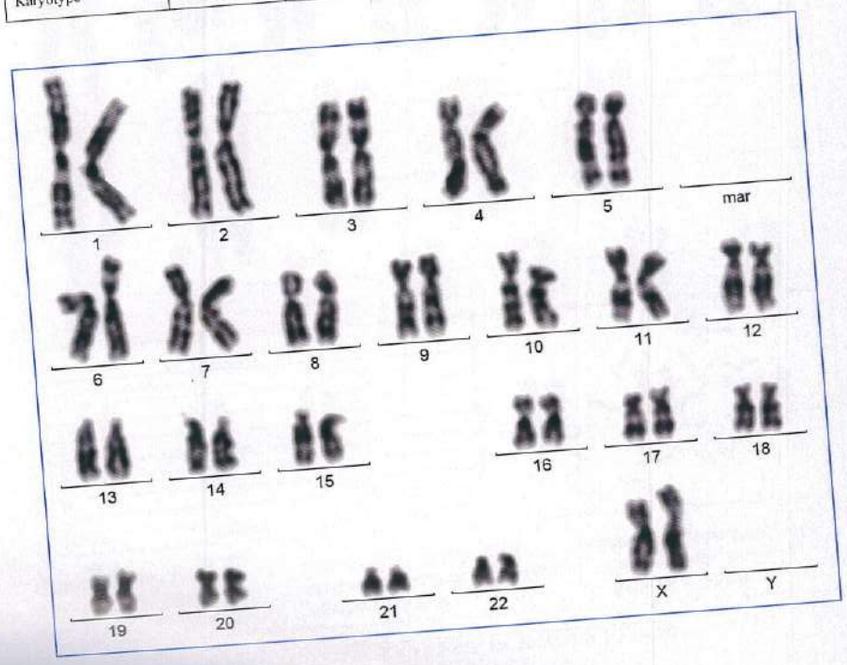
Area/ICMR No.: 7/263
Sample: Whole Blood
Collection Date: 05/05/2016

Age/Sex: 46 Y/F
Method: Cell culture and G-banding
Culture Date: 7/05/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal





MGM New Bombay Hospital, Navi Mumbai

genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December
1984 No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

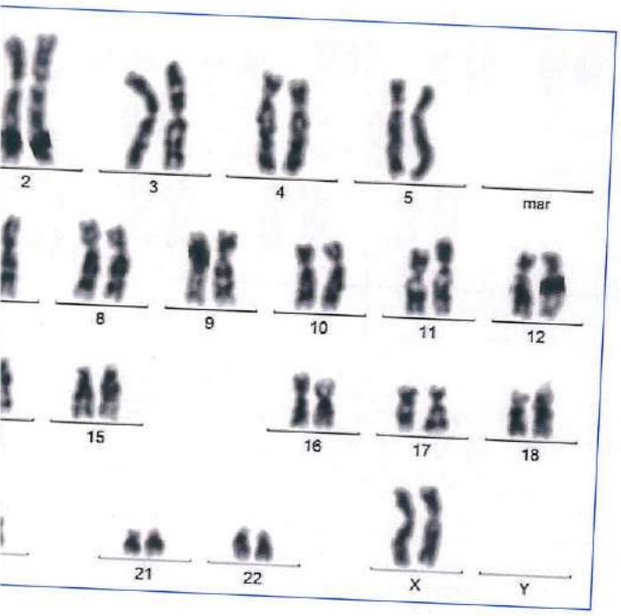
CHROMOSOME ANALYSIS IN WHOLE BLOOD

: 16/269
lood
3/04/2016

Age/Sex: 50 Y/F
Method: Cell culture and G-banding
Culture Date: 27/04/2016

Exposure: Control
Cells studied: 37
Reporting Date: 07/05/2016

Number	Normal/Abnormal
6	Normal
4	Normal
14	Normal
6	Normal
6	Normal
4	Normal
4	Normal
2	Normal
46,XX	Normal



'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

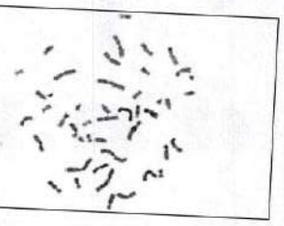
CHROMOSOME ANALYSIS IN WHOLE BLOOD

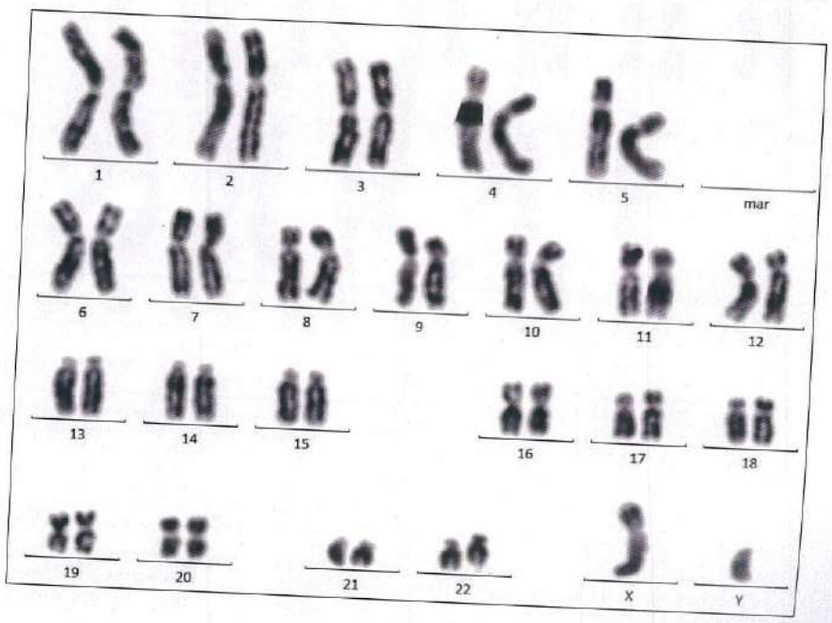
Area/ICMR No.: 16/269
Sample: Whole Blood
Collection Date: 04/02/2015

Age/Sex: 35 Y/M
Method: Cell culture and G-banding
Culture Date: 04/02/2015

Exposure: Control
Cells studied: 60
Reporting Date: 14/02/2015

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal

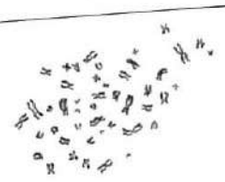


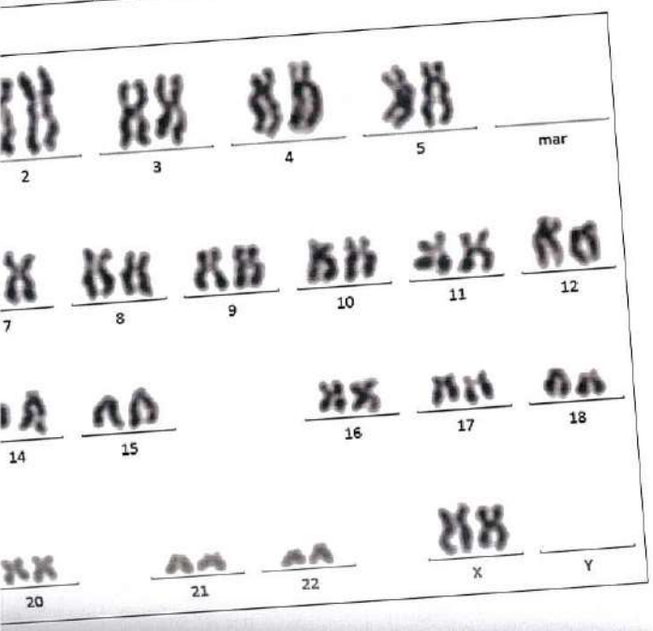


OMOSOME ANALYSIS IN WHOLE BLOOD

Age/Sex: 60 Y/F
Method: Cell culture and G-banding
Culture Date: 04/02/2015
Exposure: Control
Cells studied: 10
Reporting Date: 14/02/2015

ıber	Normal/Abnormal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
.XX	Normal

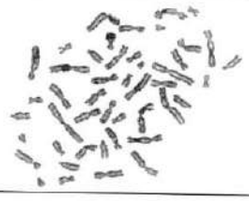


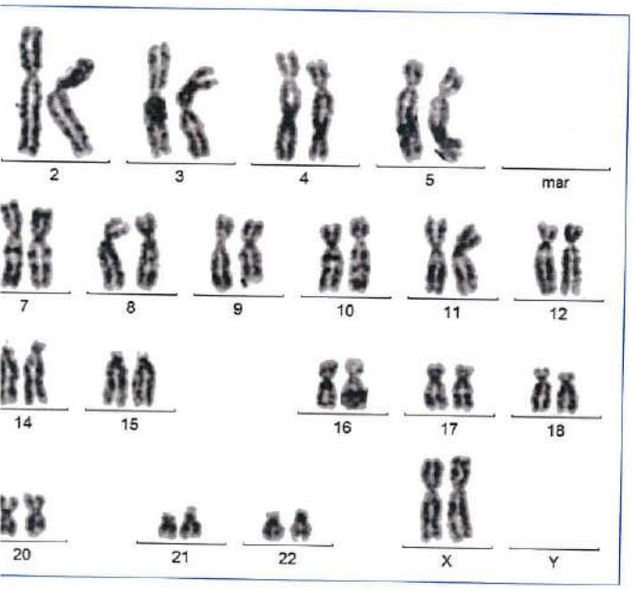


CHROMOSOME ANALYSIS IN WHOLE BLOOD

16/274
ood
3/04/2016
Age/Sex: 50 Y/F
Method: Cell culture and G-banding
Culture Date: 27/04/2016
Exposure: Control
Cells studied: 100
Reporting Date: 07/05/2016

	Number	Normal/Abnormal
	6	Normal
	4	Normal
	14	Normal
	6	Normal
	6	Normal
	4	Normal
	4	Normal
	2	Normal
	46,XX	Normal



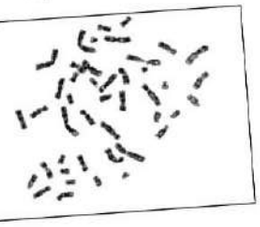


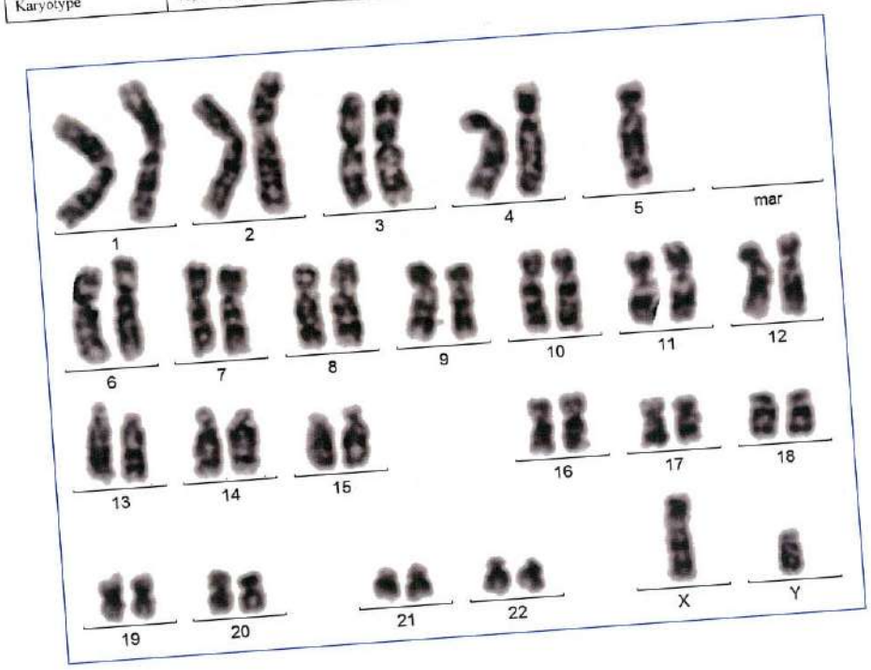
'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December
1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 16/274
Sample: Whole Blood
Collection Date: 23/04/2016
Age/Sex: 29 Y/M
Method: Cell culture and G-banding
Culture Date: 27/04/2016
Exposure: Control
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	14p+
E	6	16qh+
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY,14p+,16qh+	14p+,16qh+





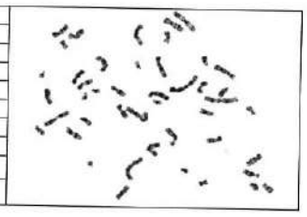
PI: Dr. Bani Bandana Ganguly
MGM New Bombay Hospital, Navi Mumbai

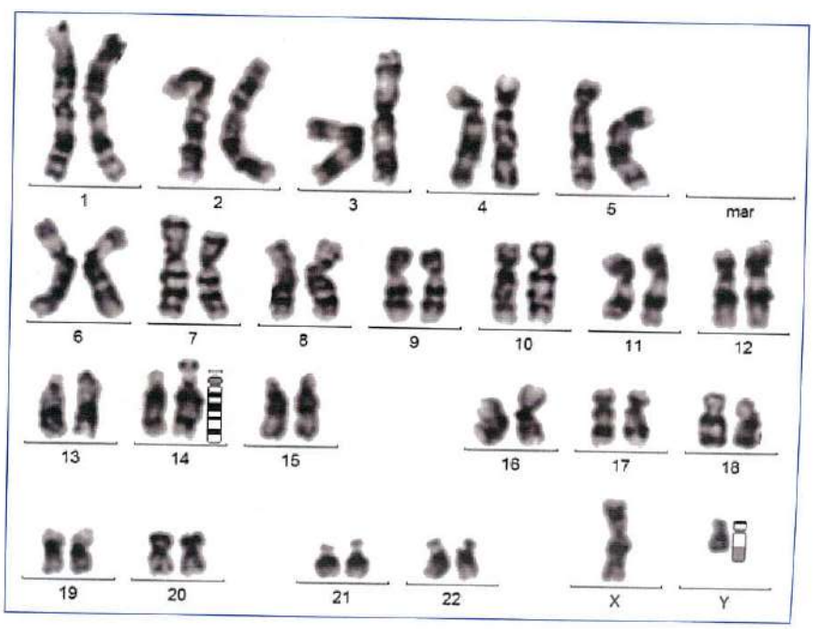
'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December
1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 7/284
Sample: Whole Blood
Collection Date: 25/04/2016
Age/Sex: 60 Y/M
Method: Cell culture and G-banding
Culture Date: 27/04/2016
Exposure: Severe
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	14p+
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Small Y
Karyotype	46,XY,small Y,14p+	Small Y,14p+





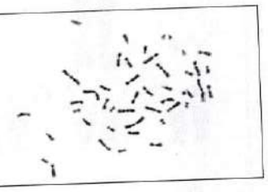
CHROMOSOME ANALYSIS IN WHOLE BLOOD

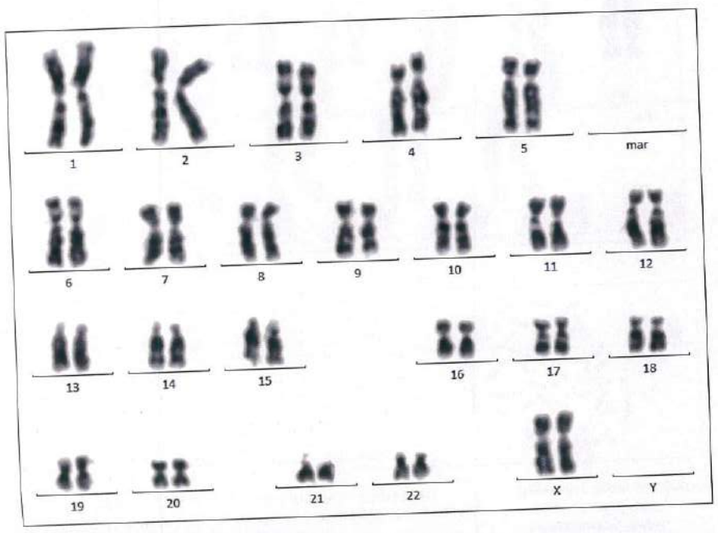
Area/ICMR No.: 7/289
Sample: Whole Blood
Collection Date: 17/02/2016

Age/Sex: 50Y/F
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 27/02/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal





'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

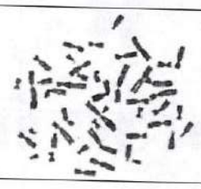
CHROMOSOME ANALYSIS IN WHOLE BLOOD

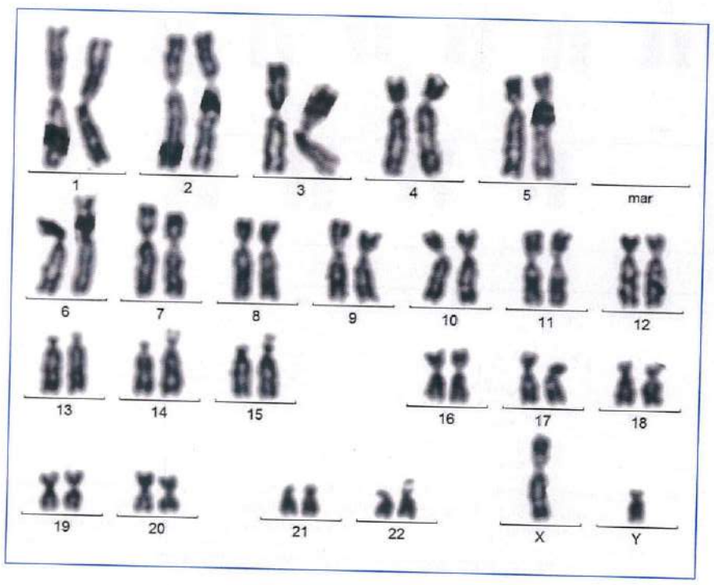
Area/ICMR No.: 1/290
Sample: Whole Blood
Collection Date: 27/04/016

Age/Sex: 58 Y/M
Method: Cell culture and G-banding
Culture Date: 30/04/2016

Exposure: Severe
Cells studied: 80
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Small Y
Karyotype	46,XY,small Y	Small Y





'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

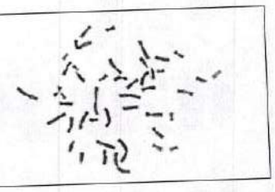
CHROMOSOME ANALYSIS IN WHOLE BLOOD

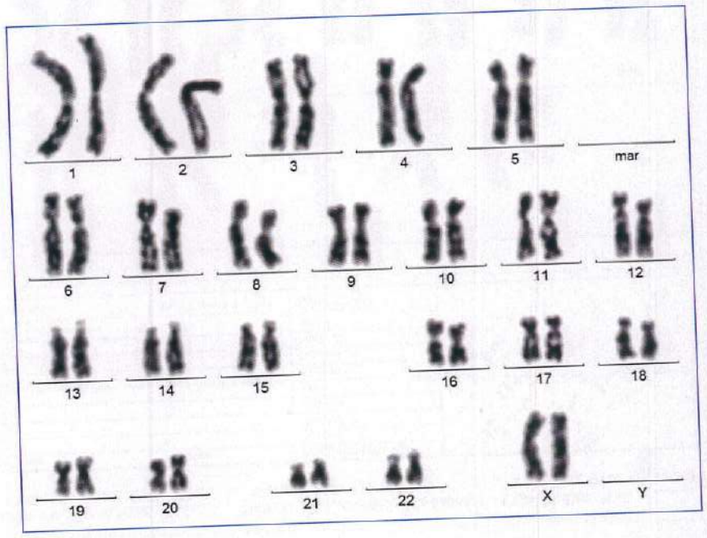
Area/ICMR No.: 1/290
Sample: Whole Blood
Collection Date: 27/04/2016

Age/Sex: 52 Y/F
Method: Cell culture and G-banding
Culture Date: 30/04/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal





'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

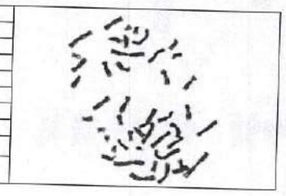
CHROMOSOME ANALYSIS IN WHOLE BLOOD

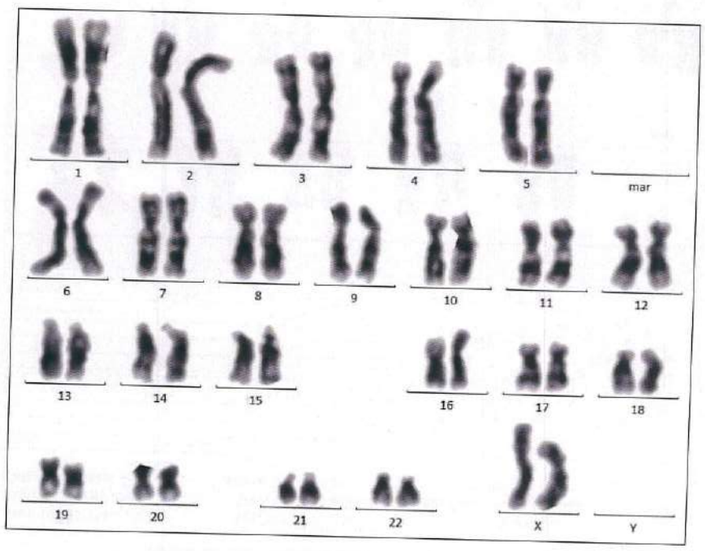
Area/ICMR No.: 1/300
Sample: Whole Blood
Collection Date: 16/02/2016

Age/Sex: 67 Y/F
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 27/02/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal



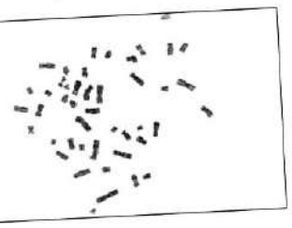


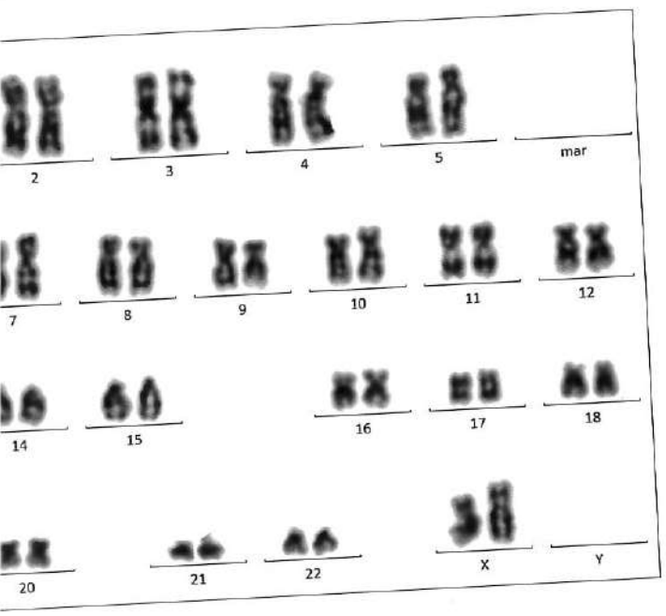
IROMOSOME ANALYSIS IN WHOLE BLOOD

Age/Sex: 35 Y/F
Method: Cell culture and G-banding
Culture Date: 27/04/2016
Exposure: Control
Cells studied: 50
Reporting Date: 07/05/2016

016

umber	Normal/Abnormal
	Normal
	Normal
4	Normal
	Normal
	Normal
4	Normal
4	Normal
2	Normal
46,XX	Normal

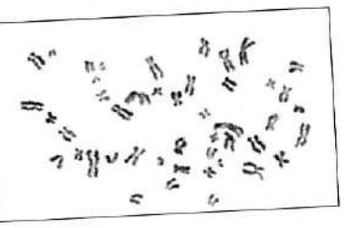


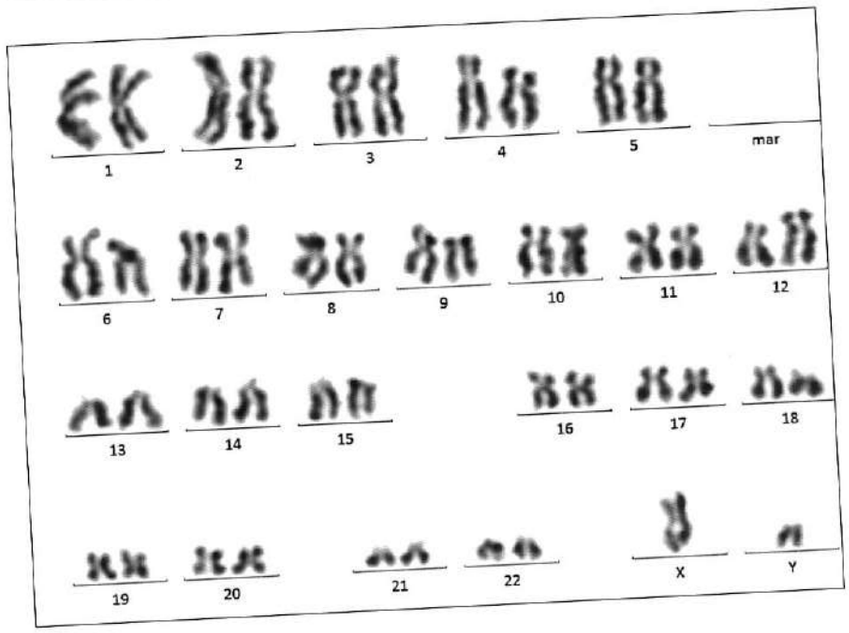


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 16/312
Sample: Whole Blood
Collection Date: 4/02/2015
Age/Sex: 37 Y/M
Method: Cell culture and G-banding
Culture Date: 4/02/2015
Exposure: Control
Cells studied: 10
Reporting Date: 14/02/2015

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal

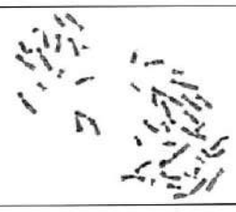


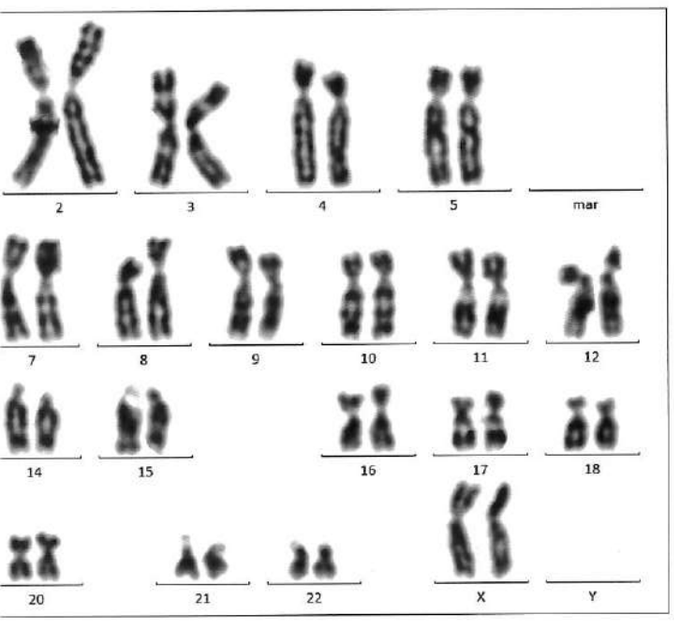


CHROMOSOME ANALYSIS IN WHOLE BLOOD

16/315
ood
8/04/2016
Age/Sex: 55 Y/F
Method: Cell culture and G-banding
Culture Date: 30/04/2016
Exposure: Control
Cells studied: 100
Reporting Date: 07/05/2016

	Number	Normal/Abnormal
	6	Normal
	4	Normal
	14	Normal
	6	Normal
	6	Normal
	4	Normal
	4	Normal
	2	Normal
	46,XX	Normal

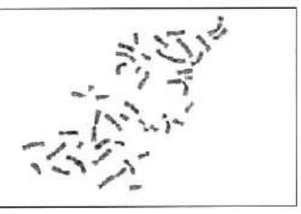


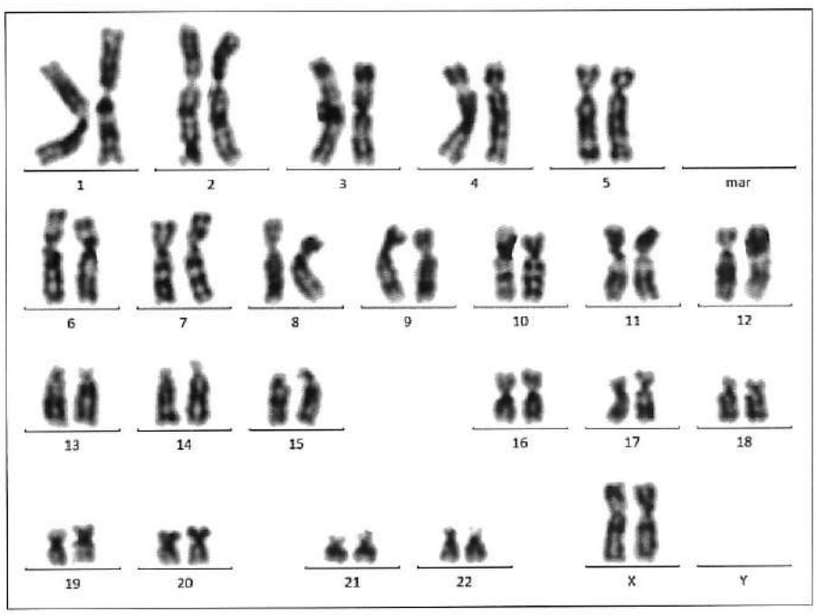


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 16/320
Sample: Whole Blood
Collection Date: 28/04/2016
Age/Sex: 61 Y/F
Method: Cell culture and G-banding
Culture Date: 30/04/2016
Exposure: Control
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal

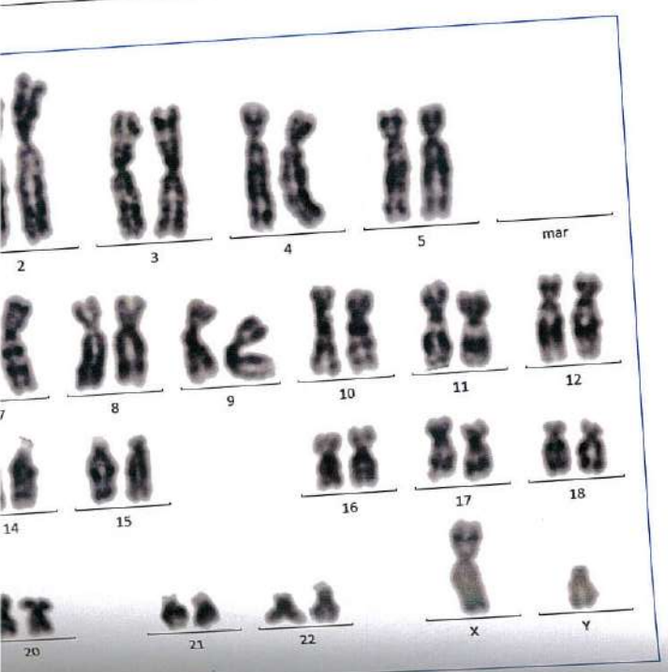




OMOSOME ANALYSIS IN WHOLE BLOOD

Age/Sex: 70 Y/M
Method: Cell culture and G-banding
Culture Date: 30/04/2016
Exposure: Control
Cells studied: 100
Reporting Date: 07/05/2016

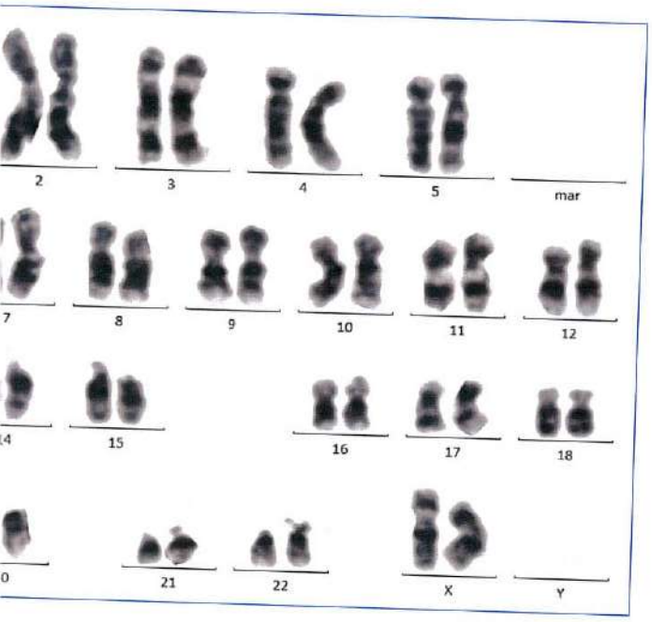
ber	Normal/Abnormal
	1qh+
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
XY,1qh+	1qh+



CHROMOSOME ANALYSIS IN WHOLE BLOOD

7/346
lood
28/04/2016
Age/Sex: 60 Y/F
Method: Cell culture and G-banding
Culture Date: 30/04/2016
Exposure: Severe
Cells studied: 100
Reporting Date: 07/05/2016

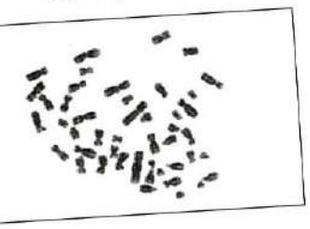
	Number	Normal/Abnormal
	6	Normal
	4	Normal
	14	Normal
	6	Normal
	6	Normal
	4	Normal
	4	Normal
	2	Normal
	46,XX	Normal

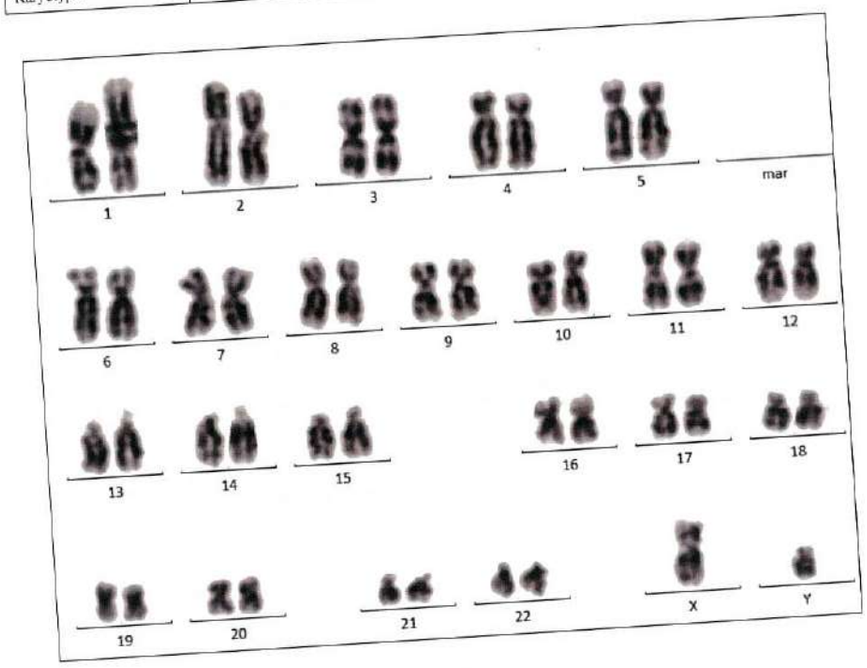


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 7/340
Sample: Whole Blood
Collection Date: 23/02/2016
Age/Sex: 42 Y/M
Method: Cell culture and G-banding
Culture Date: 25/02/16
Exposure: Severe
Cells studied: 100
Reporting Date: 05/03/16

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Small Y
Karyotype	46,XY,small Y	Small Y

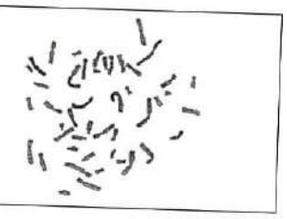


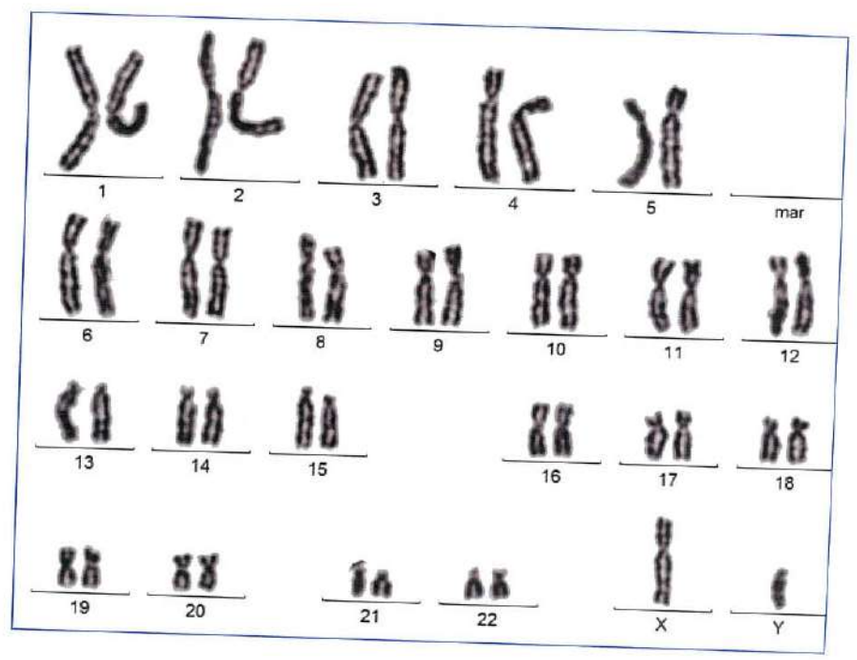


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 16/346
Sample: Whole Blood
Collection Date: 05/05/2016
Age/Sex: 41 Y/M
Method: Cell culture and G-banding
Culture Date: 07/05/2016
Exposure: Control
Cells studied: 37
Reporting Date: 17/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal

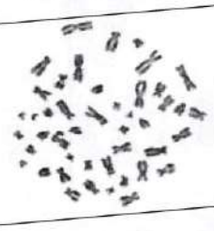


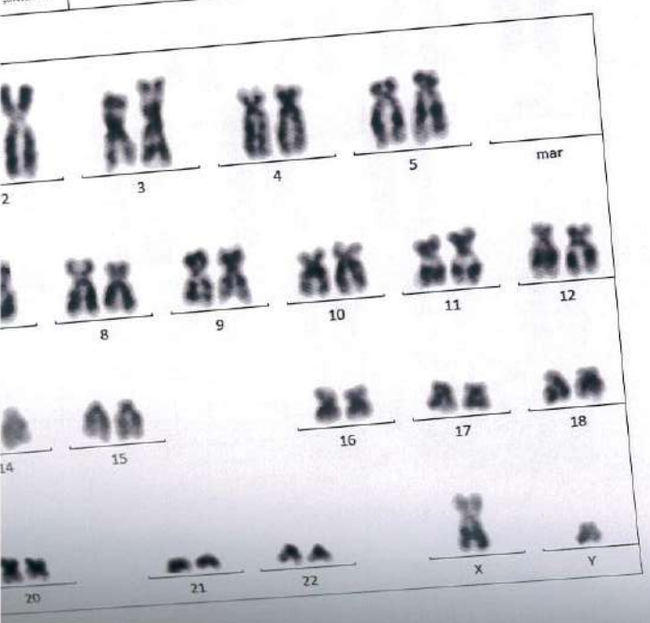


IOSOME ANALYSIS IN WHOLE BLOOD

Age/Sex: 74 Y/M
Method: Cell culture and G-banding
Culture Date: 27/04/2016
Exposure: Control
Cells studied: 10
Reporting Date: 07/05/2016

	Normal/Abnormal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Small Y
,small Y	Small Y

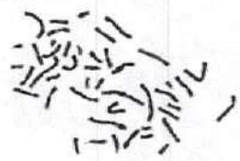


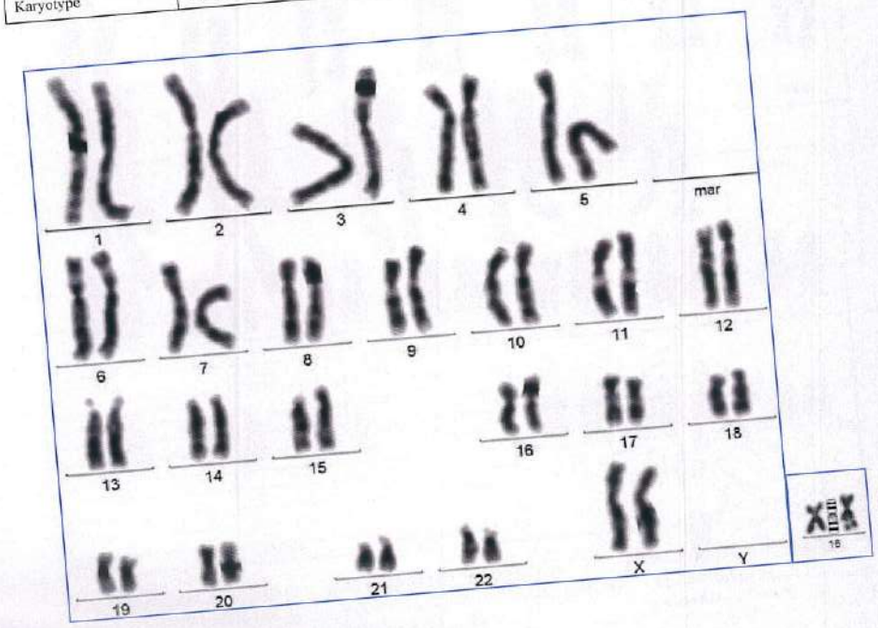


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 2/355
Sample: Whole Blood
Collection Date: 09/03/2016
Age/Sex: 40 Y/F
Method: Cell culture and G-banding
Culture Date: 12/03/2016
Exposure: Severe
Cells studied: 100
Reporting Date: 22/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Fra(16q)
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX, fra(16q)	Fra(16q)





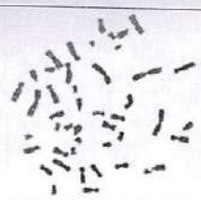
MGM New Bombay Hospital, Navi Mumbai

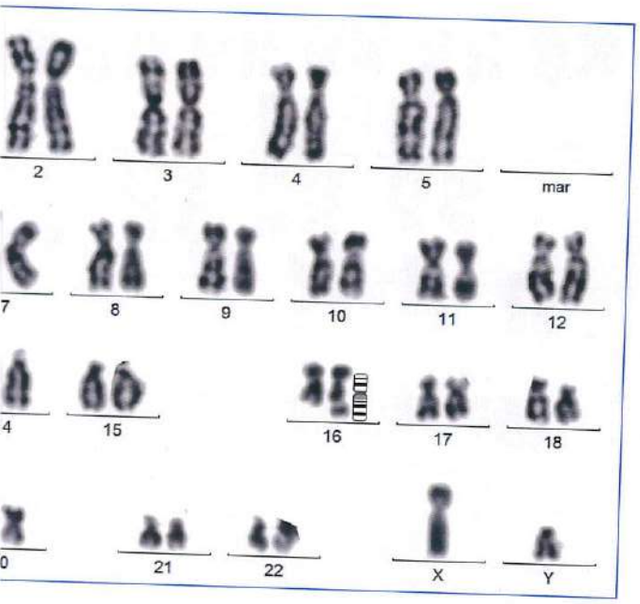
'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 7/357
Sample: Whole Blood
Collection Date: 23/02/016
Age/Sex: 40 Y/M
Method: Cell culture and G-banding
Receiving Date: 25/02/2016
Exposure: Severe
Cells studied: 76
Reporting Date: 05/03/2016

	Number	Normal/Abnormal
	6	Normal
	4	Normal
	14	Normal
	6	Normal
	6	Fra(16q22)
	4	Normal
	4	Normal
	2	Normal
	46,XY,fra(16q)	Fragile site in 16q



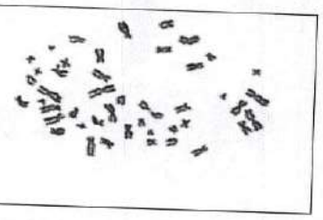


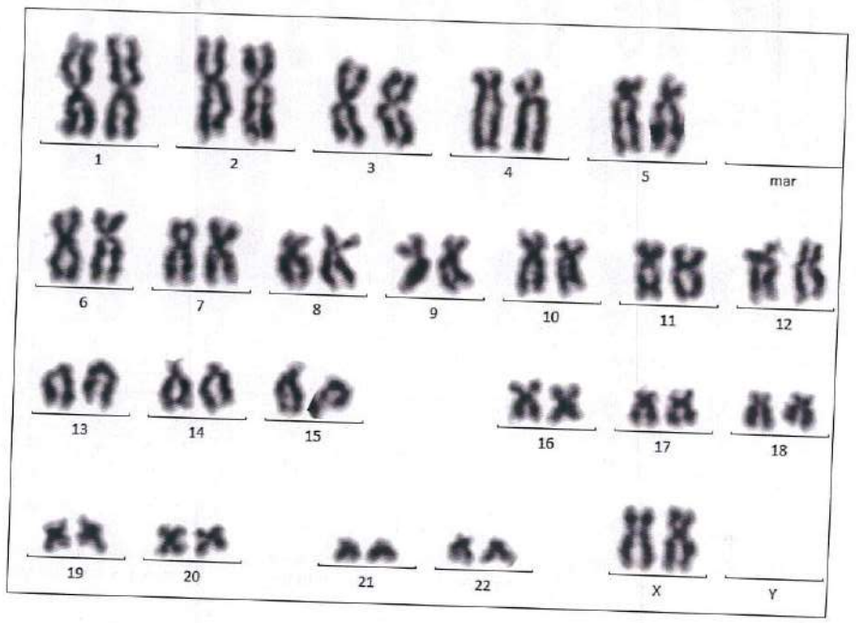
'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 16/362
Sample: Whole Blood
Collection Date: 3/02/2015
Age/Sex: 65 Y/F
Method: Cell culture and G-banding
Culture Date: 4/02/2015
Exposure: Control
Cells studied: 10
Reporting Date: 14/02/2015

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal



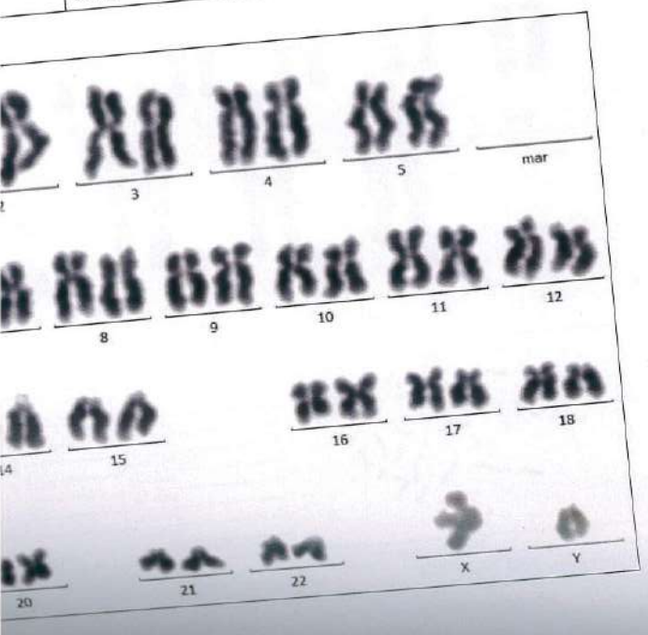


OSOME ANALYSIS IN WHOLE BLOOD

Age/Sex: 35Y/M
Method: Cell culture and G-banding
Culture Date: 4/02/2015

Exposure: Control
Cells studied: 30
Reporting Date: 14/02/2015

	Normal/Abnormal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal



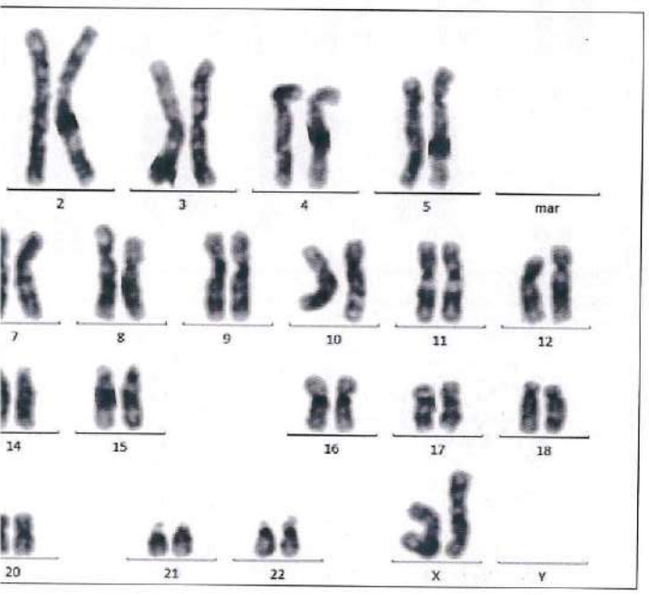
CHROMOSOME ANALYSIS IN WHOLE BLOOD

: 16/380
lood
23/04/2016

Age/Sex: 53Y/F
Method: Cell culture and G-banding
Culture Date: 27/04/2016

Exposure: Control
Cells studied: 50
Reporting Date: 07/05/2016

	Number	Normal/Abnormal
	6	1qh+
	4	Normal
	14	Normal
	6	Normal
	6	Normal
	4	Normal
	4	Normal
	2	Normal
	46,XX,1qh+	1qh+



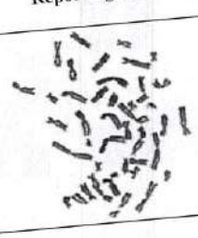
CHROMOSOME ANALYSIS IN WHOLE BLOOD

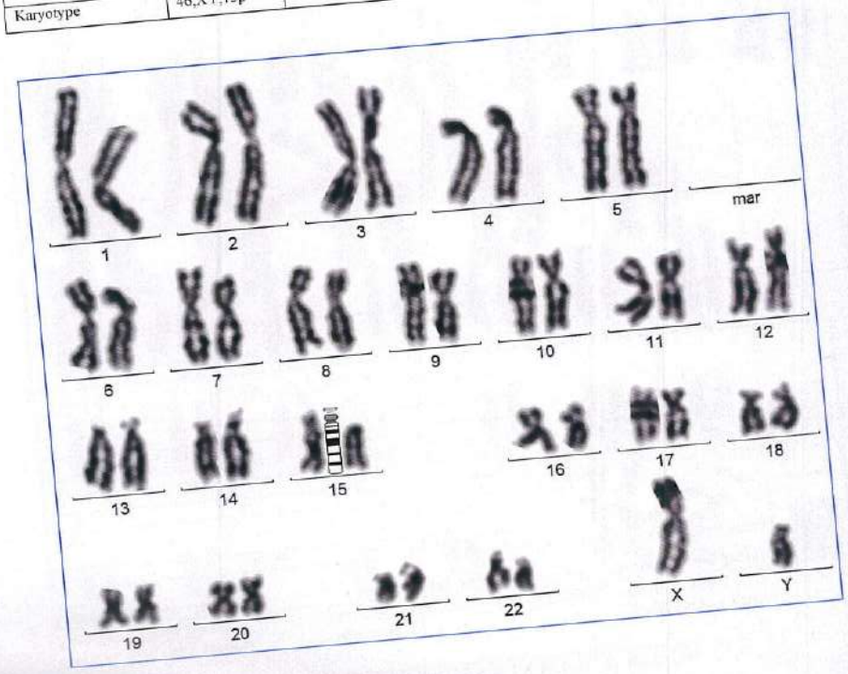
Area/ICMR No.: 05/375
Sample: Whole Blood
Collection Date: 06/05/2016

Age/Sex: 45Y/M
Method: Cell culture and G-banding
Culture Date: 07/05/2016

Exposure: Moderate
Cells studied: 100
Reporting Date: 17/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	15p+
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY,15p+	15p+





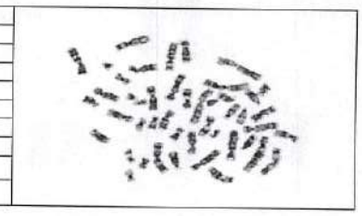
CHROMOSOME ANALYSIS IN WHOLE BLOOD

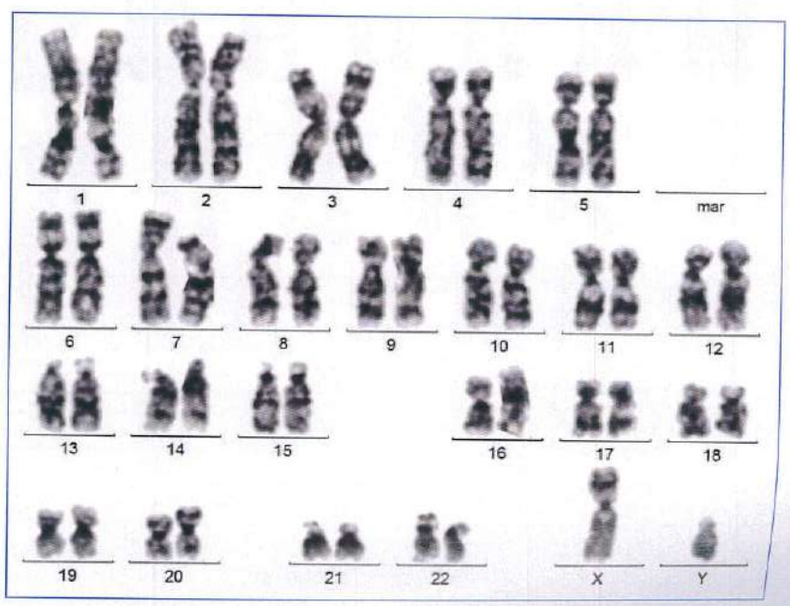
Area/ICMR No: 16/382
Sample: Whole Blood
Collection Date: 28/04/2016

Age/Sex: 37 Y/M
Method: Cell culture and G-banding
Culture Date: 30/04/2016

Exposure: Control
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal



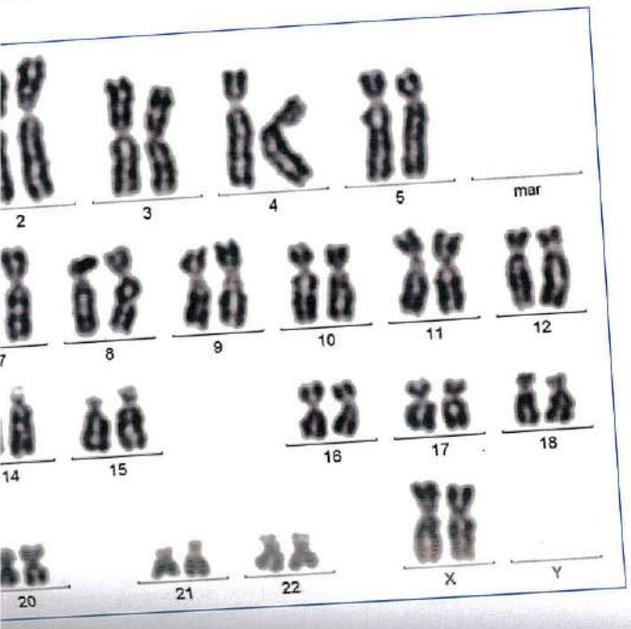


OMOSOME ANALYSIS IN WHOLE BLOOD

Age/Sex: 53Y/F
Method: Cell culture and G-banding
Culture Date: 30/04/2016

Exposure: Control
Cells studied: 100
Reporting Date: 07/05/2016

ber	Normal/Abnormal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
XX	Normal



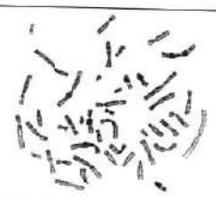
CHROMOSOME ANALYSIS IN WHOLE BLOOD

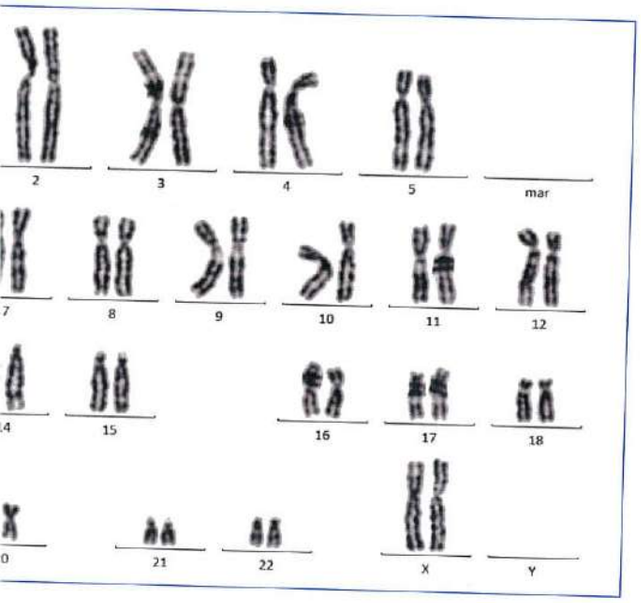
07/392
ood
3/02/2016

Age/Sex: 46 Y/F
Method: Cell culture and G-banding
Culture Date: 25/02/2016

Exposure: Severe
Cells studied: 111
Reporting Date: 05/03/2016

	Number	Normal/Abnormal
	6	Normal
	4	Normal
	14	9qh+
	6	Normal
	6	Normal
	4	Normal
	4	Normal
	2	Normal
	46,XX,9qh+	9qh+





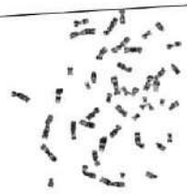
CHROMOSOME ANALYSIS IN WHOLE BLOOD

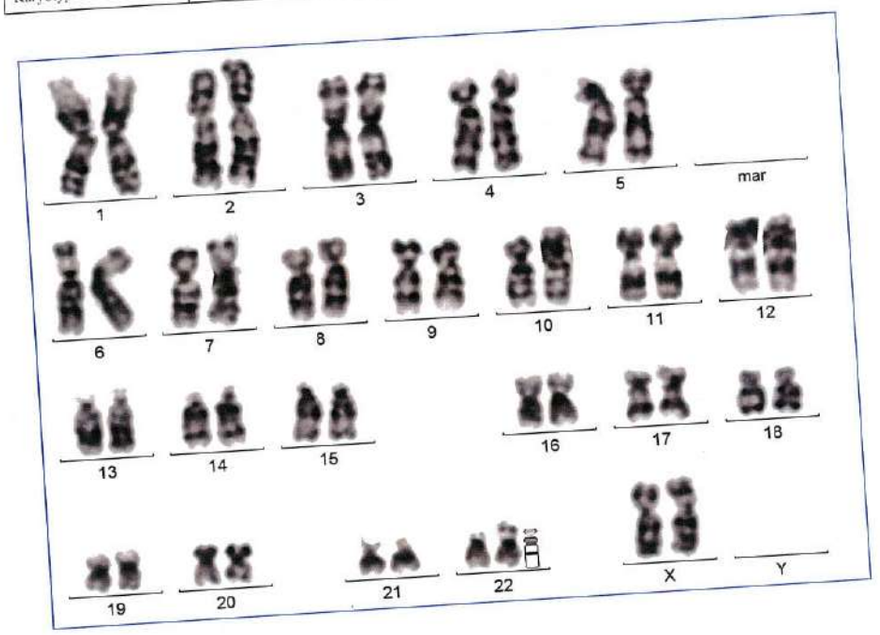
Area/ICMR No.: 07/389
Sample: Whole Blood
Collection Date: 17/02/2016

Age/Sex: 50 Y/F
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 27/02/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	22p+
Sex Chromosomes	2	Normal
Karyotype	46,XX, 22p+	22p+





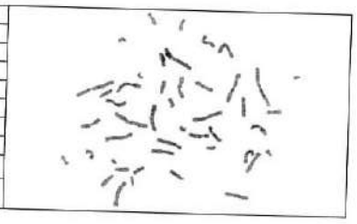
CHROMOSOME ANALYSIS IN WHOLE BLOOD

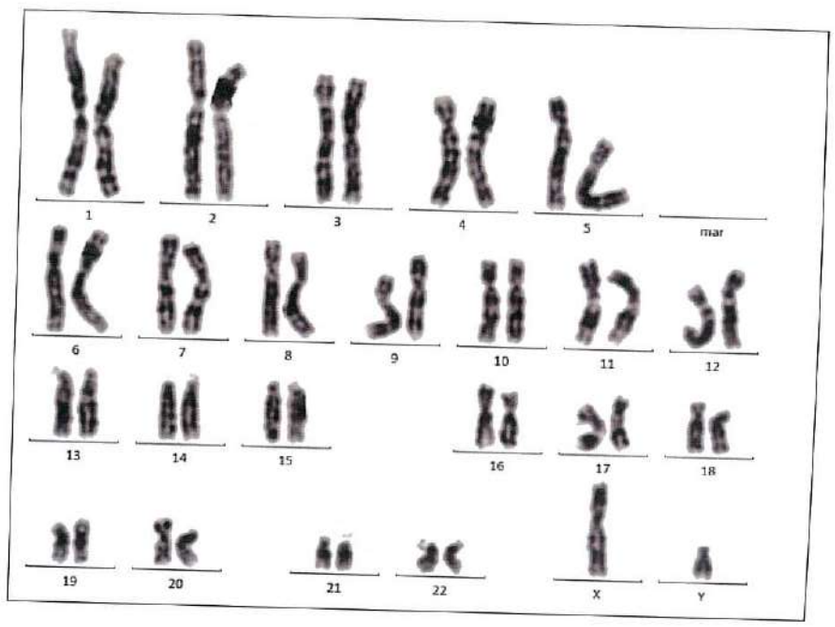
Area/ICMR No.: 07/392
Sample: Whole Blood
Collection Date: 16/02/2016

Age/Sex: 56 Y /M
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 120
Reporting Date: 27/02/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Small Y
Karyotype	46,XY,small Y	Small Y



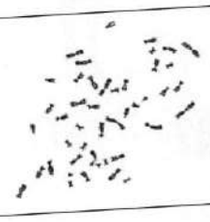


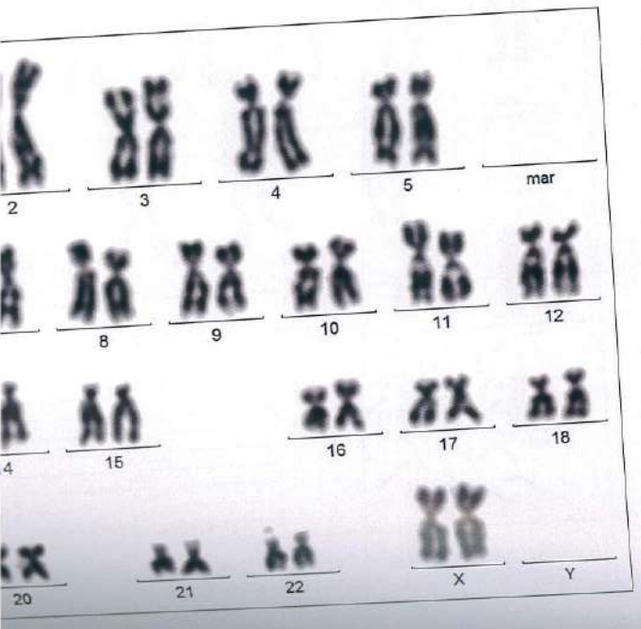
OMOSOME ANALYSIS IN WHOLE BLOOD

Age/Sex: 60 Y/F
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 70
Reporting Date: 27/02/2016

ber	Normal/Abnormal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
XX	Normal





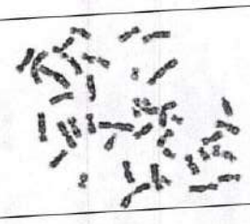
CHROMOSOME ANALYSIS IN WHOLE BLOOD

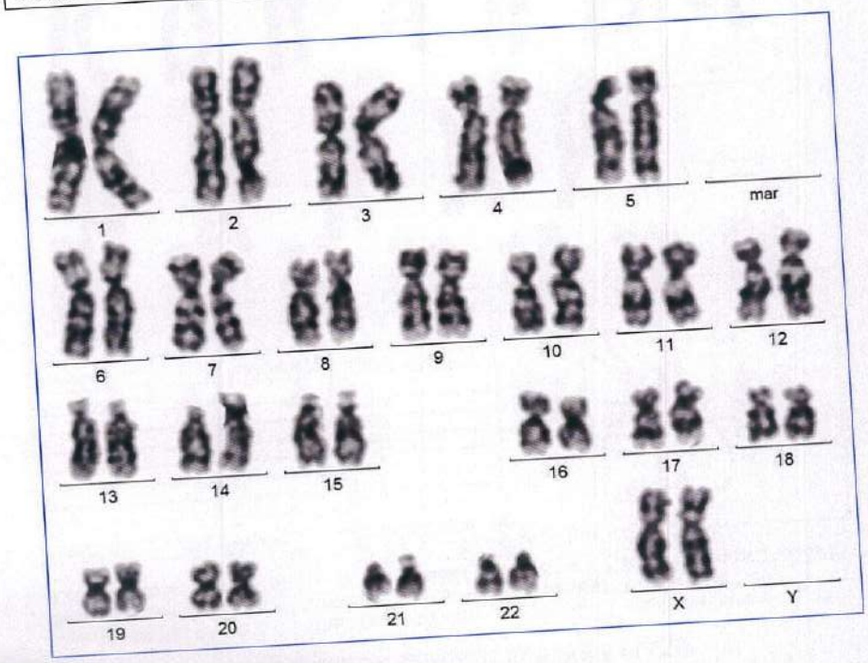
Area/ICMR No: 5/397
Sample: Whole Blood
Collection Date: 28/04/2016

Age/Sex: 57 Y/F
Method: Cell culture and G-banding
Culture Date: 30/04/2016

Exposure: Control
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal





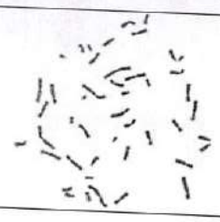
CHROMOSOME ANALYSIS IN WHOLE BLOOD

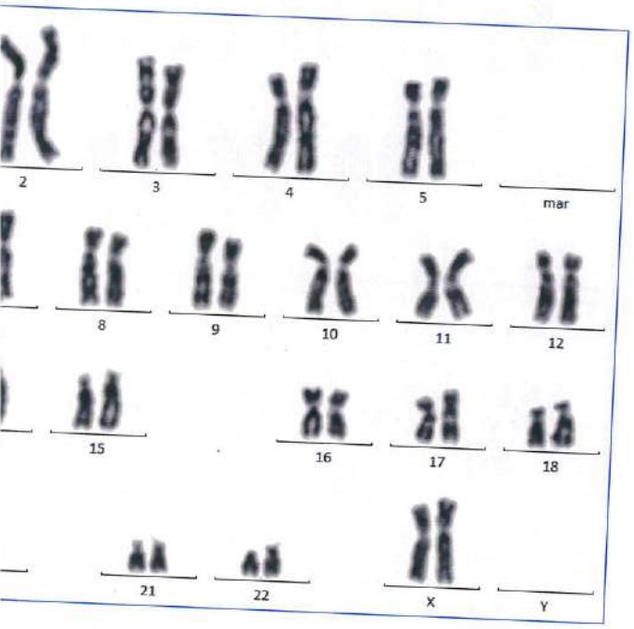
7/399
od
3/02/2016

Age/Sex: 50 Y/F
Method: Cell culture and G-banding
Culture Date: 25/02/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 05/03/2016

Number	Normal/Abnormal
6	Normal
4	Normal
14	Normal
6	Normal
6	Normal
4	Normal
4	Normal
2	Normal
46,XX	Normal





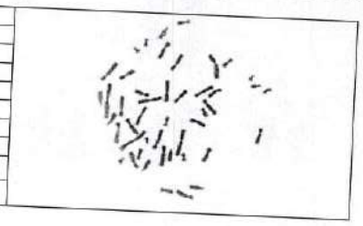
CHROMOSOME ANALYSIS IN WHOLE BLOOD

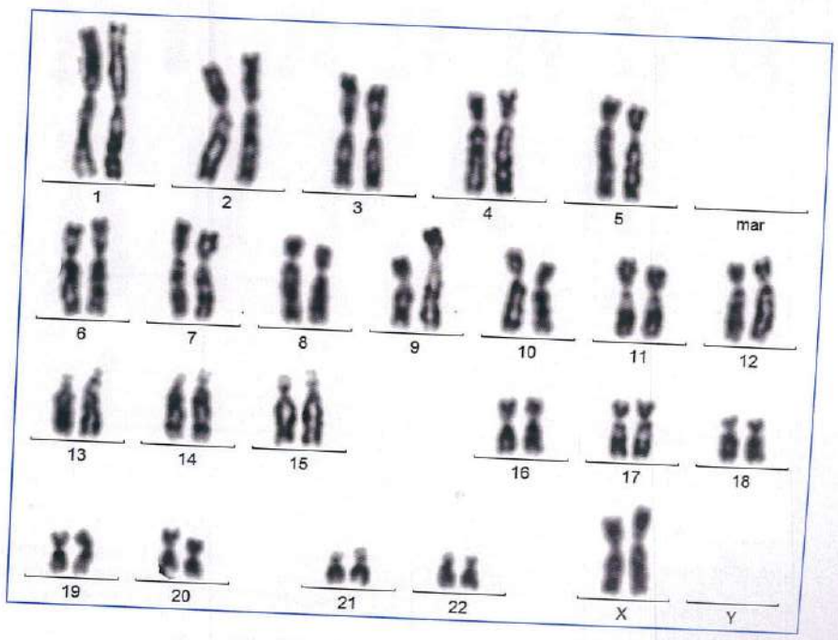
Area/ICMR No.: 01/404
Sample: Whole Blood
Collection Date: 25/04/2016

Age/Sex: 65 Y/F
Method: Cell culture and G-banding
Culture Date: 27/04/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	9qh+
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX,9qh+	9qh+

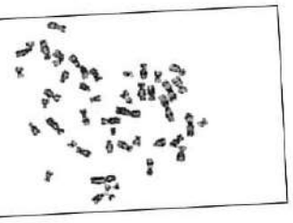


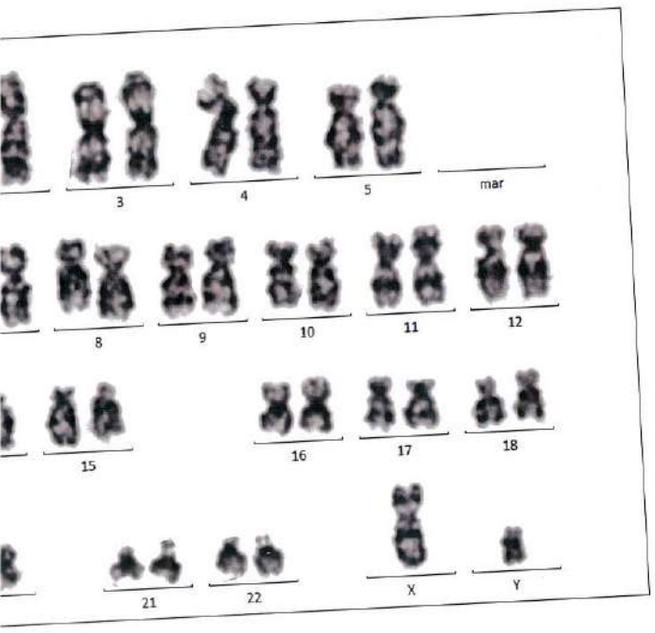


OMOSOME ANALYSIS IN WHOLE BLOOD

Age/Sex: 36 Y/M
Method: Cell culture and G-banding
Culture Date: 27/04/2016
Exposure: Severe
Cells studied: 100
Reporting Date: 07/05/2016

ber	Normal/Abnormal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
XY	Normal

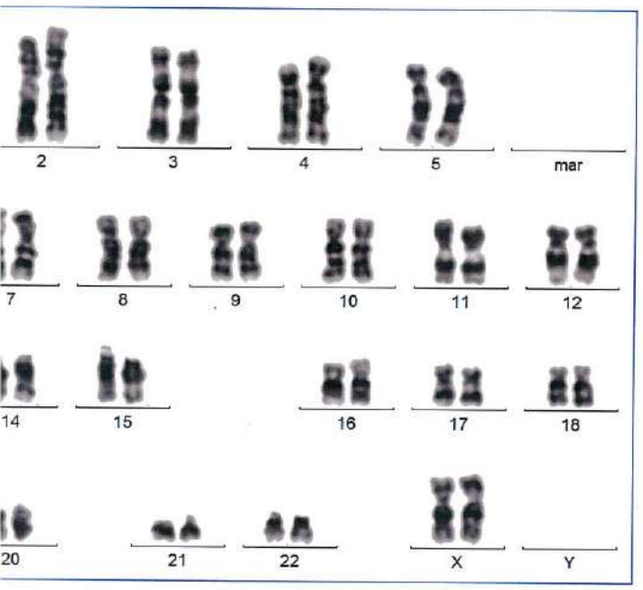




CHROMOSOME ANALYSIS IN WHOLE BLOOD

/433
d
02/2016
Age/Sex: 60 Y/F
Method: Cell culture and G-banding
Culture Date: 17/02/2016
Exposure: Severe
Cells studied: 100
Reporting Date: 27/02/2016

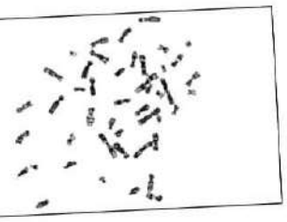
Number	Normal/Abnormal
6	Normal
4	Normal
14	Normal
6	Normal
6	Normal
4	Normal
4	Normal
2	Normal
46,XX	Normal

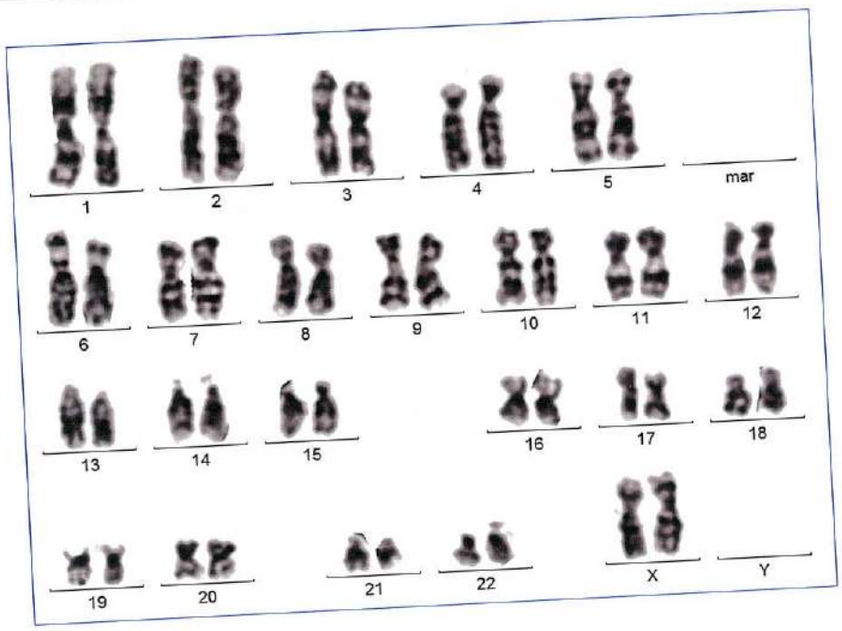


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 07/423
Sample: Whole Blood
Collection Date: 17/02/2016
Age/Sex: 45 Y/F
Method: Cell culture and G-banding
Culture Date: 17/02/2016
Exposure: Severe
Cells studied: 100
Reporting Date: 27/02/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	9qh+
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX,9qh+	9qh+

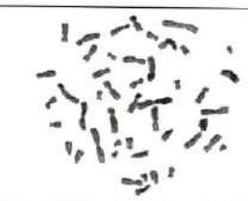


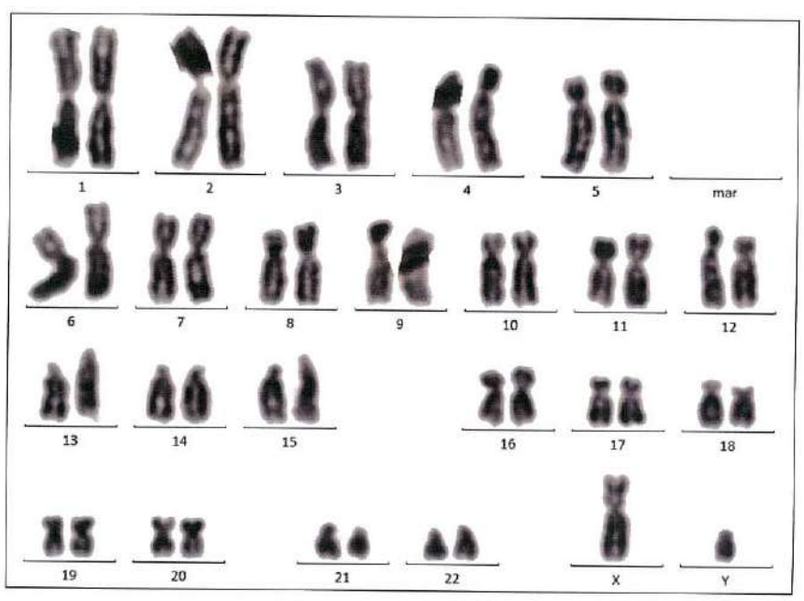


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 16/441
Sample: Whole Blood
Collection Date: 29/04/2016
Age/Sex: 45 Y/M
Method: Cell culture and G-banding
Culture Date: 30/04/2016
Exposure: Control
Cells studied: 10
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal



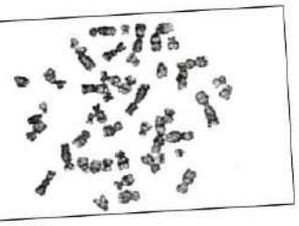


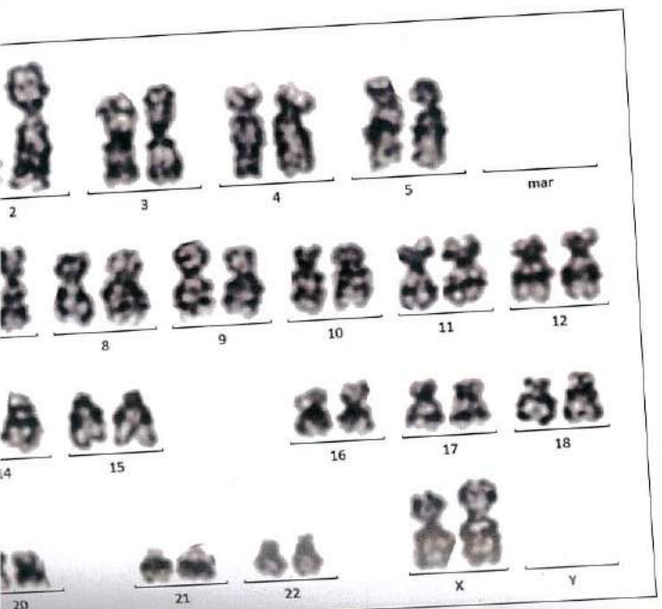
OMOSOME ANALYSIS IN WHOLE BLOOD

Age/Sex: 61 Y/F
Method: Cell culture and G-banding
Culture Date: 30/04/2016

Exposure: Control
Cells studied: 100
Reporting Date: 07/05/2016

ber	Normal/Abnormal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
XX	Normal





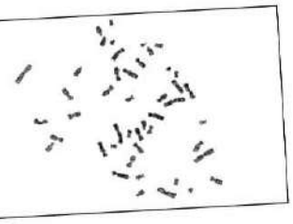
CHROMOSOME ANALYSIS IN WHOLE BLOOD

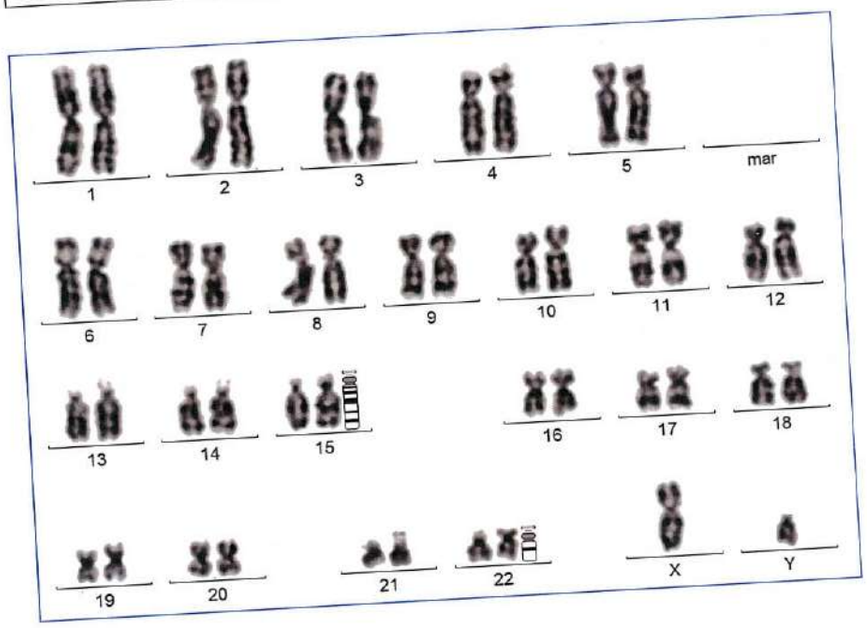
Area/ICMR No: 16/452
Sample: Whole Blood
Collection Date: 28/04/2016

Age/Sex: 44 Y/M
Method: Cell culture and G-banding
Culture Date: 30/04/2016

Exposure: Control
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	15p+
E	6	Normal
F	4	Normal
G	4	22p+
Sex Chromosomes	2	Normal
Karyotype	46,XY,15p+,22p+	15p+,22p+





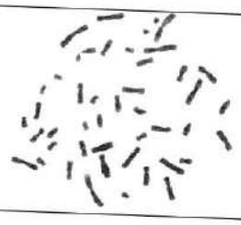
CHROMOSOME ANALYSIS IN WHOLE BLOOD

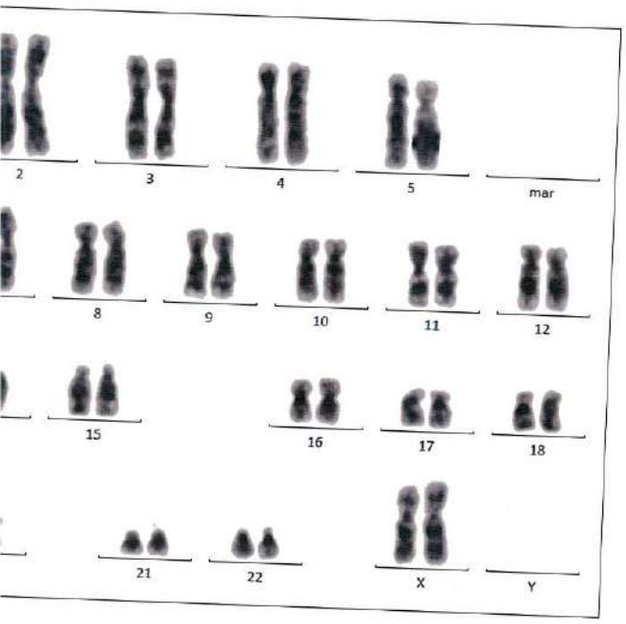
7/458
od
7/02/2016

Age/Sex: 50 Y/F
Method: Cell culture and G-banding
Receiving Date: 17/02/2016

Exposure: Severe
Cells studied: 60
Reporting Date: 27/02/2016

Number	Normal/Abnormal
6	1qh+
4	Normal
14	Normal
6	Normal
6	Normal
4	Normal
4	Normal
2	Normal
46,XX,1qh+	1qh+





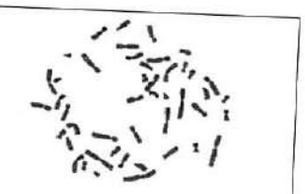
CHROMOSOME ANALYSIS IN WHOLE BLOOD

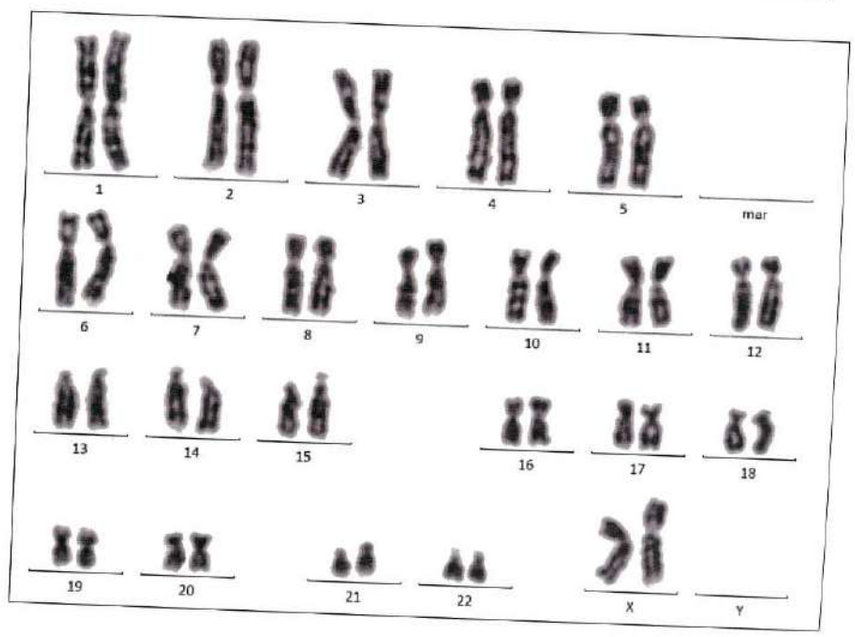
Area/ICMR No.: 01/474
Sample: Whole Blood
Collection Date: 16/02/2016

Age/Sex: 61 Y/F
Method: Cell culture and G-banding
Culture Date: 17/02/2017

Exposure: Severe
Cells studied: 100
Reporting Date: 27/02/2017

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	15p+
E	6	Normal
F	4	Normal
G	4	21p+
Sex Chromosomes	2	Normal
Karyotype	46,XX,15p+,21p+	15p+,21p+

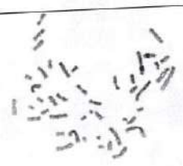


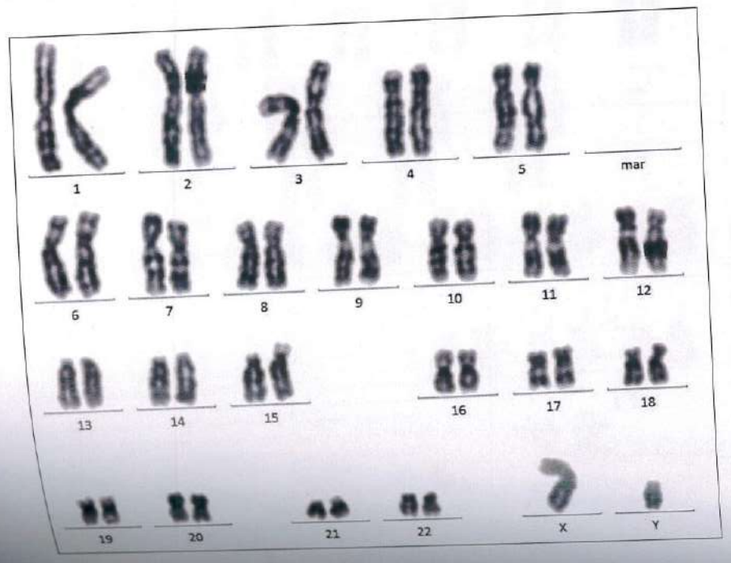


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.:16/486 **Age/Sex:** 38 Y/M **Exposure:** Control
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied:** 10
Collection Date: 04/02/2015 **Culture Date:** 04/02/2015 **Reporting Date:** 10/02/2015

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal



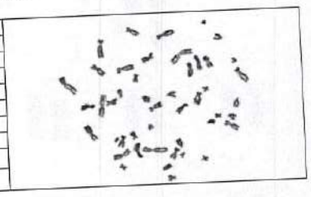


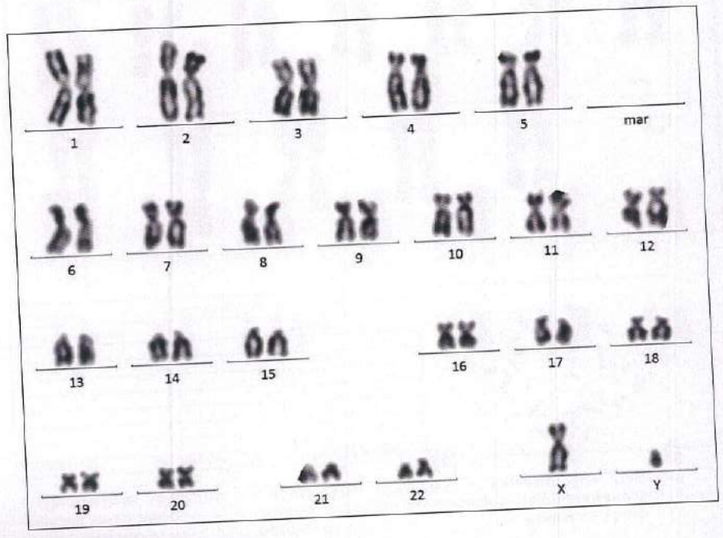
'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.:16/518 **Age/Sex:** 60Y/M **Exposure:** Control
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied:** 10
Collection Date: 03/02/2015 **Receiving Date:** 04/02/2015 **Reporting Date:** 13/02/2015

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Small Y
Karyotype	46,XY,small Y	Small Y



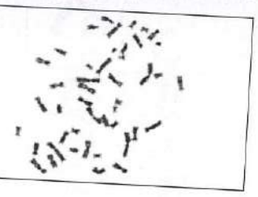


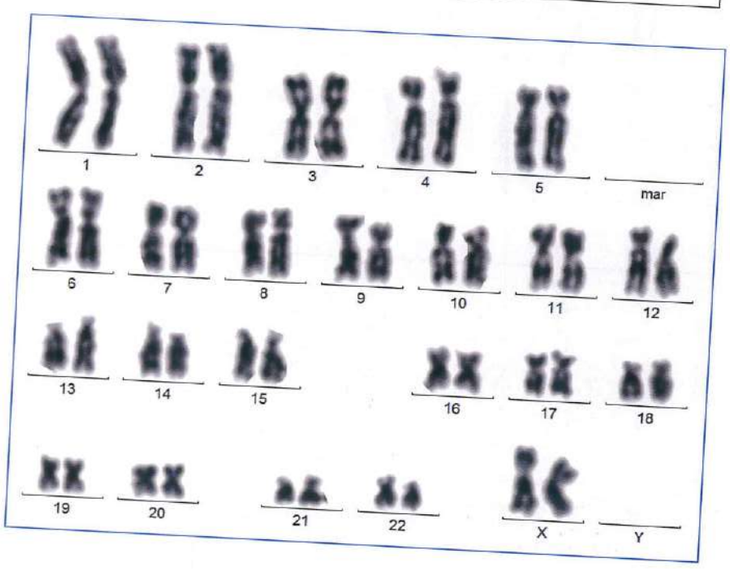
'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 01/493 **Age/Sex:** 60 Y/F **Exposure:** Control
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied:** 70
Collection Date: 27/04/2016 **Culture Date:** 30/04/2016 **Reporting Date:** 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal



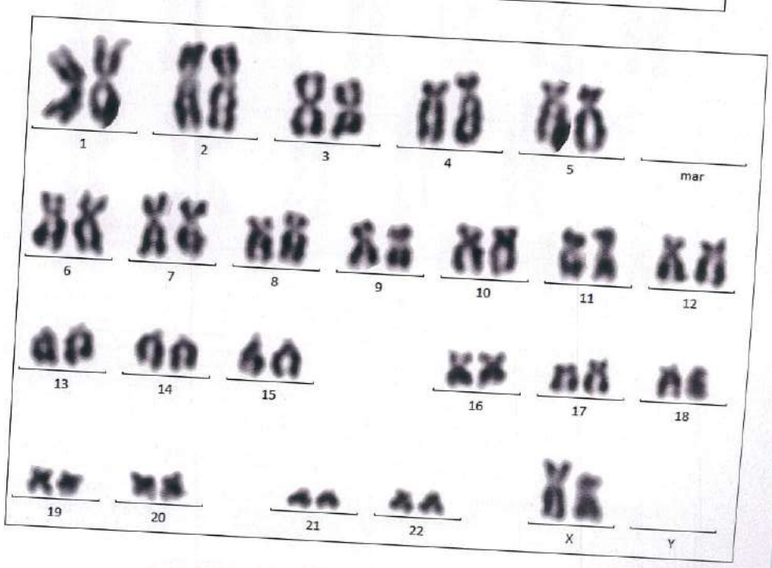


'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 16/493 **Age/Sex:** 53 Y/F **Exposure:** Control
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied:** 25
Collection Date: 03/02/2015 **Culture Date:** 04/02/2015 **Reporting Date:** 10/02/2015

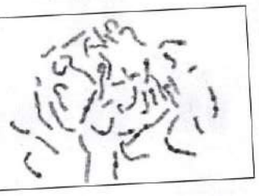
Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal

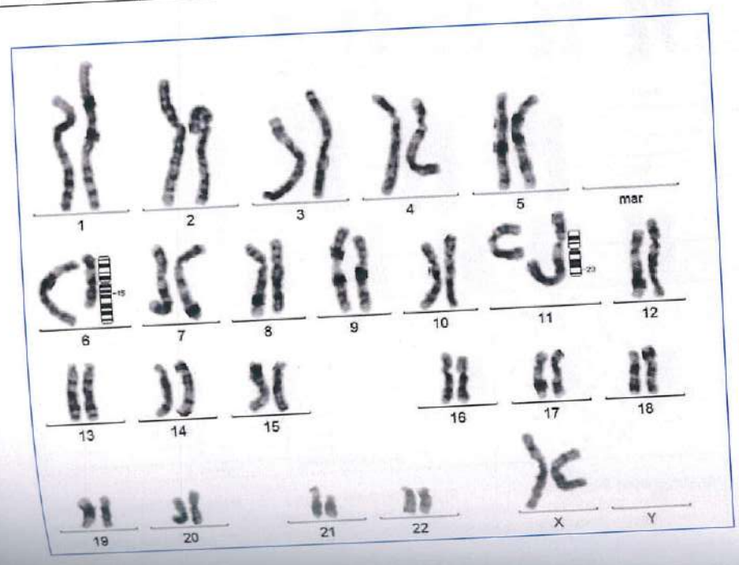


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 16/496
Sample: Whole Blood
Collection Date: 28/04/2016
Age/Sex: 71Y/F
Method: Cell culture and G-banding
Culture Date: 30/04/2016
Exposure: Control
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	T(6,11)
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX,t(6;11)(q15;q23)	t(6;11)(q15;q23)

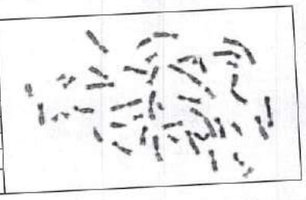


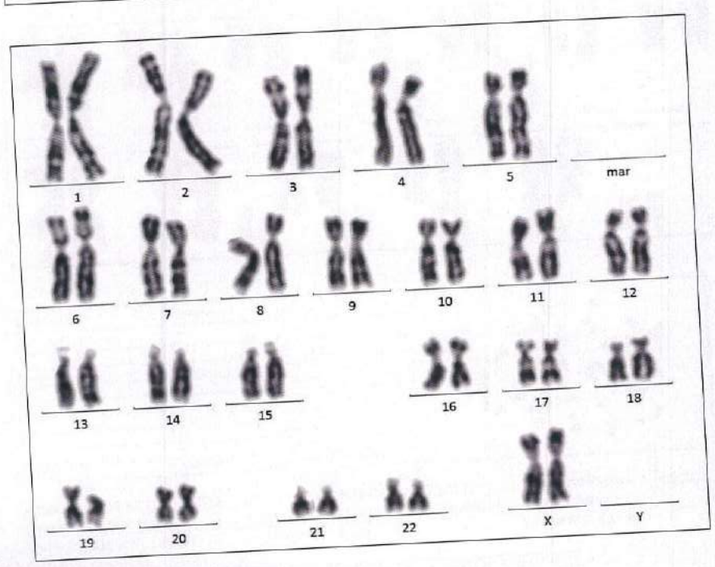


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 16/498
Sample: Whole Blood
Collection Date: 29/04/2016
Age/Sex: 60 Y/F
Method: Cell culture and G-banding
Culture Date: 30/04/2016
Exposure: Control
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal

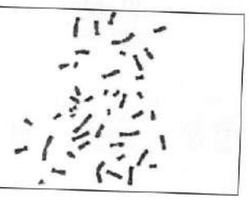


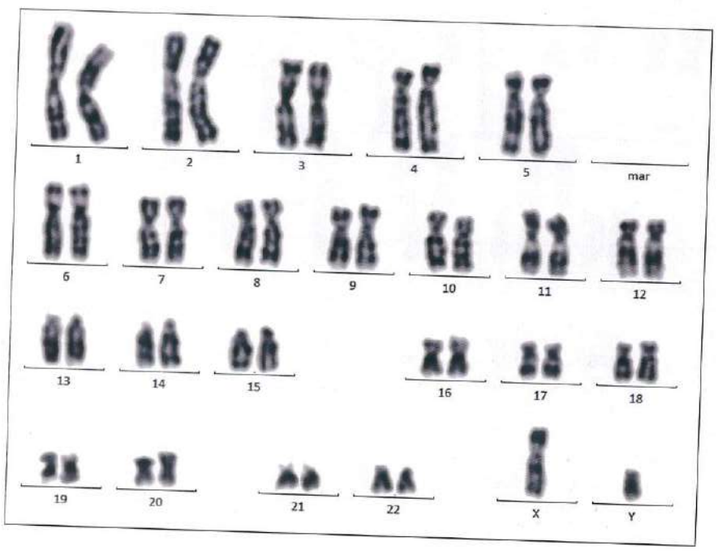


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 16/498
Sample: Whole Blood
Collection Date: 29/04/2016
Age/Sex: 38 Y/M
Method: Cell culture and G-banding
Receiving Date: 30/04/2016
Exposure: Control
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal

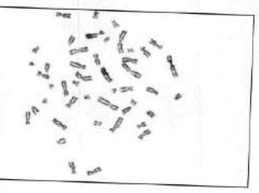


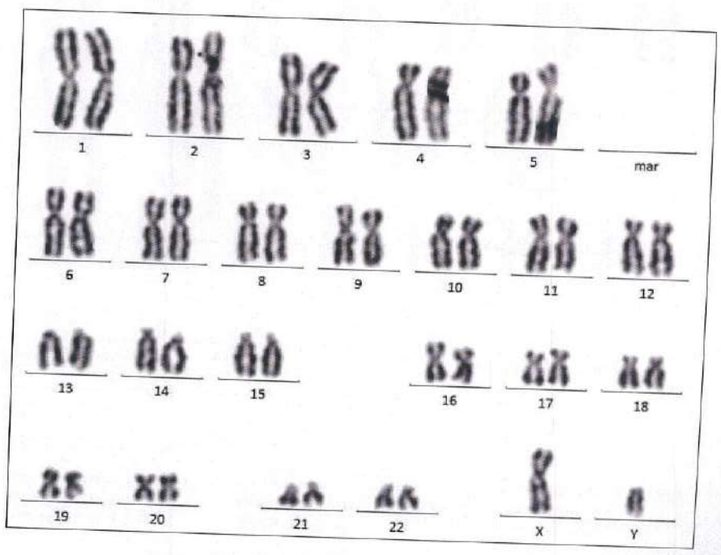


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 16/518
Sample: Whole Blood
Collection Date: 23/04/2016
Age/Sex: 54Y/M
Method: Cell culture and G-banding
Receiving Date: 27/04/2016
Exposure: Control
Cells studied: 10
Reporting Date: 05/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal





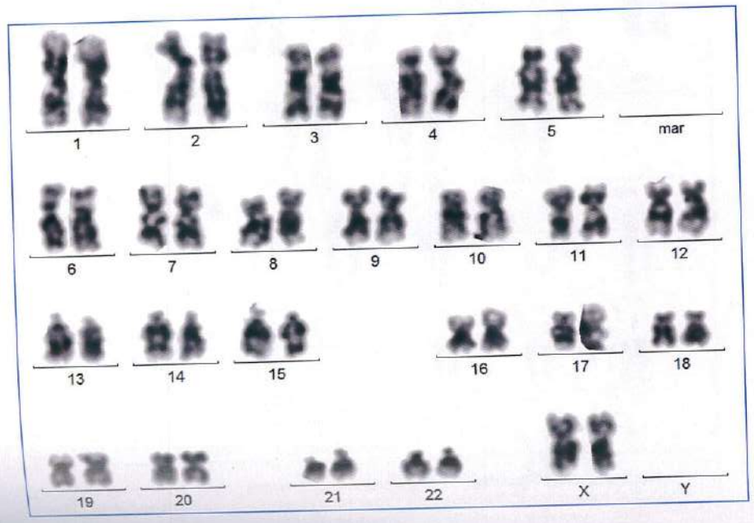
CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 16/518
Sample: Whole Blood
Collection Date: 29/04/2016

Age/Sex: 49 Y/F
Method: Cell culture and G-banding
Culture Date: 30/04/2016

Exposure: Control
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal



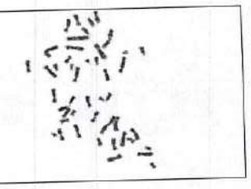
CHROMOSOME ANALYSIS IN WHOLE BLOOD

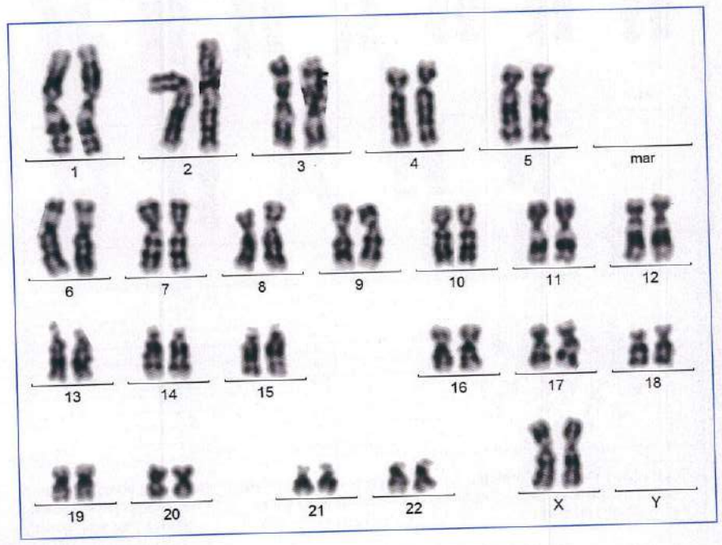
Area/ICMR No.: 1/523
Sample: Whole Blood
Collection Date: 25/04/2016

Age/Sex: 50Y/F
Method: Cell culture and G-banding
Culture Date: 27/04/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal





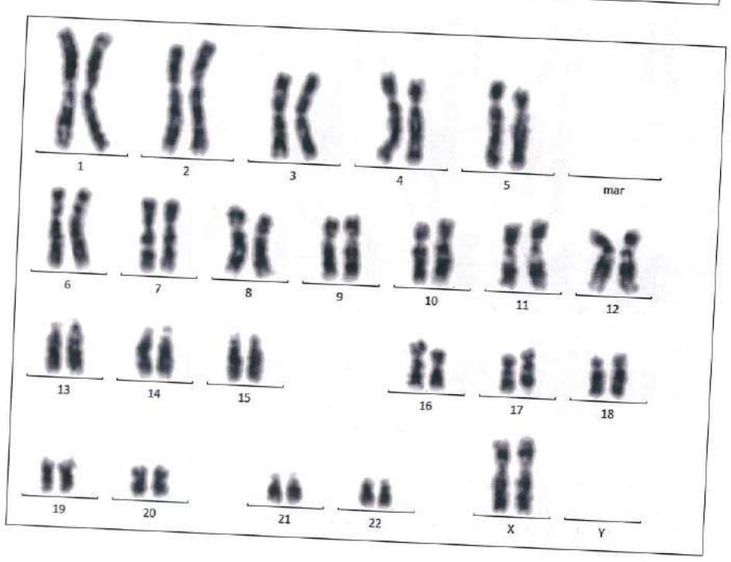
CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 3/542
Sample: Whole Blood
Collection Date: 24/02/2016

Age/Sex: 50Y/F
Method: Cell culture and G-banding
Culture Date: 25/02/2016

Exposure: Moderate
Cells studied: 100
Reporting Date: 07/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal



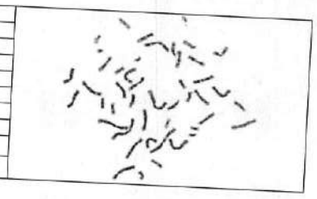
CHROMOSOME ANALYSIS IN WHOLE BLOOD

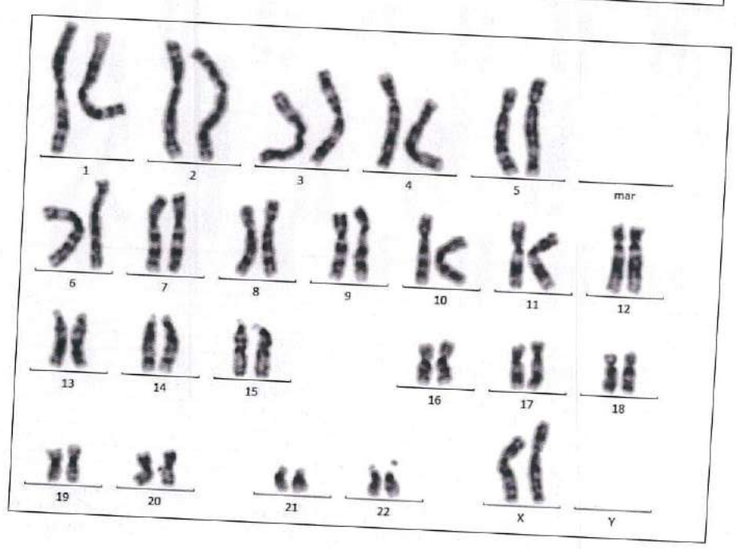
Area/ICMR No.: 02/550
Sample: Whole Blood
Collection Date: 09/03/2016

Age/Sex: 70 Y/F
Method: Cell culture and G-banding
Culture Date: 12/03/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 22/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	22s+
Sex Chromosomes	2	Normal
Karyotype	46,XX,22s+	22s+





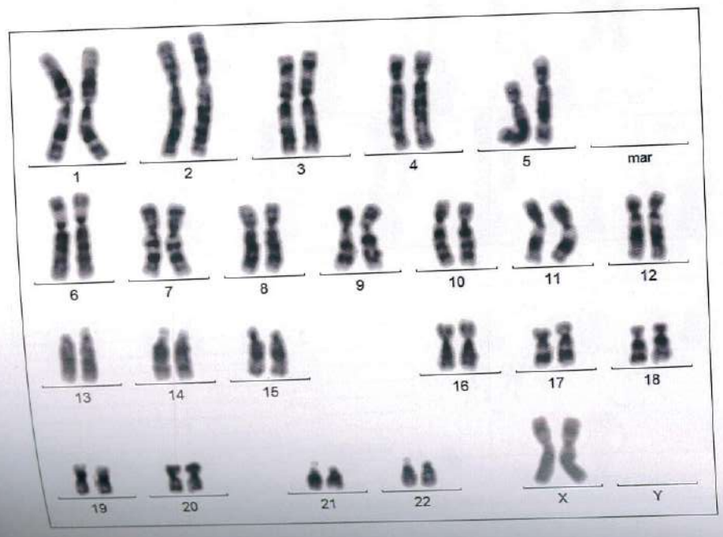
CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 3/564
Sample: Whole Blood
Collection Date: 09/03/2016

Age/Sex: 62 Y/F
Method: Cell culture and G-banding
Culture Date: 12/03/2016

Exposure: Moderate
Cells studied: 75
Reporting Date: 22/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	14p+
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX,14p+	14p+



'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

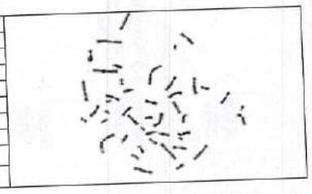
CHROMOSOME ANALYSIS IN WHOLE BLOOD

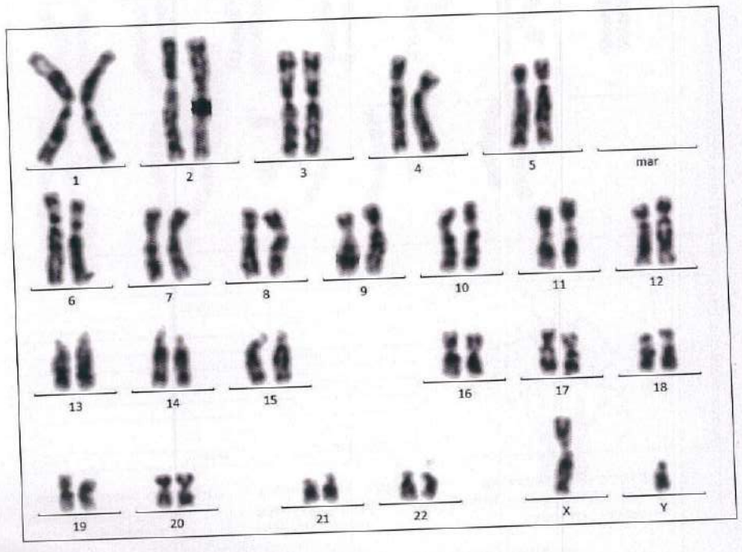
Area/ICMR No.: 3/576
Sample: Whole Blood
Collection Date: 24/02/2016

Age/Sex: 36 Y/M
Method: Cell culture and G-banding
Culture Date: 25/02/2016

Exposure: Moderate
Cells studied: 111
Reporting Date: 05/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Small Y
Karyotype	46,XY,small Y	Small Y





'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

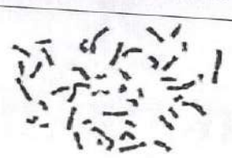
CHROMOSOME ANALYSIS IN WHOLE BLOOD

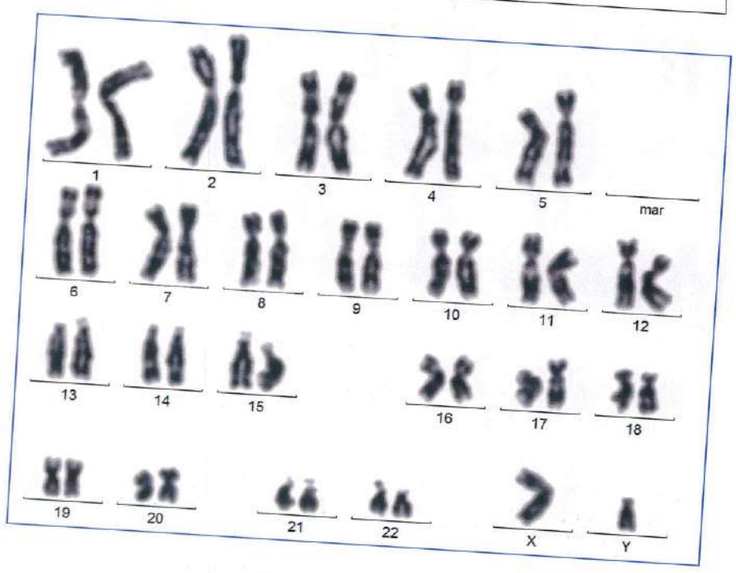
Area/ICMR No.: 10/587
Sample: Whole Blood
Collection Date: 05/05/2016

Age/Sex: 46 Y/M
Method: Cell culture and G-banding
Culture Date: 07/05/2016

Exposure: Moderate
Cells studied: 100
Reporting Date: 17/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal





'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

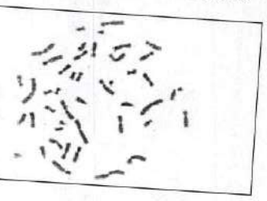
CHROMOSOME ANALYSIS IN WHOLE BLOOD

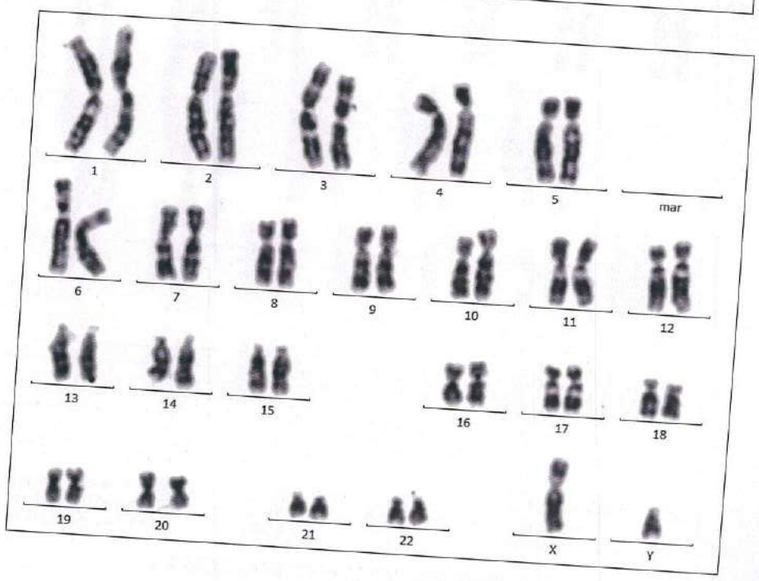
Area/ICMR No.: 03/590
Sample: Whole Blood
Collection Date: 24/02/2016

Age/Sex: 51 Y/M
Method: Cell culture and G-banding
Culture Date: 25/02/2016

Exposure: Moderate
Cells studied: 100
Reporting Date: 05/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal



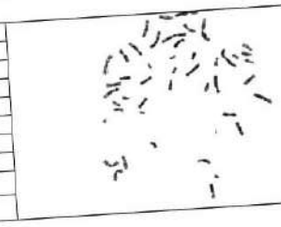


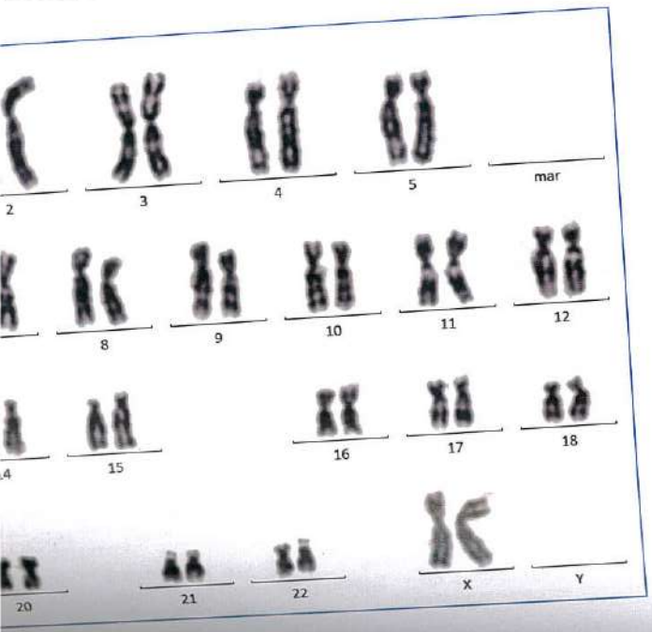
MOSOME ANALYSIS IN WHOLE BLOOD

Age/Sex: 47 Y/F
Method: Cell culture and G-banding
Receiving Date: 25/02/2016

Exposure: Moderate
Cells studied: 100
Reporting Date: 05/03/2016

er	Normal/Abnormal
	Normal
	Normal
	9qh+
	Normal
	Normal
	Normal
	Normal
	Normal
X,9qh+	9qh+





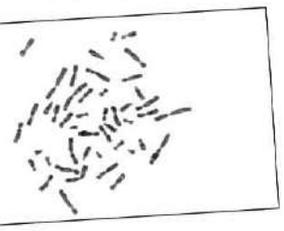
CHROMOSOME ANALYSIS IN WHOLE BLOOD

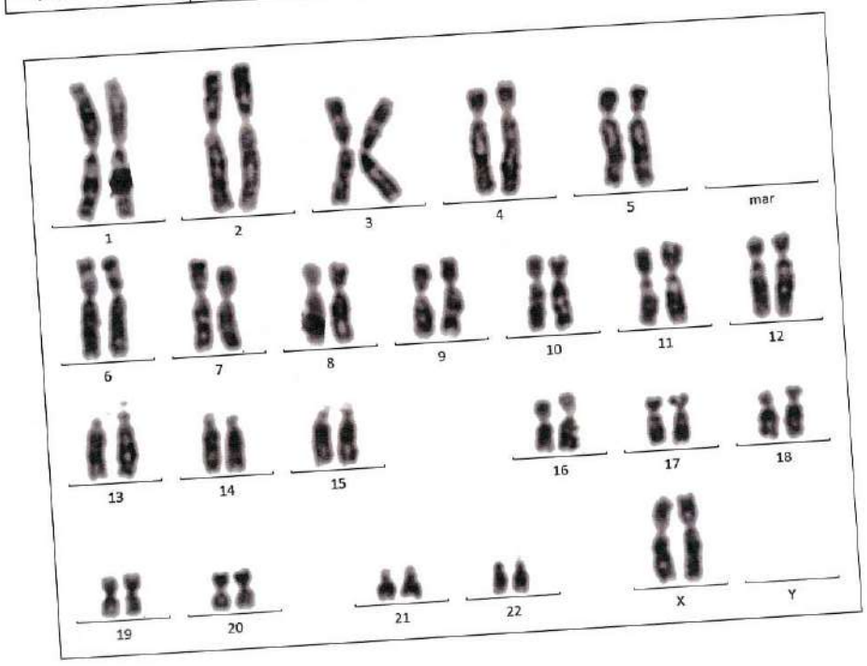
Area/ICMR No.: 1/621
Sample: Whole Blood
Collection Date: 16/02/2016

Age/Sex: 56 Y/F
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 115
Reporting Date: 27/02/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal





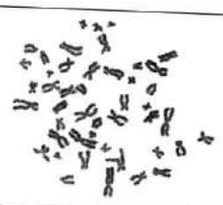
CHROMOSOME ANALYSIS IN WHOLE BLOOD

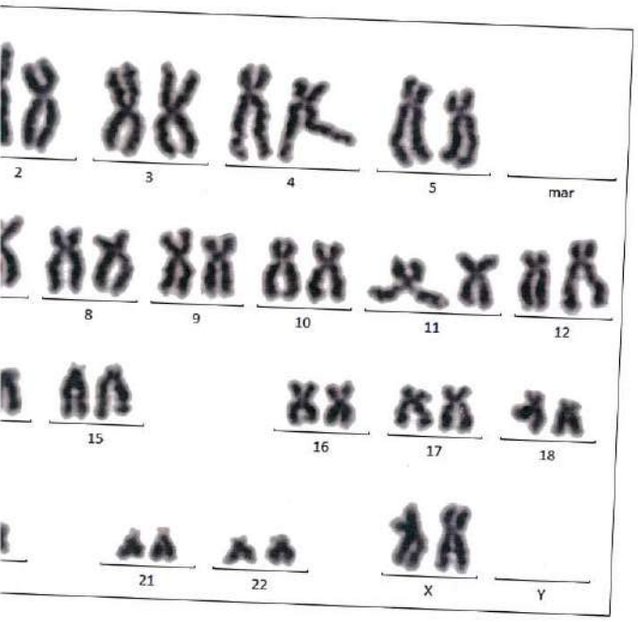
3/624
od
/02/2016

Age/Sex: 63 Y/F
Method: Cell culture and G-banding
Culture Date: 25/02/2016

Exposure: Moderate
Cells studied: 100
Reporting Date: 05/03/2016

Number	Normal/Abnormal
6	Normal
4	Normal
14	Normal
6	Normal
6	Normal
4	Normal
4	Normal
2	Normal
46,XX	Normal





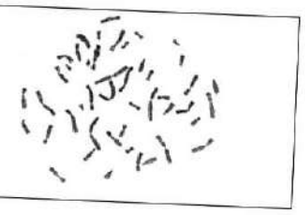
CHROMOSOME ANALYSIS IN WHOLE BLOOD

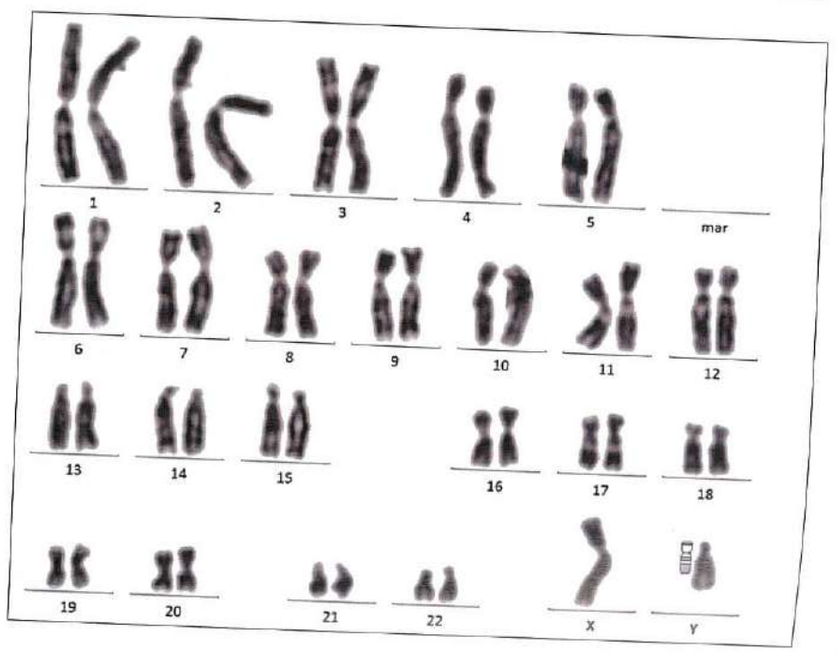
Area/ICMR No.: 03/624
Sample: Whole Blood
Collection Date: 24/02/2016

Age/Sex: 68 Y/M
Method: Cell culture and G-banding
Culture Date: 25/02/2016

Exposure: Moderate
Cells studied: 100
Reporting Date: 05/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Long Y
Karyotype	46,XY,long Y	Long Y

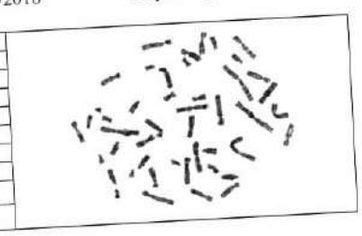


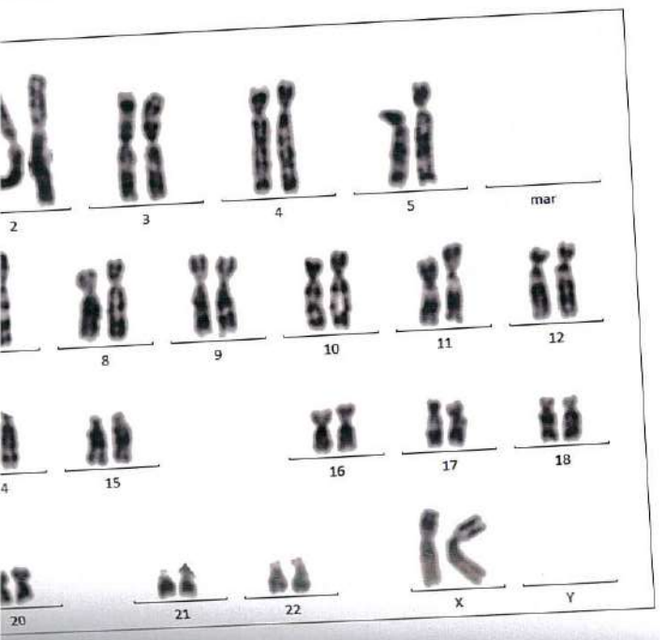


OMOSOME ANALYSIS IN WHOLE BLOOD

6 **Age/Sex:** 55 Y/F **Exposure:** Severe
Method: Cell culture and G-banding **Cells studied**: 66
Culture Date: 27/04/2016 **Reporting Date:** 07/05/2016

ber	Normal/Abnormal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
XX	Normal

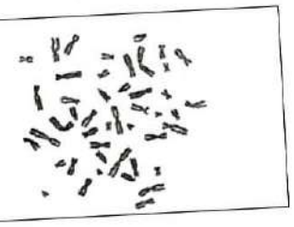


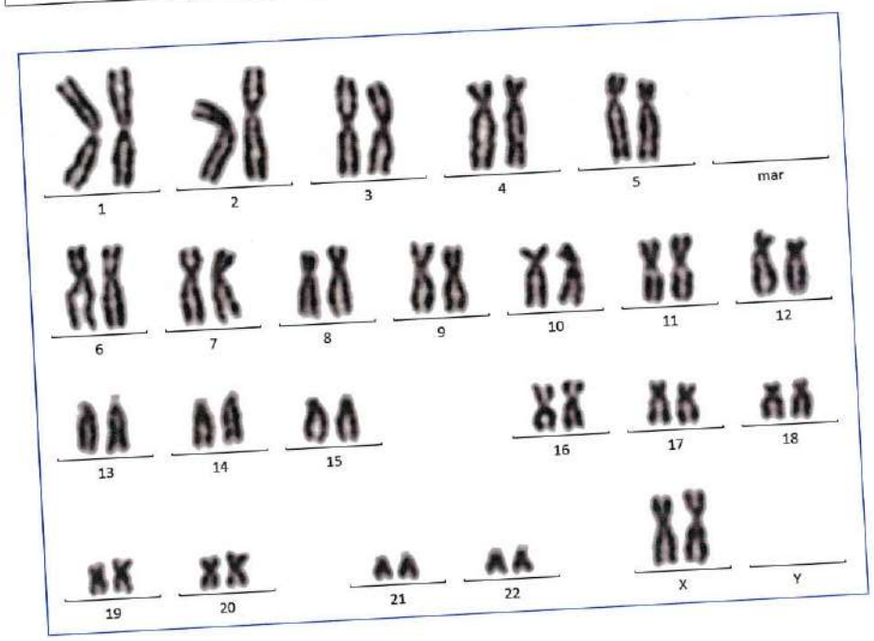


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 7/663 **Age/Sex:** 45 Y/F **Exposure:** Severe
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied**:104
Collection Date: 25/04/2016 **Culture Date**: 27/04/2016 **Reporting Date:** 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal

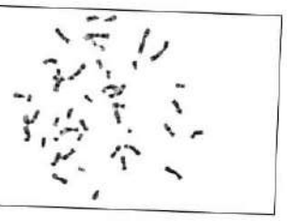


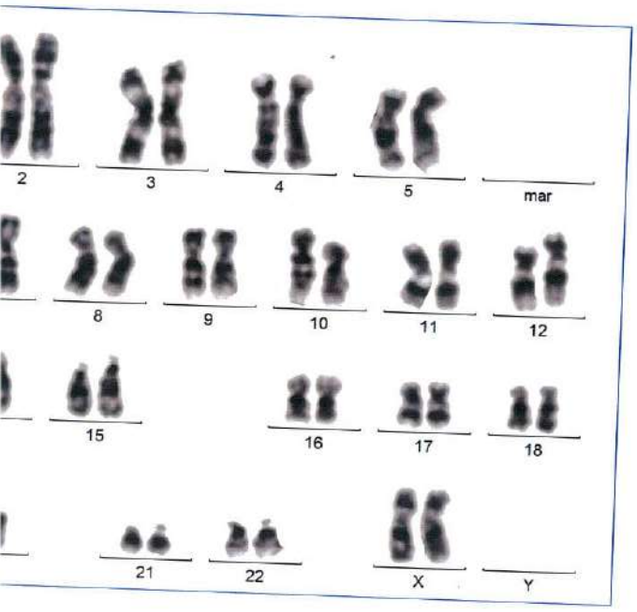


CHROMOSOME ANALYSIS IN WHOLE BLOOD

7/664 **Age/Sex:** 54 Y/F **Exposure:** Severe
od **Method:** Cell culture and G-banding **Cells studied**: 100
02/2016 **Receiving Date**: 17/02/2016 **Reporting Date:** 27/02/2016

Number	Normal/Abnormal
6	Normal
4	Normal
14	Normal
6	Normal
6	Normal
4	Normal
4	Normal
2	Normal
46,XX	Normal

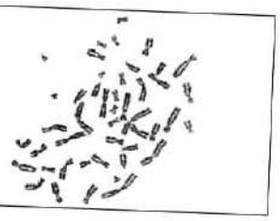


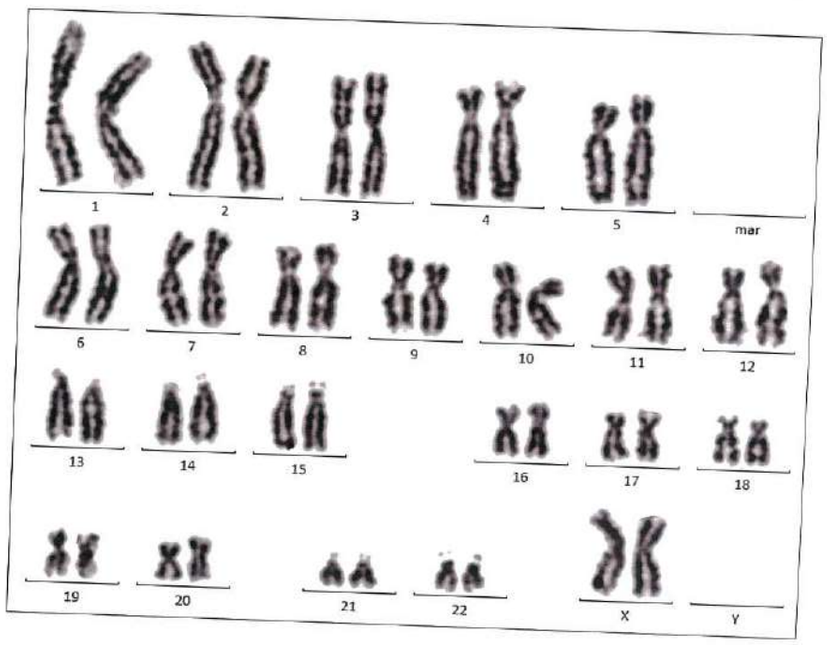


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 03/721 **Age/Sex:** 60 Y/F **Exposure:** Moderate
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied**: 100
Collection Date: 24/02/2016 **Culture Date**: 25/02/2016 **Reporting Date:** 05/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal





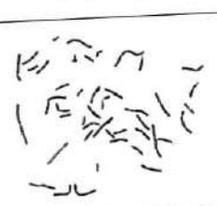
OMOSOME ANALYSIS IN WHOLE BLOOD

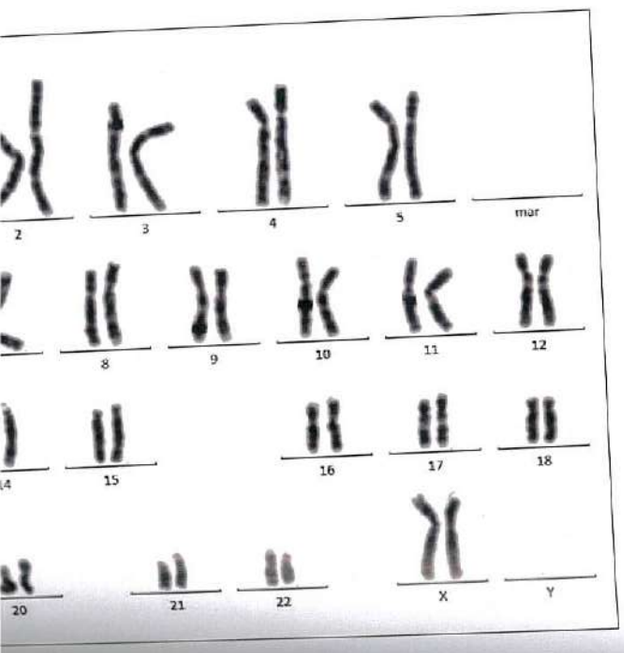
Age/Sex: 55 Y/F
Method: Cell culture and G-banding
Culture Date: 25/02/2016

Exposure: Moderate
Cells studied: 100
Reporting Date: 05/03/2016

6

ber	Normal/Abnormal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
XX	Normal





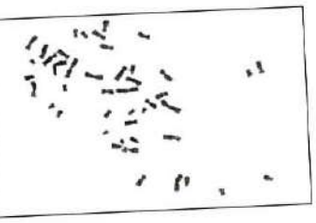
CHROMOSOME ANALYSIS IN WHOLE BLOOD

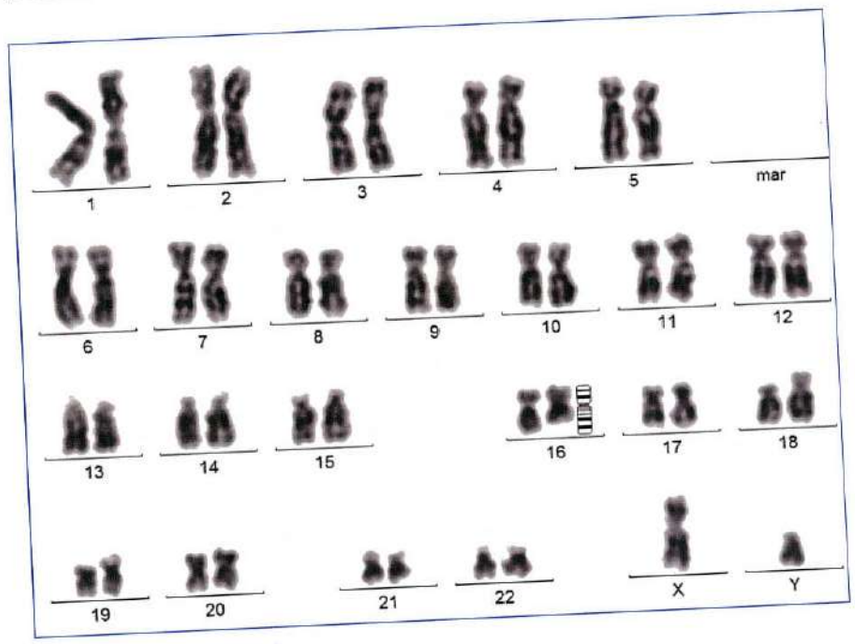
Area/ICMR No.: 05/759
Sample: Whole Blood
Collection Date: 27/04/2016

Age/Sex: 86Y/M
Method: Cell culture and G-banding
Culture Date: 30/04/2016

Exposure: Moderate
Cells studied: 100
Reporting Date: 10/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	?Del(16q)
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY,?del(16q)	?del(16q)





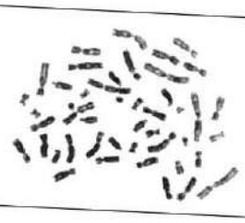
CHROMOSOME ANALYSIS IN WHOLE BLOOD

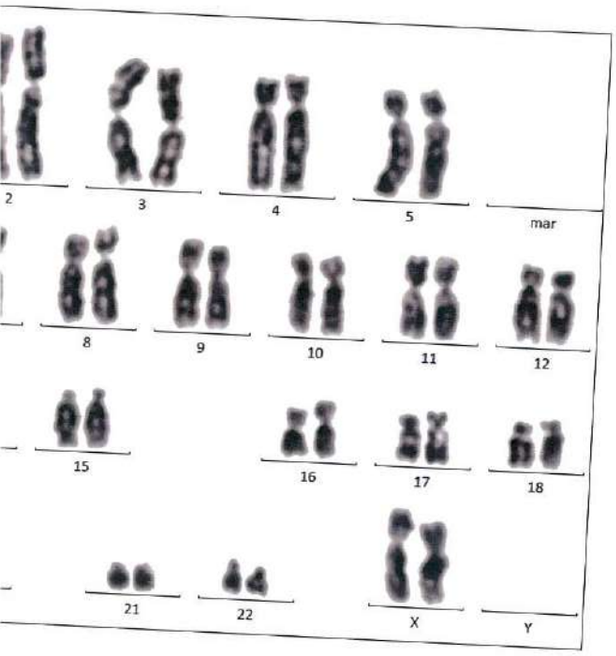
/759
d
02/2016

Age/Sex: 55 Y/F
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 27/02/2016

Number	Normal/Abnormal
6	Normal
4	Normal
14	Normal
6	Normal
6	Normal
4	Normal
4	Normal
2	Normal
46,XX	Normal





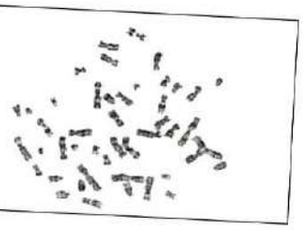
CHROMOSOME ANALYSIS IN WHOLE BLOOD

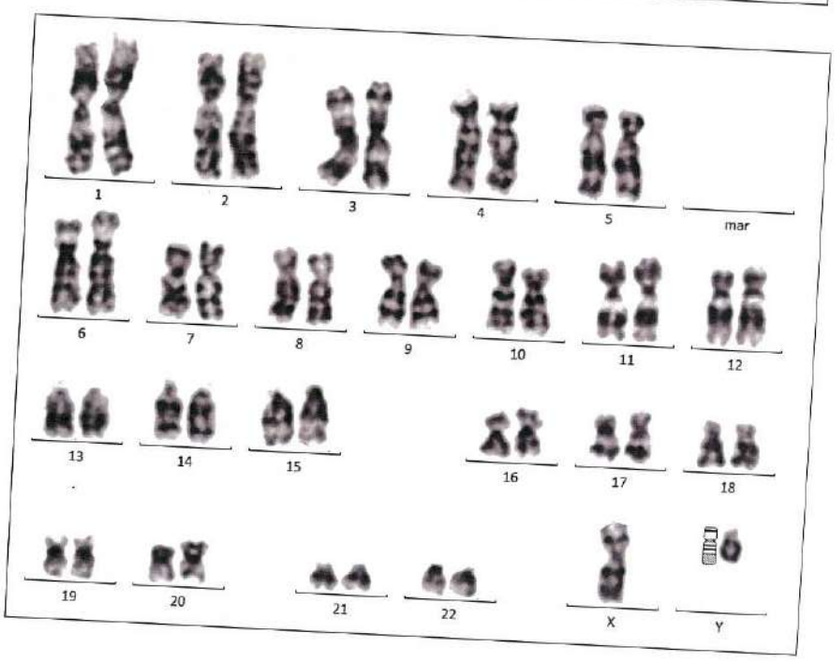
Area/ICMR No.: 07/760
Sample: Whole Blood
Collection Date: 25/04/2016

Age/Sex: 60 Y/M
Method: Cell culture and G-banding
Culture Date: 27/04/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal





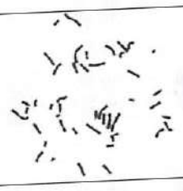
CHROMOSOME ANALYSIS IN WHOLE BLOOD

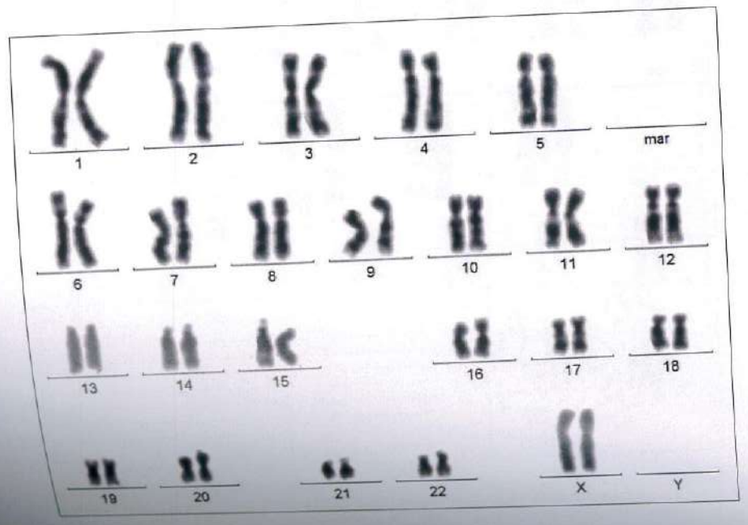
Area/ICMR No.: 07/767
Sample: Whole Blood
Collection Date: 17/02/2016

Age/Sex: 54 Y/F
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 70
Reporting Date: 27/02/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal





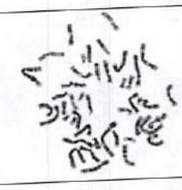
CHROMOSOME ANALYSIS IN WHOLE BLOOD

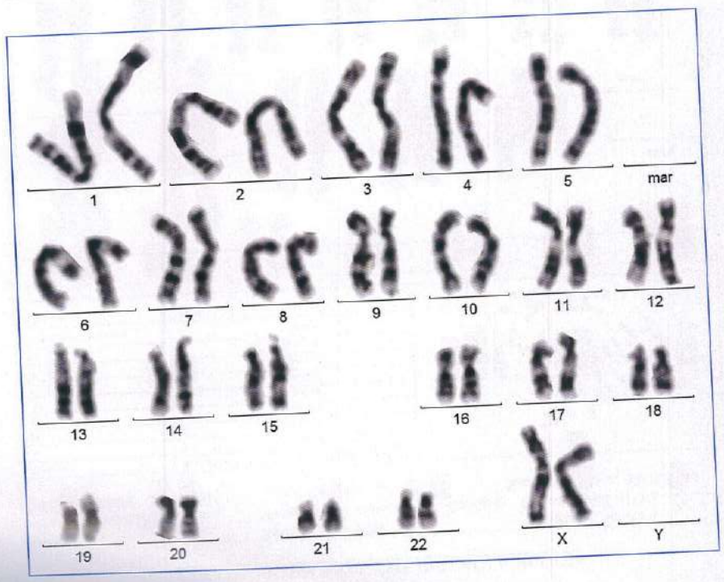
Area/ICMR No.: 10/771
Sample: Whole Blood
Collection Date: 11/03/2016

Age/Sex: 50 Y/F
Method: Cell culture and G-banding
Culture Date: 12/03/2016

Exposure: Moderate
Cells studied: 45
Reporting Date: 22/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal





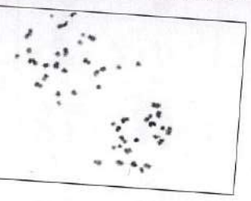
CHROMOSOME ANALYSIS IN WHOLE BLOOD

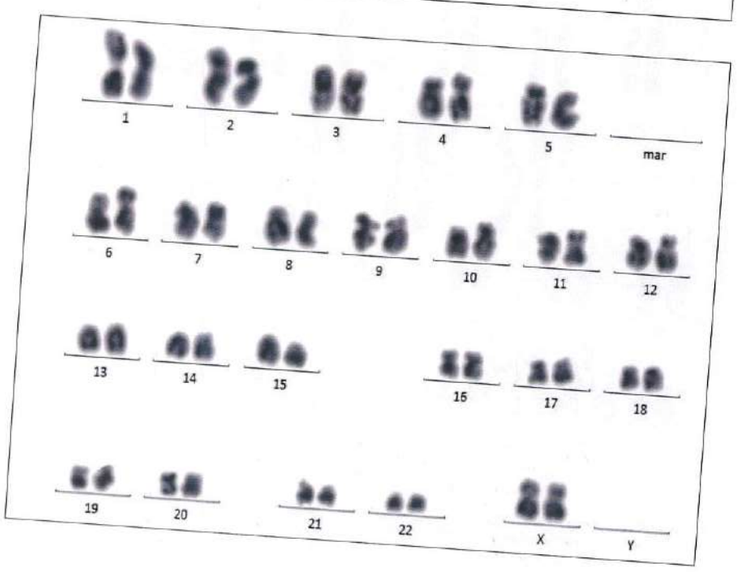
Area/ICMR No.: 16/775
Sample: Whole Blood
Collection Date: 04/02/2015

Age/Sex: 47 Y/F
Method: Cell culture and G-banding
Culture Date: 07/02/2015

Exposure: Control
Cells studied: 10
Reporting Date: 17/02/2015

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal





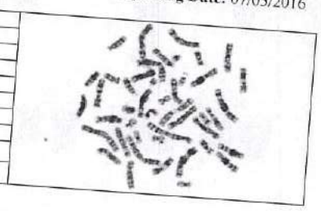
CHROMOSOME ANALYSIS IN WHOLE BLOOD

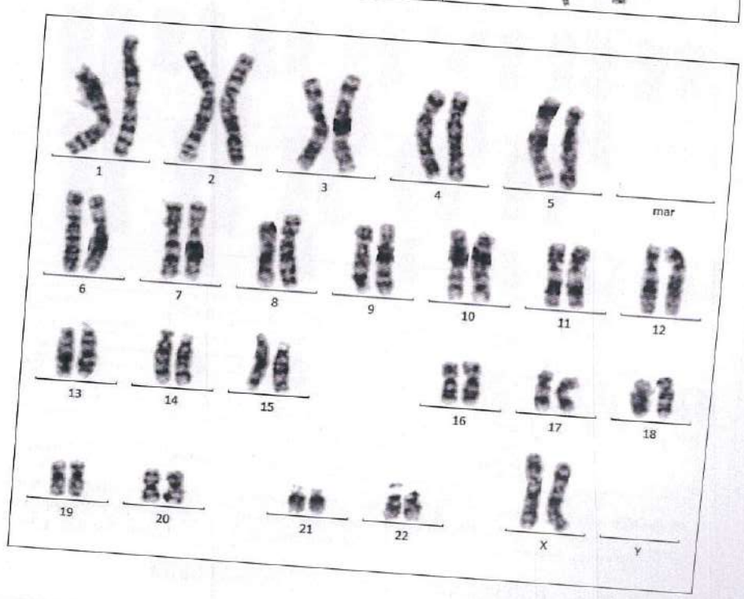
Area/ICMR No.: 03/824
Sample: Whole Blood
Collection Date: 22/04/2016

Age/Sex: 35 Y/F
Method: Cell culture and G-banding
Culture Date: 27/04/2016

Exposure: Moderate
Cells studied: 50
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	22p+
Karyotype	46,XX,22p+	Normal
		22p+





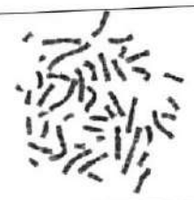
ROMOSOME ANALYSIS IN WHOLE BLOOD

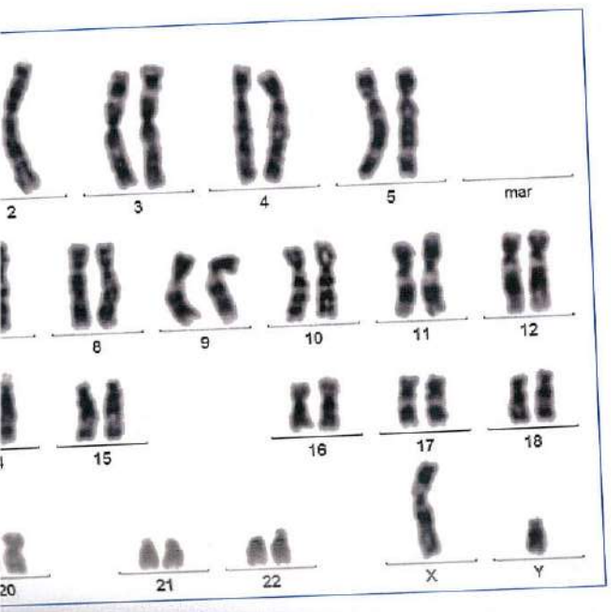
016

Age/Sex: 34 Y/M
Method: Cell culture and G-banding
Culture Date: 12/03/2016

Exposure: Moderate
Cells studied: 100
Reporting Date: 22/03/2016

mber	Normal/Abnormal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
	Normal
.XY	Normal





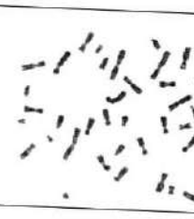
CHROMOSOME ANALYSIS IN WHOLE BLOOD

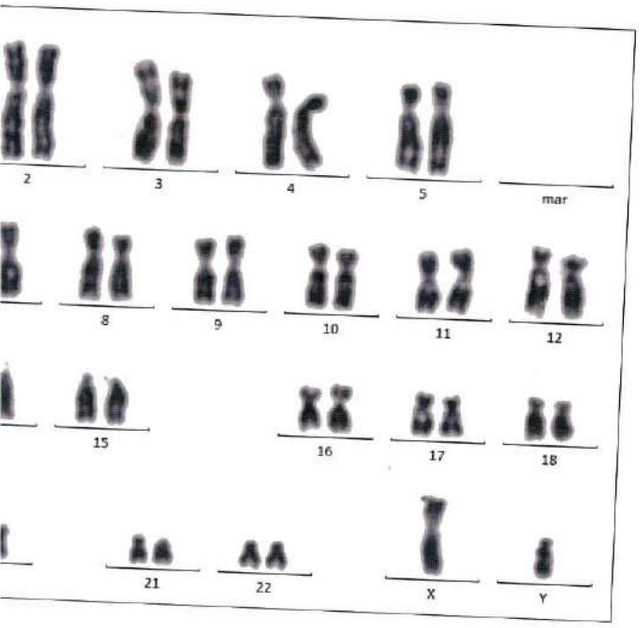
3/879
od
/02/2016

Age/Sex: 35 Y/M
Method: Cell culture and G-banding
Culture Date: 25/02/2016

Exposure: Moderate
Cells studied: 112
Reporting Date: 05/03/2016

Number	Normal/Abnormal
6	Normal
4	Normal
14	Normal
6	Normal
6	Normal
4	Normal
4	Normal
2	Long Y
46,XY,long Y	Long Y





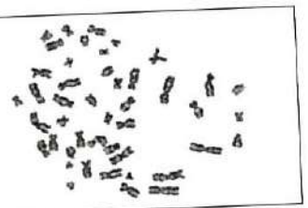
CHROMOSOME ANALYSIS IN WHOLE BLOOD

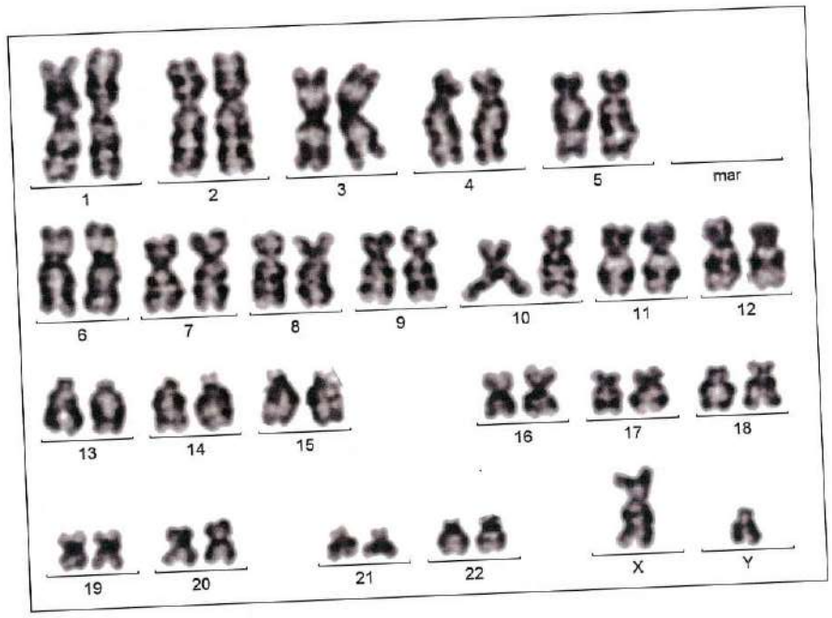
Area/ICMR No.: 03/847
Sample: Whole Blood
Collection Date: 22/04/2016

Age/Sex: 40 Y/M
Method: Cell culture and G-banding
Culture Date: 27/04/2016

Exposure: Moderate
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal





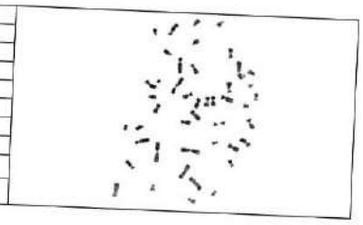
CHROMOSOME ANALYSIS IN WHOLE BLOOD

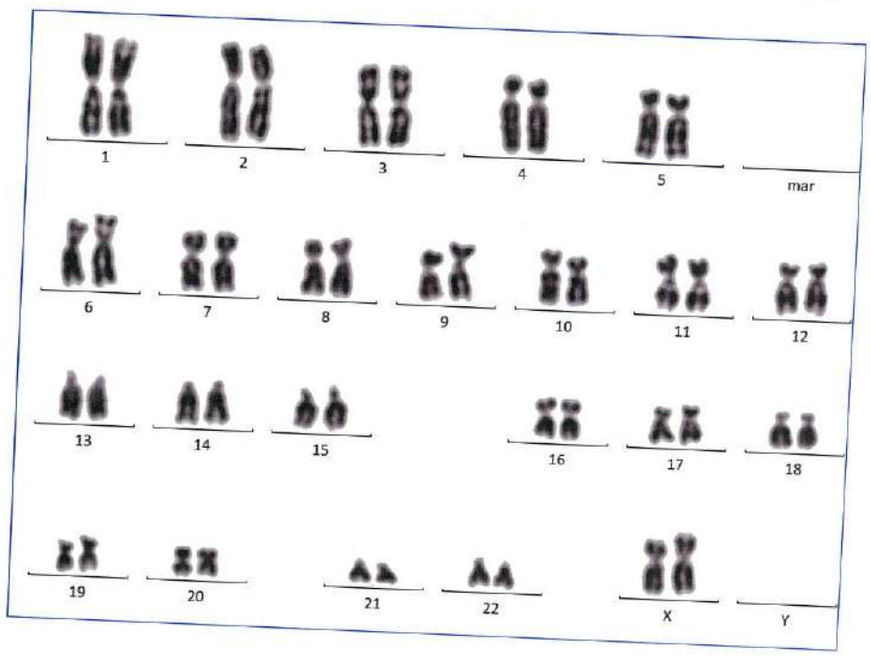
Area/ICMR No.: 03/880
Sample: Whole Blood
Collection Date: 24/02/2016

Age/Sex: 65 Y/F
Method: Cell culture and G-banding
Culture Date: 25/02/2016

Exposure: Moderate
Cells studied: 100
Reporting Date: 05/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal





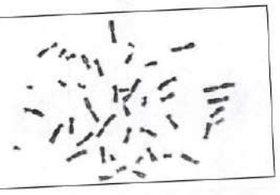
CHROMOSOME ANALYSIS IN WHOLE BLOOD

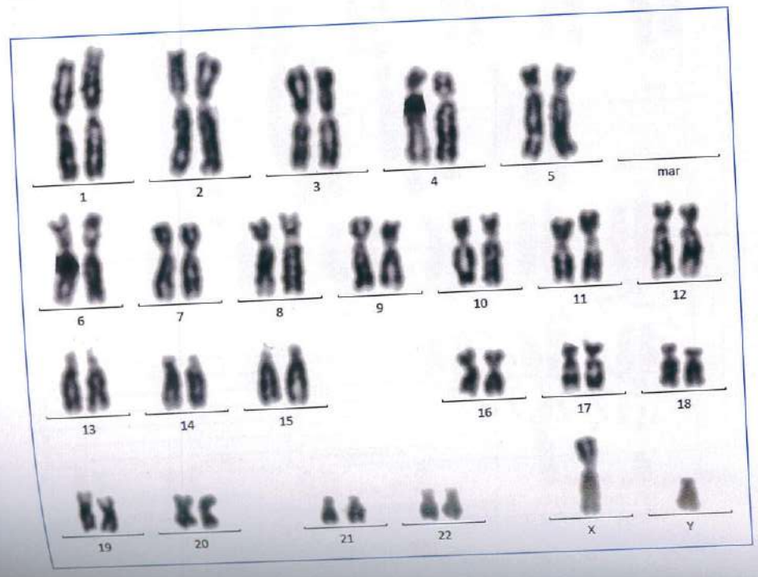
Area/ICMR No.: 03/882
Sample: Whole Blood
Collection Date: 24/02/2016

Age/Sex: 68 Y/M
Method: Cell culture and G-banding
Culture Date: 25/02/2016

Exposure: Moderate
Cells studied: 137
Reporting Date: 05/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal





'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

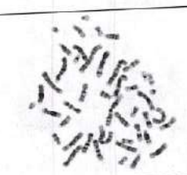
CHROMOSOME ANALYSIS IN WHOLE BLOOD

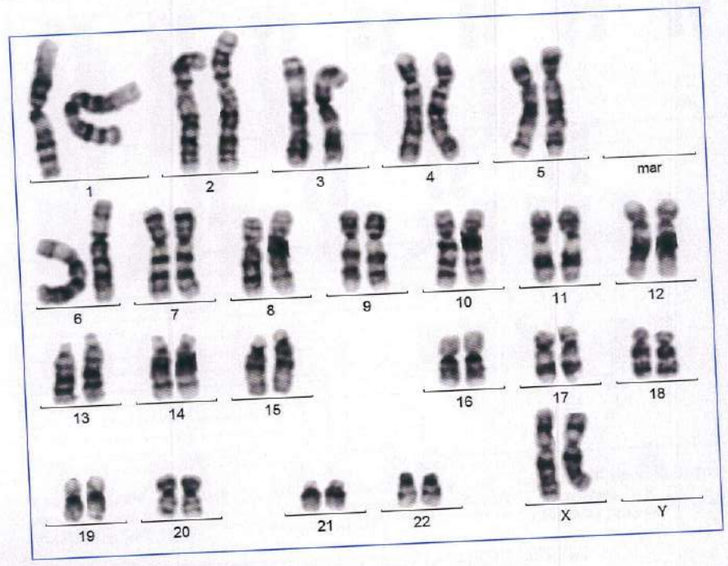
Area/ICMR No.: 03/905
Sample: Whole Blood
Collection Date: 22/04/2016

Age/Sex: 70 Y/F
Method: Cell culture and G-banding
Culture Date: 24/04/2016

Exposure: Moderate
Cells studied: 100
Reporting Date: 04/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal





PI: Dr. Bani Bandana Ganguly MGM New Bombay Hospital, Navi Mumbai

'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

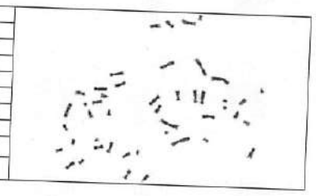
CHROMOSOME ANALYSIS IN WHOLE BLOOD

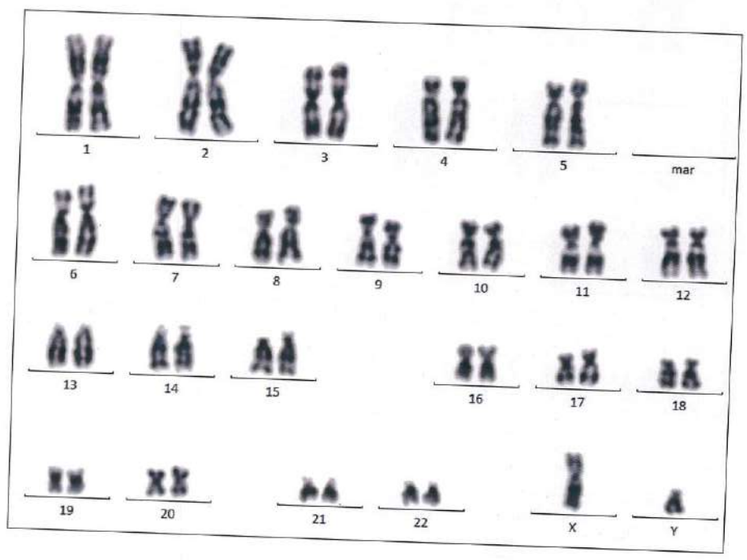
Area/ICMR No.: 02/940
Sample: Whole Blood
Collection Date: 09/03/2016

Age/Sex: 32 Y/M
Method: Cell culture and G-banding
Culture Date: 12/03/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 22/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal





PI: Dr. Bani Bandana Ganguly MGM New Bombay Hospital, Navi Mumbai

'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

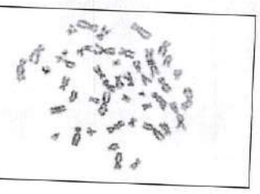
CHROMOSOME ANALYSIS IN WHOLE BLOOD

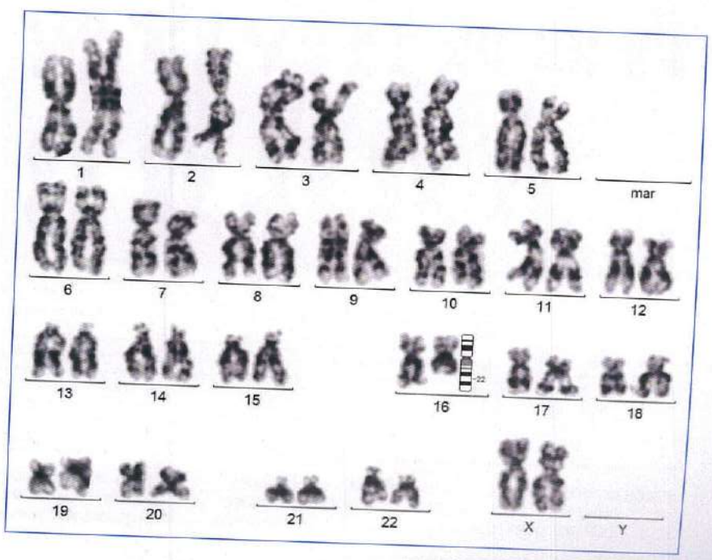
Area/ICMR No.: 03/1009
Sample: Whole Blood
Collection Date: 22/04/2016

Age/Sex: 45 Y/F
Method: Cell culture and G-banding
Culture Date: 27/04/2016

Exposure: Moderate
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Del(16q)
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX,del(16q)	Del(16q)





PI: Dr. Bani Bandana Ganguly MGM New Bombay Hospital, Navi Mumbai

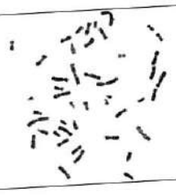
CHROMOSOME ANALYSIS IN WHOLE BLOOD

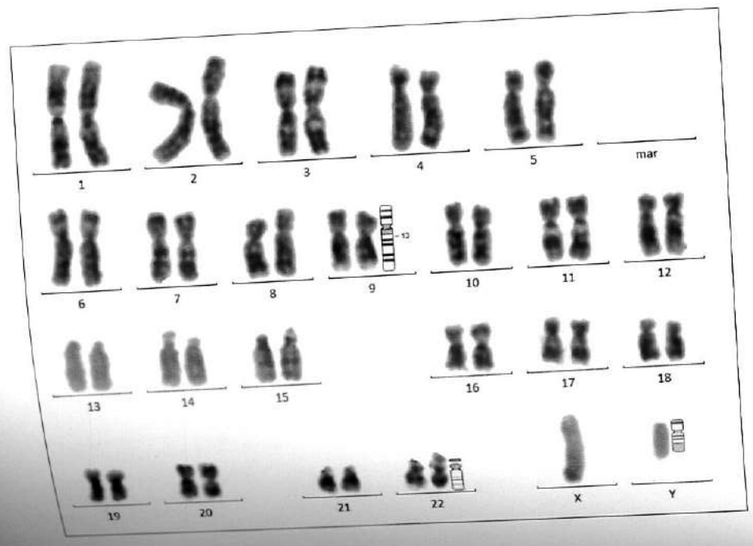
Area/ICMR No.: 01/1009
Sample: Whole Blood
Collection Date: 09/05/2014

Age/Sex: 38 Y/M
Method: Cell culture and G-banding
Culture Date: 09/05/2014

Exposure: Severe
Cells studied: 30
Reporting Date: 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	?del9(q13)
C	14	Normal
D	6	Normal
E	6	Normal
F	4	22p+
G	4	Normal
Sex Chromosomes	2	?del9(q13),22p+
Karyotype	46,XY,?del9(q13),22p+	





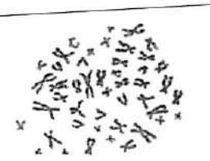
CHROMOSOME ANALYSIS IN WHOLE BLOOD

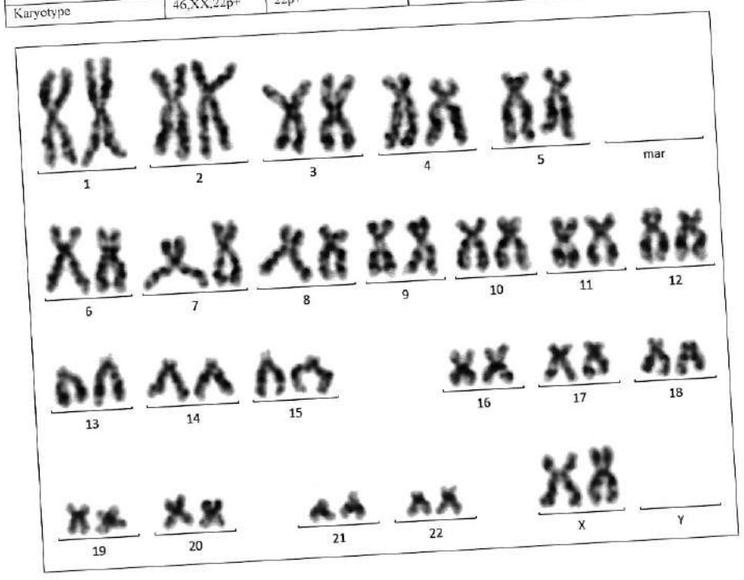
Area/ICMR No.: 02/1034
Sample: Whole Blood
Collection Date: 09/03/016

Age/Sex: 50 Y/F
Method: Cell culture and G-banding
Culture Date: 12/03/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 22/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	22p+
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX,22p+	22p+





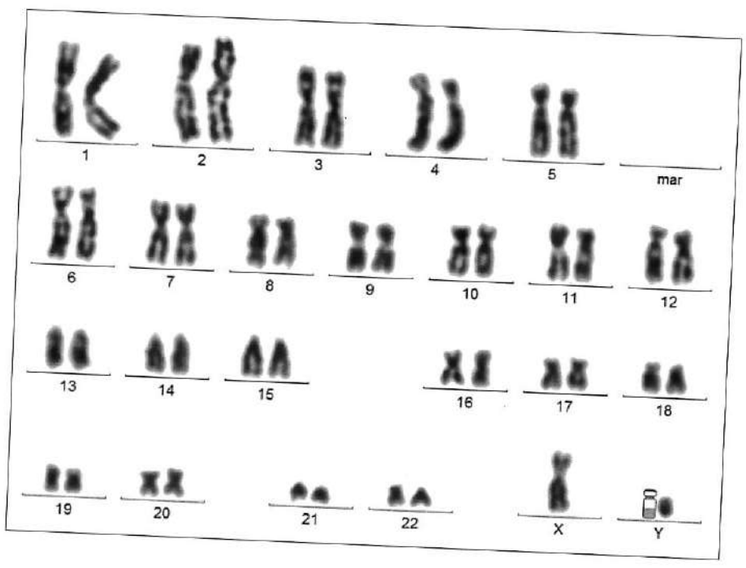
CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 07/1043
Sample: Whole Blood
Collection Date: 17/02/2016

Age/Sex: 58 Y/M
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 60
Reporting Date: 27/02/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Small Y
Karyotype	46,XY,small Y	Small Y



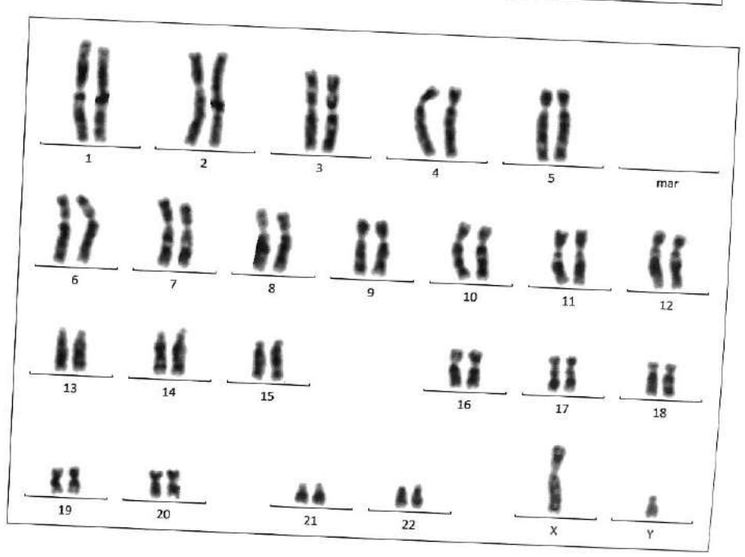
CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 07/1034
Sample: Whole Blood
Collection Date: 17/02/2016

Age/Sex: 33 Y/M
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 27/02/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Small Y
Karyotype	46,XY,small Y	Small Y



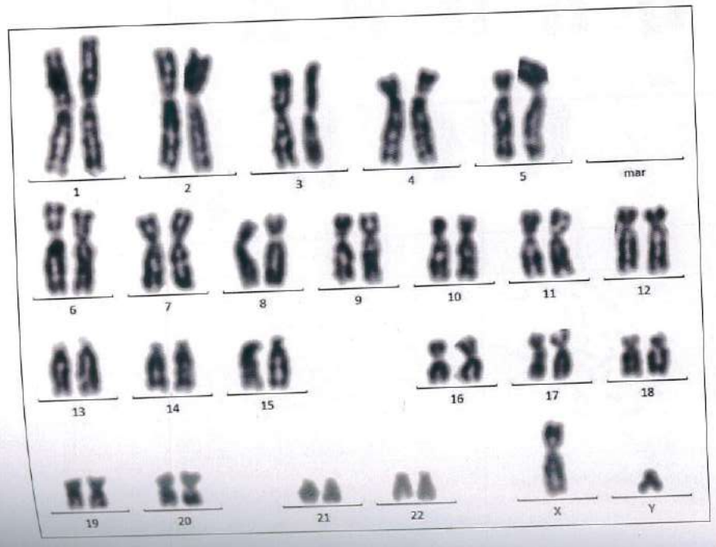
CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 07/1043
Sample: Whole Blood
Collection Date: 17/02/2016

Age/Sex: 38 Y/M
Method: Cell culture and G-banding
Culture Date: 17/02/2016

Exposure: Severe
Cells studied: 110
Reporting Date: 27/02/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Small Y
Karyotype	46,XY,small Y	Small Y



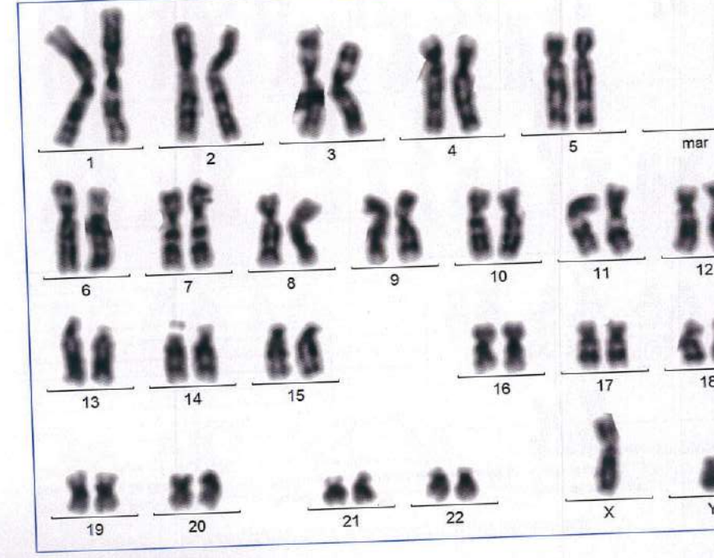
CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 02/1053
Sample: Whole Blood
Collection Date: 07/05/2016

Age/Sex: 45 Y/M
Method: Cell culture and G-banding
Culture Date: 07/05/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 17/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal



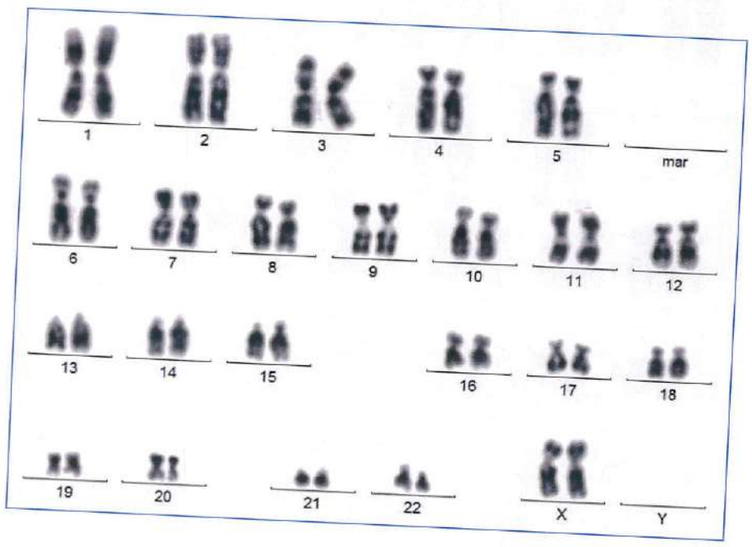
CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 03/1059
Sample: Whole Blood
Collection Date: 09/03/2016

Age/Sex: 61 Y/F
Method: Cell culture and G-banding
Culture Date: 12/03/2016

Exposure: Moderate
Cells studied: 50
Reporting Date: 22/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal



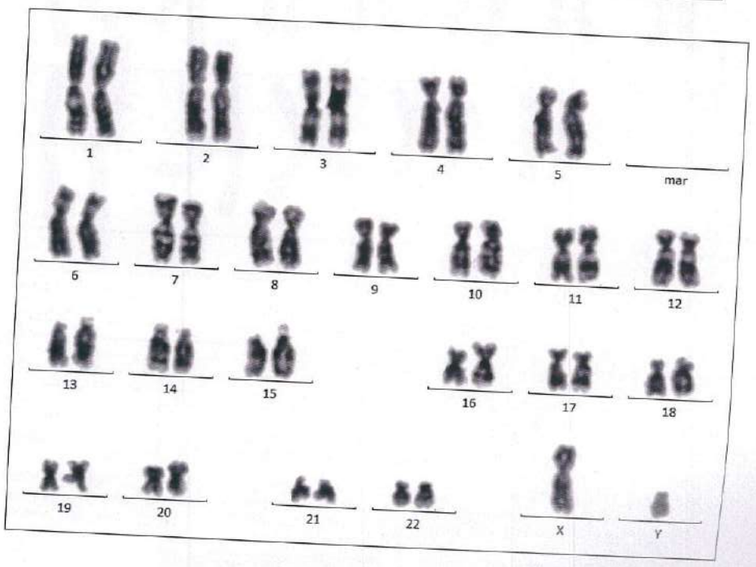
CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 03/1063
Sample: Whole Blood
Collection Date: 22/04/2016

Age/Sex: 39 Y/M
Method: Cell culture and G-banding
Culture Date: 24/04/2016

Exposure: Moderate
Cells studied: 95
Reporting Date: 04/05/2016

Group	Number	Normal/Abnormal
A	6	1qh+
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY,1qh+	1qh+



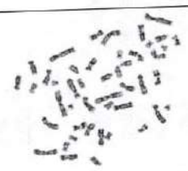
CHROMOSOME ANALYSIS IN WHOLE BLOOD

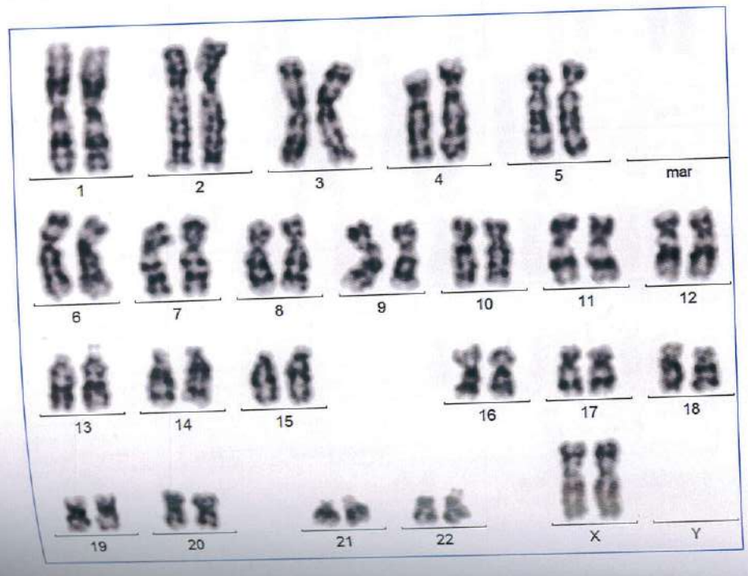
Area/ICMR No.: 03/1063
Sample: Whole Blood
Collection Date: 22/04/2016

Age/Sex: 60 Y/F
Method: Cell culture and G-banding
Culture Date: 27/04/2016

Exposure: Moderate
Cells studied: 40
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal





'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

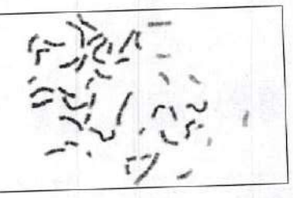
CHROMOSOME ANALYSIS IN WHOLE BLOOD

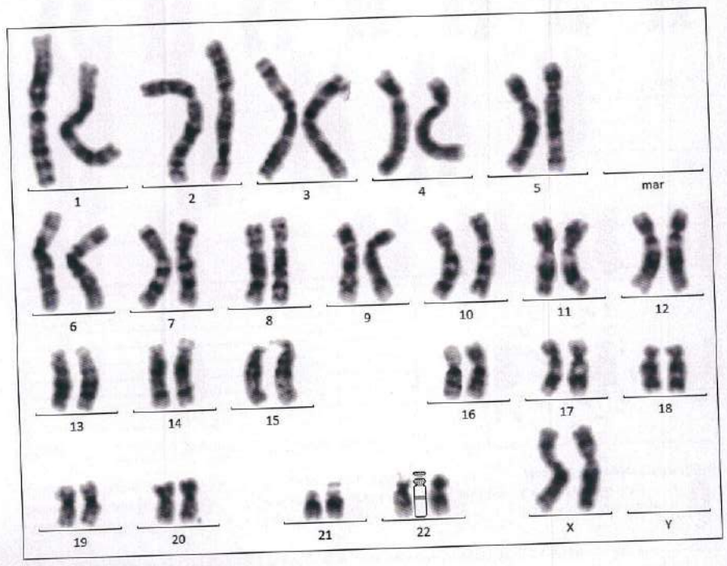
Area/ICMR No.: 01/1072
Sample: Whole Blood
Collection Date: 09/05/2014

Age/Sex: 36 Y/F
Method: Cell culture and G-banding
Culture Date: 09/05/2014

Exposure: Severe
Cells studied: 35
Reporting Date: 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	22p+
Sex Chromosomes	2	Normal
Karyotype	46,XX,22p+	22p+





'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

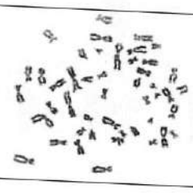
CHROMOSOME ANALYSIS IN WHOLE BLOOD

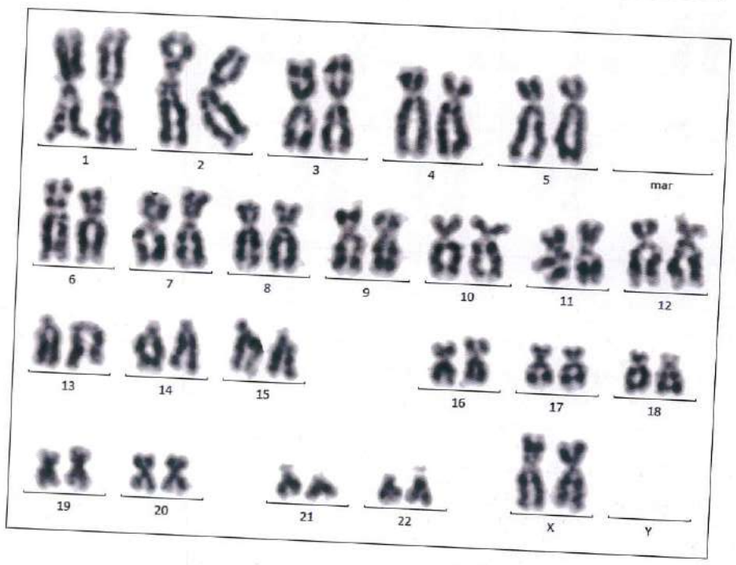
Area/ICMR No.: 03/1103
Sample: Whole Blood
Collection Date: 24/02/2016

Age/Sex: 50 Y/F
Method: Cell culture and G-banding
Culture Date: 25/02/2016

Exposure: Moderate
Cells studied: 115
Reporting Date: 05/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal





'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

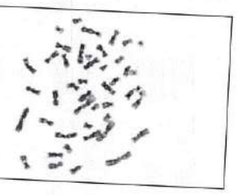
CHROMOSOME ANALYSIS IN WHOLE BLOOD

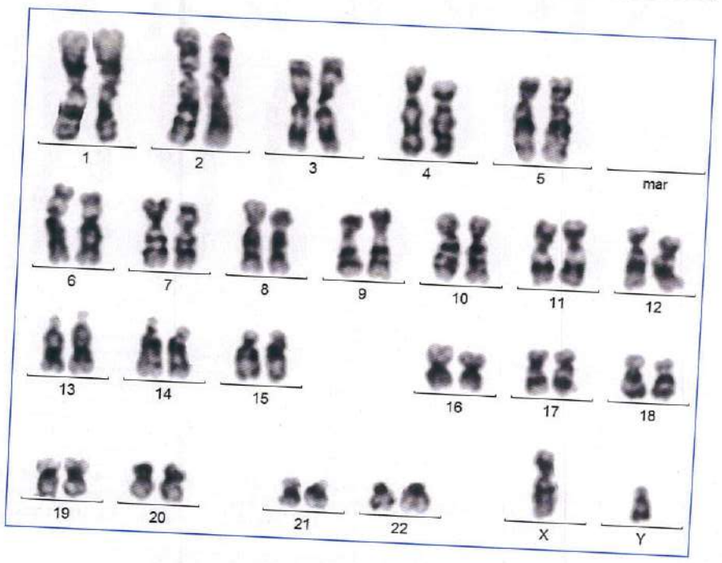
Area/ICMR No.: 02/1112
Sample: Whole Blood
Collection Date: 09/03/2016

Age/Sex: 48 Y/M
Method: Cell culture and G-banding
Culture Date: 12/03/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 22/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	9qh+
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY,9qh+	9qh+





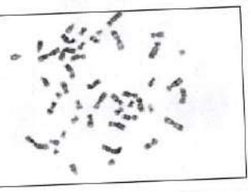
CHROMOSOME ANALYSIS IN WHOLE BLOOD

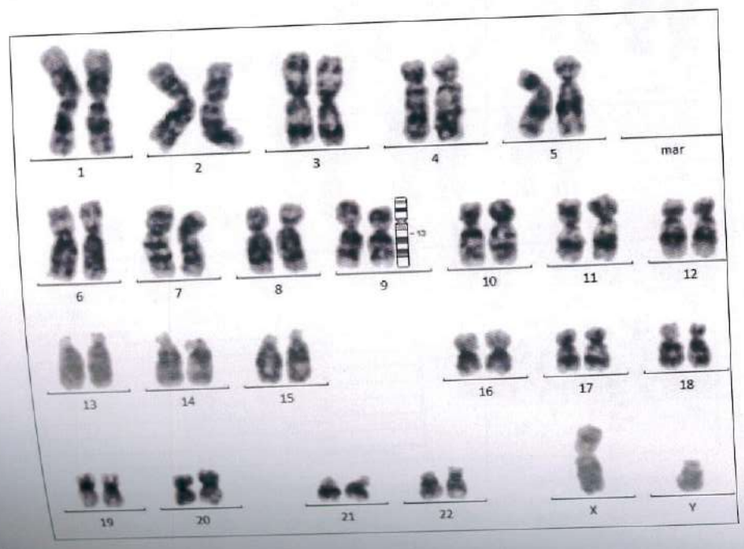
Area/ICMR No.: 01/1138
Sample: Whole Blood
Collection Date: 09/05/2014

Age/Sex: 59 Y/M
Method: Cell culture and G-banding
Culture Date: 09/05/2014

Exposure: Severe
Cells studied: 84
Reporting Date: 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	?del9(q13)
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX,?del9(q13)	?del9(q13)





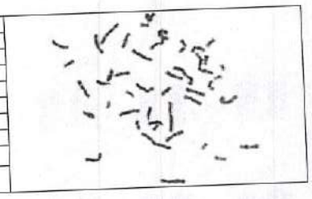
CHROMOSOME ANALYSIS IN WHOLE BLOOD

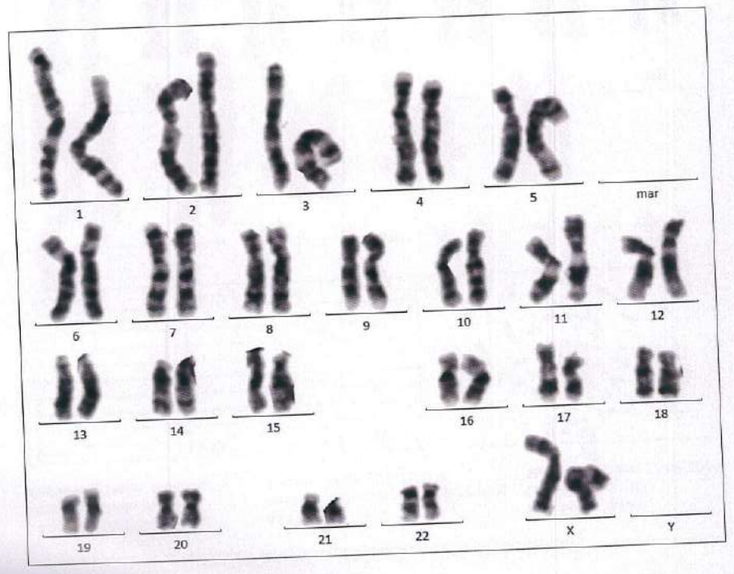
Area/ICMR No. 1/1138
Sample: Whole Blood
Collection Date: 09/05/2014

Age/Sex: 50 Y/F
Method: Cell culture and G-banding
Culture Date: 09/05/2014

Exposure: Severe
Cells studied: 35
Reporting Date: 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	15p+
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX,15p+	15p+





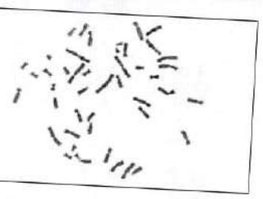
CHROMOSOME ANALYSIS IN WHOLE BLOOD

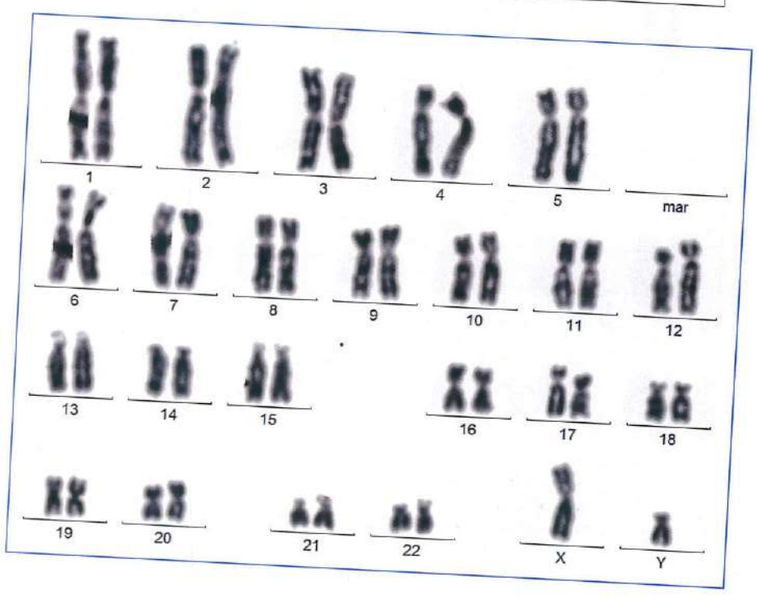
Area/ICMR No.: 03/1156
Sample: Whole Blood
Collection Date: 22/04/2016

Age/Sex: 32 Y/M
Method: Cell culture and G-banding
Culture Date: 27/04/2016

Exposure: Moderate
Cells studied: 200
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal





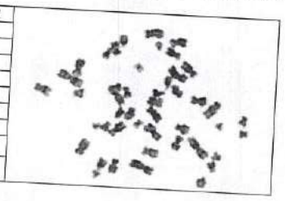
CHROMOSOME ANALYSIS IN WHOLE BLOOD

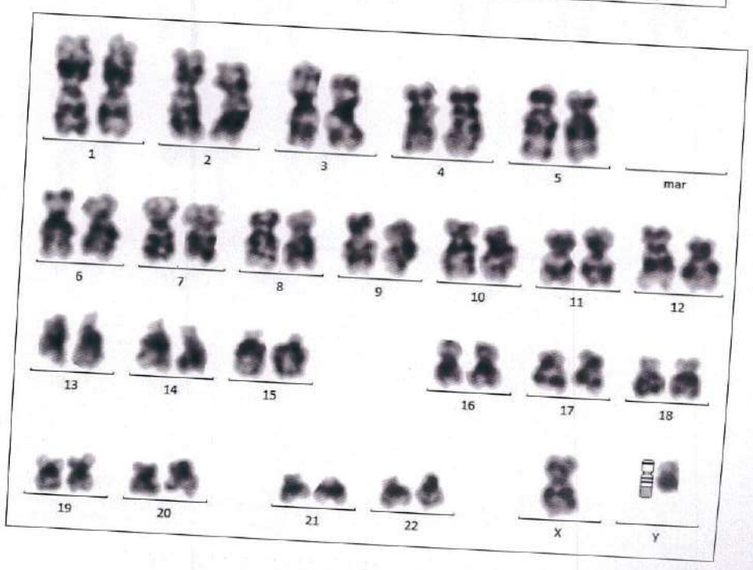
Area/ICMR No.: 03/1156
Sample: Whole Blood
Collection Date: 22/04/2016

Age/Sex: 52 Y/M
Method: Cell culture and G-banding
Culture Date: 27/04/2016

Exposure: Moderate
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	15p+
E	6	Normal
F	4	Normal
G	4	22p+
Sex Chromosomes	2	small Y
Karyotype	46,XY,22p+,15p+,small Y	22p+,15p+,small Y





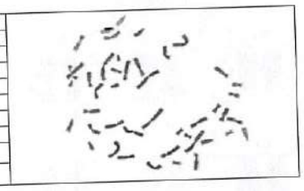
CHROMOSOME ANALYSIS IN WHOLE BLOOD

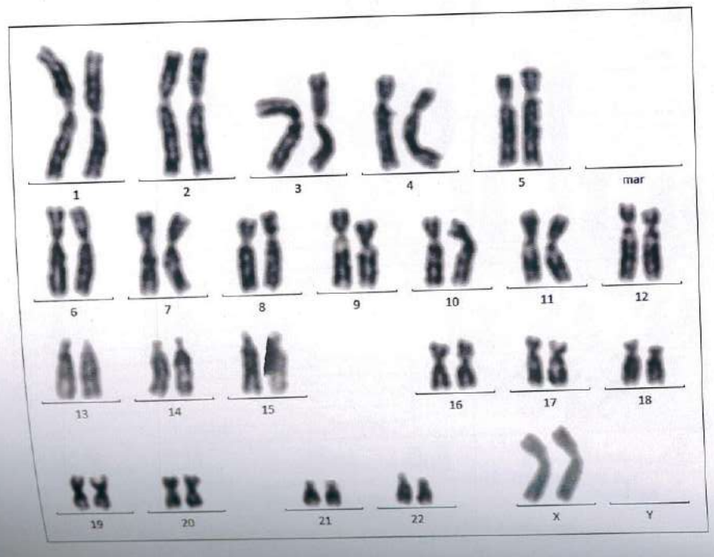
Area/ICMR No.: 03/1162
Sample: Whole Blood
Collection Date: 24/02/2016

Age/Sex: 50 Y/F
Method: Cell culture and G-banding
Culture Date: 25/02/2016

Exposure: Moderate
Cells studied: 100
Reporting Date: 05/03/2016

Group	Number	Normal/Abnormal
A	6	1qh+
B	4	Normal
C	14	9qh+
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX,1qh+,9qh+	1qh+,9qh+





'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

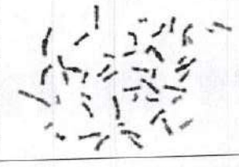
CHROMOSOME ANALYSIS IN WHOLE BLOOD

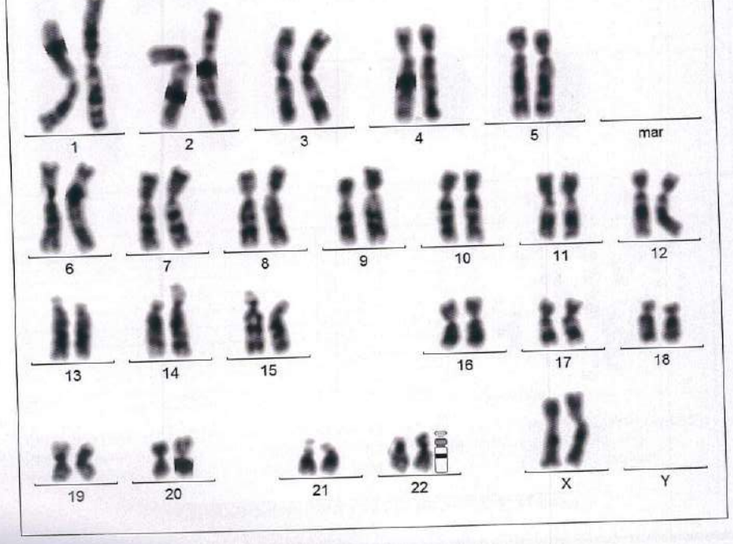
Area/ICMR No.: 03/1182
Sample: Whole Blood
Collection Date: 24/02/2016

Age/Sex: 40 Y/F
Method: Cell culture and G-banding
Culture Date: 25/02/2016

Exposure: Moderate
Cells studied: 100
Reporting Date: 05/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	9qh+
D	6	14p+
E	6	Normal
F	4	Normal
G	4	22p+
Sex Chromosomes	2	Normal
Karyotype	46,XX,9qh+,14p+,22p+	9qh+,14p+,22p+





'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

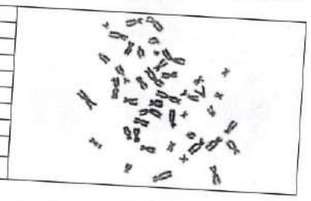
CHROMOSOME ANALYSIS IN WHOLE BLOOD

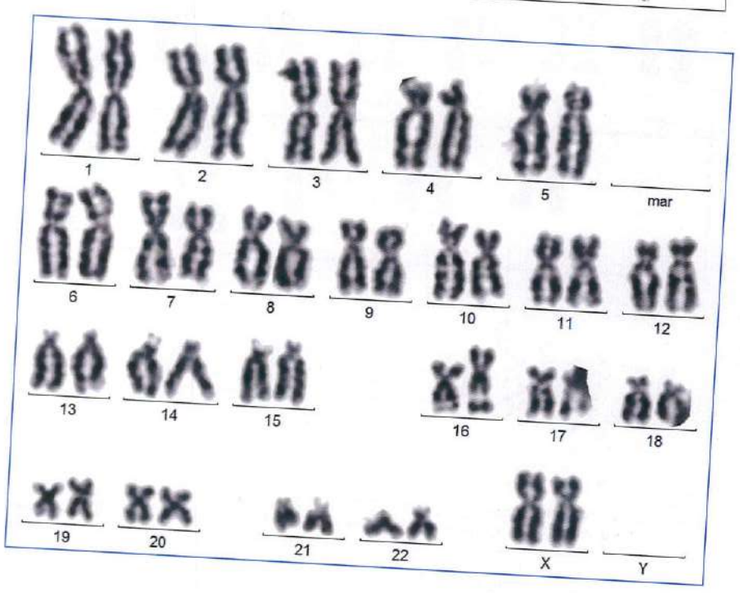
Area/ICMR No.: 01/1243
Sample: Whole Blood
Collection Date: 25/04/2016

Age/Sex: 50 Y/F
Method: Cell culture and G-banding
Culture Date: 27/04/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Fra(16q22)
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX,fra(16q22)	Fra(16q22)





'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

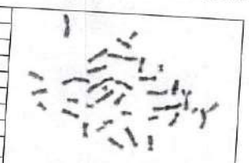
CHROMOSOME ANALYSIS IN WHOLE BLOOD

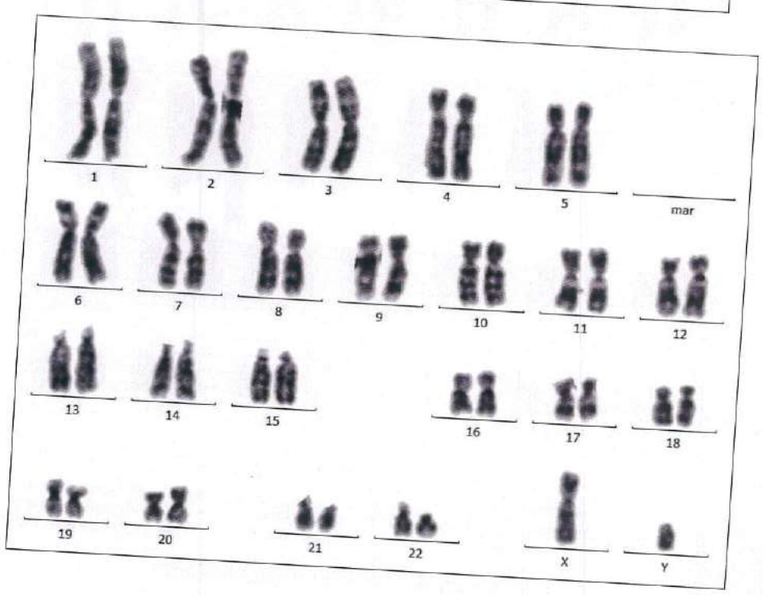
Area/ICMR No.: 1/1308
Sample: Whole Blood
Collection Date: 09/05/2014

Age/Sex: 47 Y/M
Method: Cell culture and G-banding
Culture Date: 09/05/2014

Exposure: Severe
Cells studied: 10
Reporting Date: 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Small Y
Karyotype	46,XY,small Y	Small Y





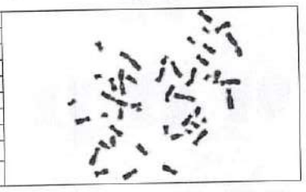
CHROMOSOME ANALYSIS IN WHOLE BLOOD

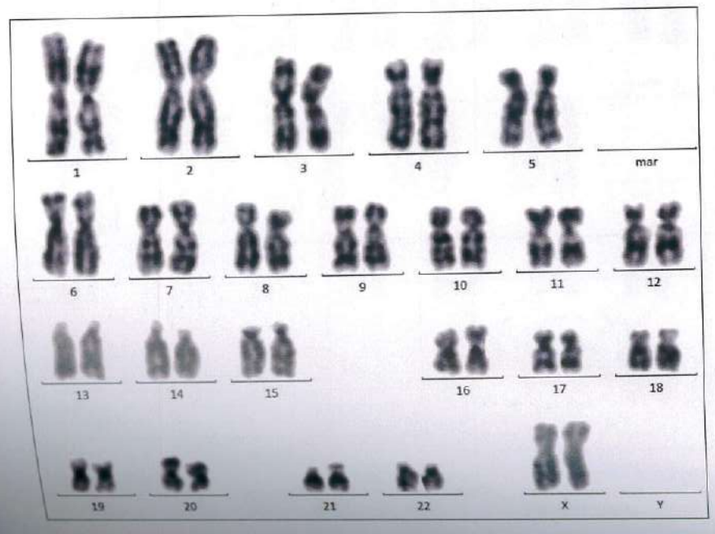
Area/ICMR No.: 1/1308
Sample: Whole Blood
Collection Date: 09/05/2014

Age/Sex: 35 Y/F
Method: Cell culture and G-banding
Culture Date: 09/05/2014

Exposure: Severe
Cells studied: 40
Reporting Date: 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	9qh+
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX,9qh+	9qh+





'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

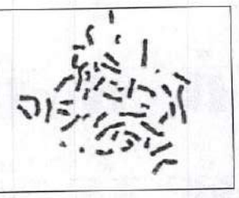
CHROMOSOME ANALYSIS IN WHOLE BLOOD

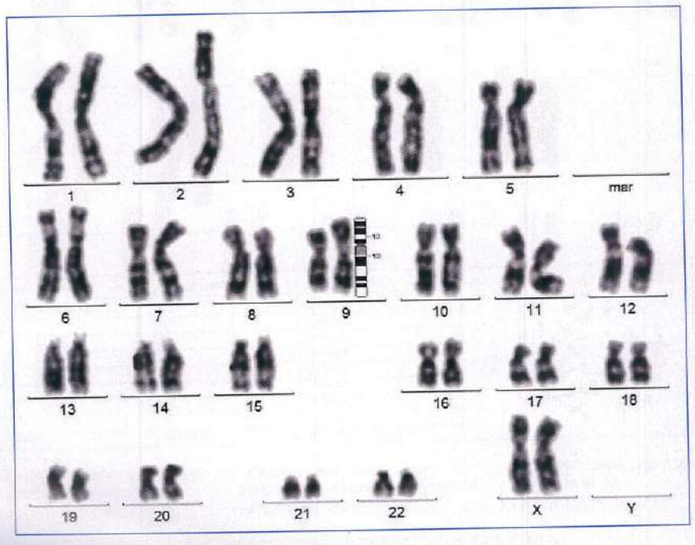
Area/ICMR No.: 01/1804
Sample: Whole Blood
Collection Date: 26/04/2016

Age/Sex: 45 Y/F
Method: Cell culture and G-banding
Culture Date: 27/04/2016

Exposure: Severe
Cells studied: 100
Reporting Date: 07/05/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	inv(9)(p11q13),9qh+
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX,inv(9)(p11q13),9qh+	inv(9)(p11q13),9qh+





PI: Dr. Bani Bandana Ganguly MGM New Bombay Hospital, Navi Mumbai

'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

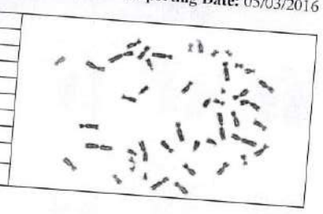
CHROMOSOME ANALYSIS IN WHOLE BLOOD

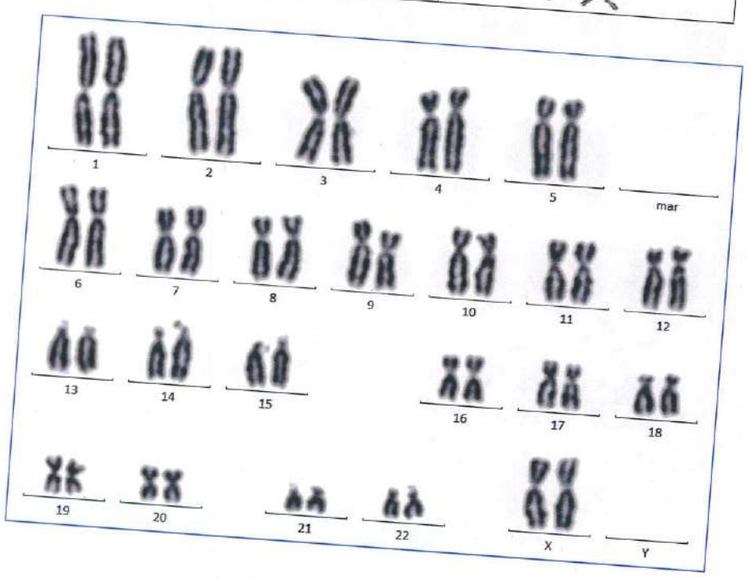
Area/ICMR No.: 01/1846
Sample: Whole Blood
Collection Date: 23/02/2016

Age/Sex: 65 Y/F
Method: Cell culture and G-banding
Culture Date: 25/02/2016

Exposure: Severe
Cells studied: 102
Reporting Date: 05/03/2016

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal





'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

CHROMOSOME ANALYSIS IN WHOLE BLOOD

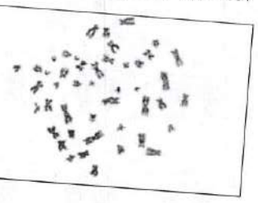
Area 1

Clinical History: Mental retardation, congenital deformities, severe mouth ulcer; brother has similar history, two elder sisters are normal
Sample: Whole Blood
Collection Date: 08/05/2014

Age/Sex: 14 Y/M
Method: Cell culture and G-banding
Culture Date: 09/05/2014

Parent's Exposure: Severe
Cells studied: 144
Reporting Date: 19/05/2014

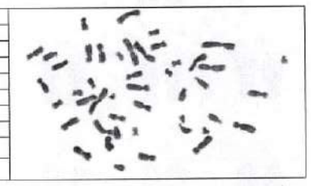
Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal

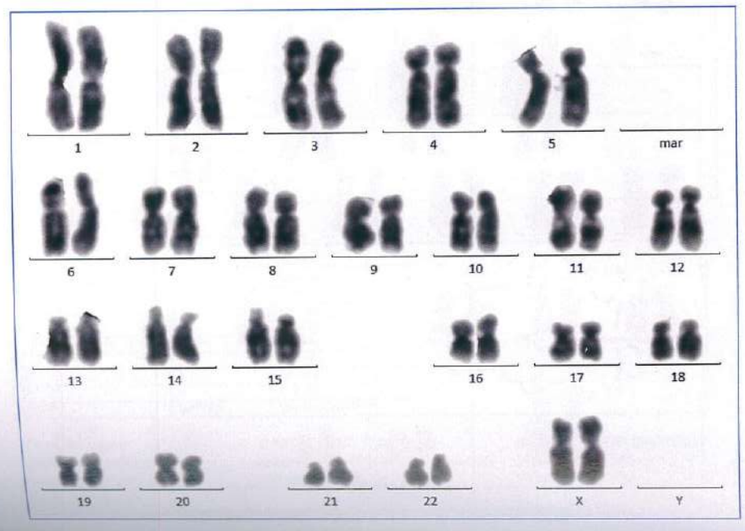


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Age/Sex: 14 Y/F **Parent's Exposure:** Severe
Clinical History: Subnormal intelligence, congenital deformities, gum hyperthrophy, Youger sister and brother do not have similar history
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied**: 58
Collection Date: 09/05/2014 **Culture Date**: 09/05/2014 **Reporting Date:** 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal

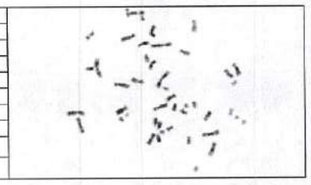


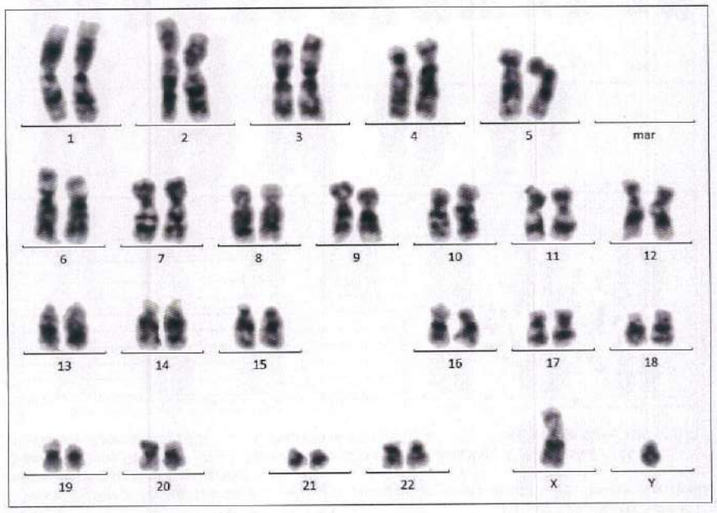


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 01/NR **Age/Sex:** 85 Y/M **Exposure:** Severe
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied**: 15
Collection Date: 08/05/2014 **Culture Date**: 09/05/2014 **Reporting Date:** 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Small Y
Karyotype	46,XY,small Y	Small Y

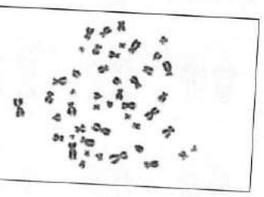


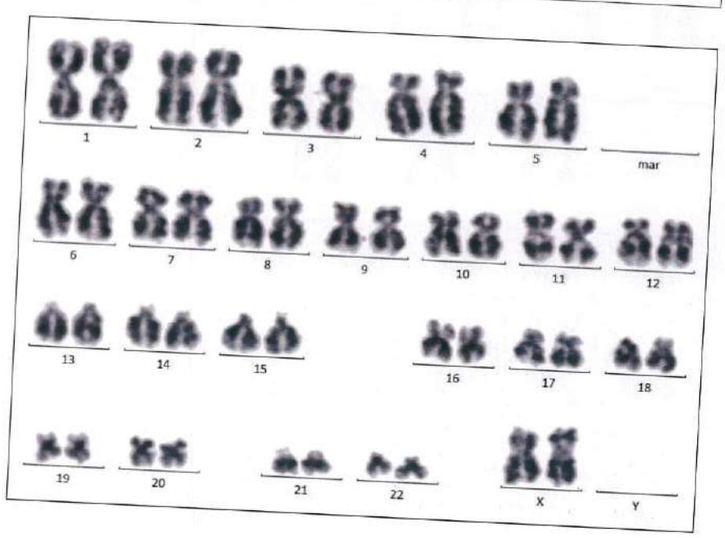


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area: 01 **Age/Sex:** Y/F **Parent's Exposure:** Severe
Clinical History: Two younger brothers have mental retardation and congenital malformation
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied**: 71
Collection Date: 08/05/2014 **Culture Date**: 09/05/2014 **Reporting Date:** 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	15p+
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX,15p+	15p+

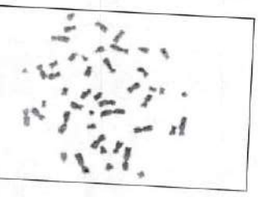


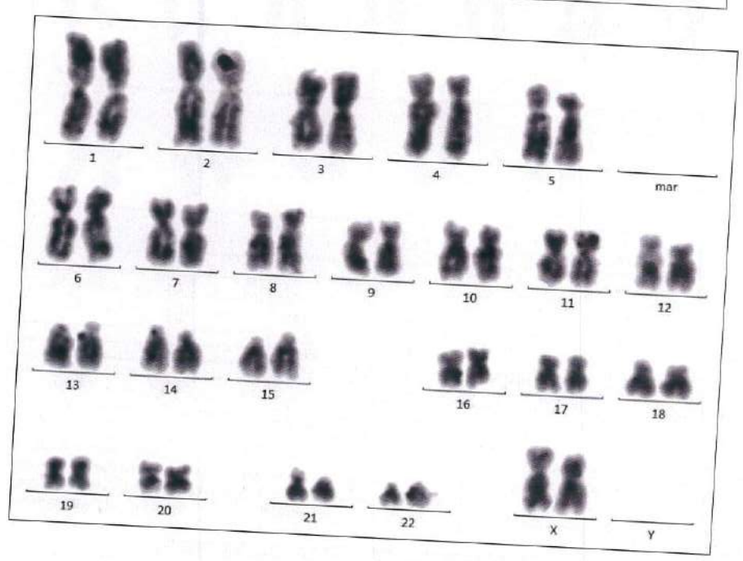


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area: 1 ICMR No: NR **Age/Sex:** 60 Y/F **Exposure:** Severe
Family history: Two male grandsons (sons of Sanjay) have mental retardation and congenital anomaly
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied**: 6
Collection Date: 08/05/2014 **Culture Date**: 09/05/2014 **Reporting Date:** 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal

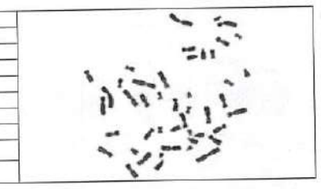


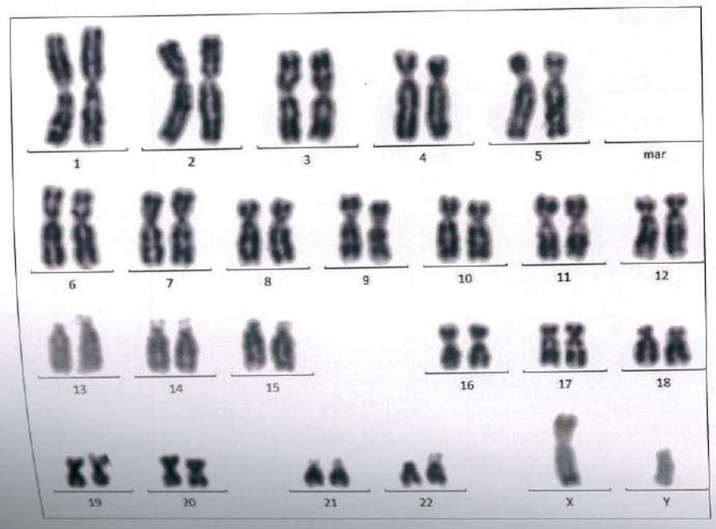


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area: I **Age/Sex:** 65 Y/M **Exposure:** Severe
Family history: Two male grandsons (sons of Sanjay) have mental retardation and congenital anomaly
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied**: 78
Collection Date: 08/05/2014 **Culture Date**: 09/05/2014 **Reporting Date:** 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal





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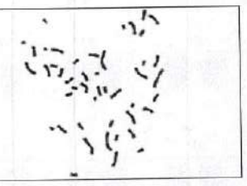
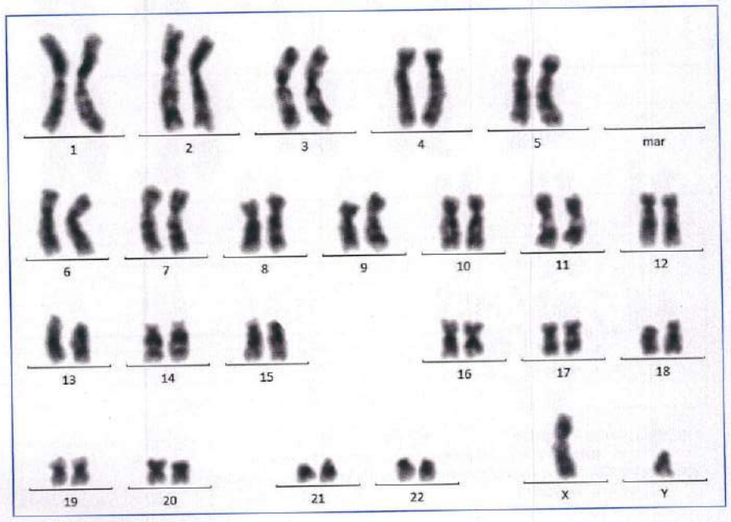
CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.: 1/NR **Age/Sex:** 40 Y/M **Exposure:** Severe
Family history: Two sons have mental retardation and congenital anomaly
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied**: 23
Collection Date: 08/05/2014 **Culture Date**: 09/05/2014 **Reporting Date:** 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal





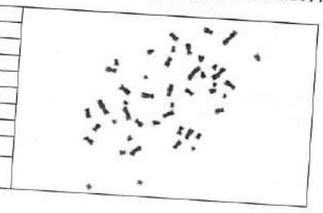
PI: Dr. Bani Bandana Ganguly MGM New Bombay Hospital, Navi Mumbai

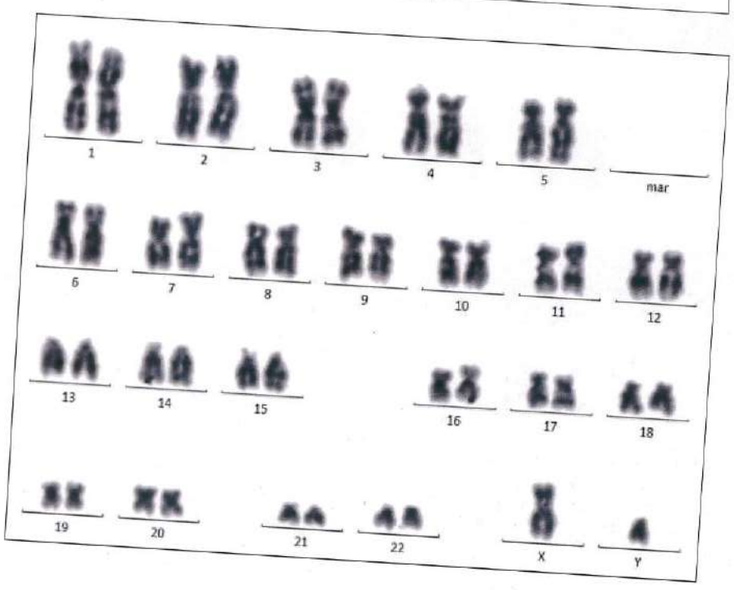
'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.:
Sample: Whole Blood **Age/Sex:** 40 Y/M **Exposure:** Severe
Collection Date: 08/05/2014 **Method:** Cell culture and G-banding **Cells studied**: 23
Culture Date: 09/05/2014 **Reporting Date:** 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY	Normal



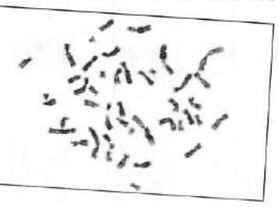


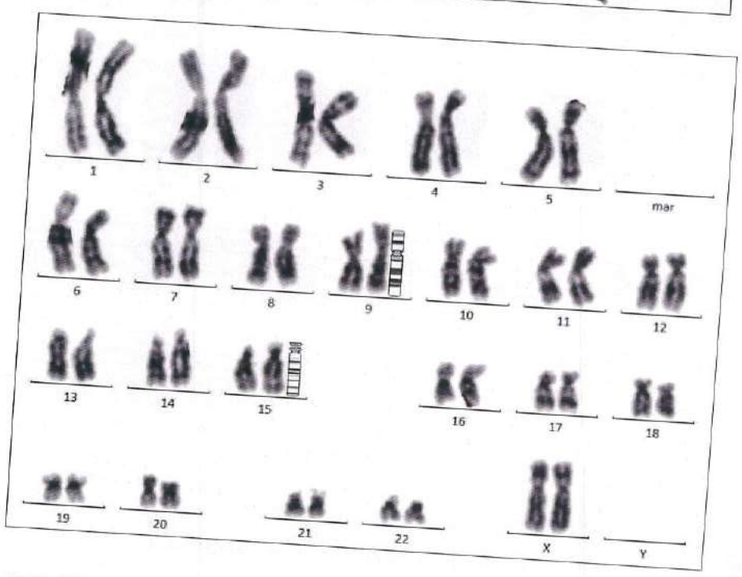
'Long term genetic effect(s) of MIC gas, if any, on the Bhopal Population exposed in December 1984'No. ICMR-65/BBG-1/NCD-II & NIREH/IMP/BBG/2013/01

CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area/ICMR No.:1/NR **Age/Sex:** 40 Y/F **Exposure:** Severe
Family history: Two sons have mental retardation and congenital anomaly
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied**: 80
Collection Date: 08/05/2014 **Culture Date**: 09/05/2014 **Reporting Date:** 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	9qh+
D	6	15p+
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX,9qh+,15p+	9qh+,15p+



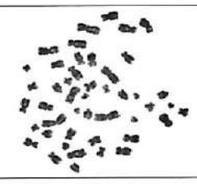


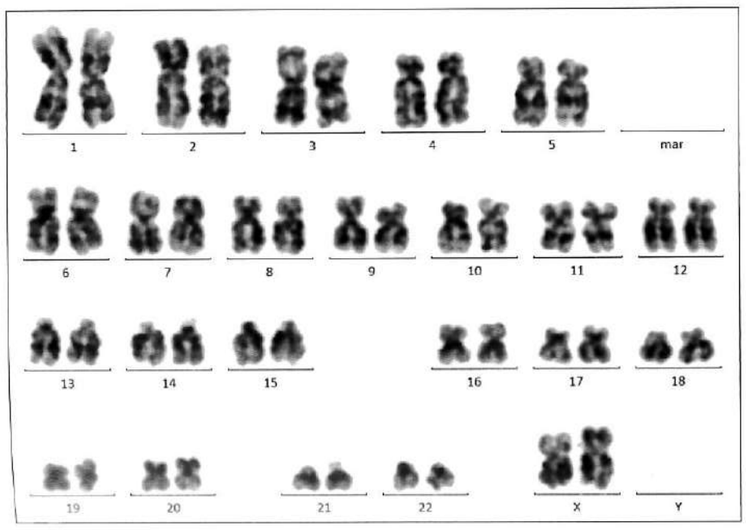
PI: Dr. Bani Bandana Ganguly MGM New Bombay Hospital, Navi Mumbai

CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area: 1/ **Age/Sex:** 15 Y/F **Parent's Exposure:** Severe
Clinical History: Two younger brothers have mental retardation and congenital malformation
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied**: 106
Collection Date: 08/05/2014 **Culture Date**: 09/05/2014 **Reporting Date:** 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	Normal
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XX	Normal

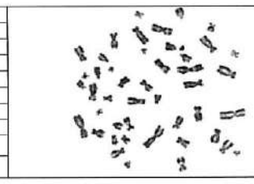


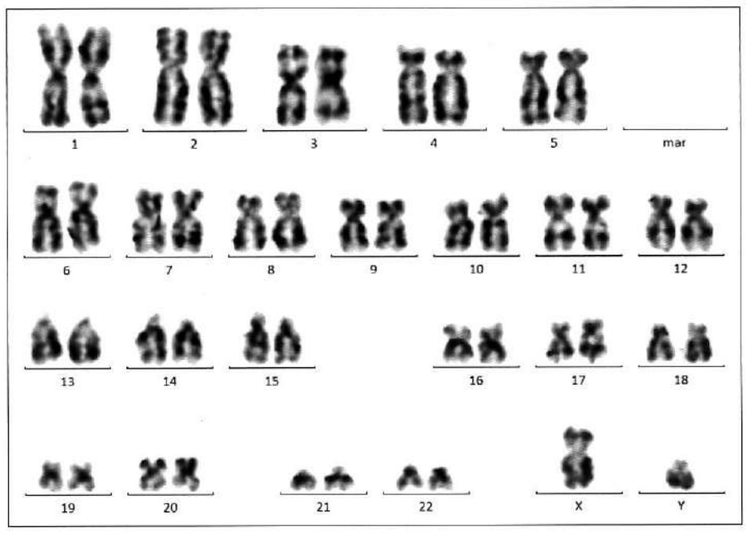


CHROMOSOME ANALYSIS IN WHOLE BLOOD

Area 1 **Age/Sex:** 15 Y/M **Parent's Exposure:** Severe
Clinical History: Mental retardation, congenital deformities, severe mouth ulcer; brother has similar history, two elder sisters are normal
Sample: Whole Blood **Method:** Cell culture and G-banding **Cells studied**: 98
Collection Date: 08/05/2014 **Culture Date**: 09/05/2014 **Reporting Date:** 19/05/2014

Group	Number	Normal/Abnormal
A	6	Normal
B	4	Normal
C	14	Normal
D	6	15p+
E	6	Normal
F	4	Normal
G	4	Normal
Sex Chromosomes	2	Normal
Karyotype	46,XY,15p+	15p+





Published review on MIC

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Journal of
Environmental & Analytical Toxicology

Ganguly et al., J Environ Anal Toxicol 2017, 7:3
DOI: 10.4172/2161-0525.1000452

Research Article

OMICS International

Genotoxic and Carcinogenic Effects of Methyl Isocyanate (MIC) Reviewed on Exposed Bhopal Population and Future Perspectives for Assessment of Long-Term MIC-Effect

Bani Bandana Ganguly[1*], Shouvik Mandal[1] and Nitin N Kadam[2]

[1]*MGM Center for Genetic Research & Diagnosis, MGM New Bombay Hospital, Navi Mumbai, India*
[2]*Department of Pediatrics, MGM Medical College, Navi Mumbai, India*

Abstract

MIC disaster has been established as one of the largest industrial disasters which claimed>10000 lives and seriously jeopardized lives of millions. Besides acute illness and multi-system complications, genetic damage at chromosomal level was indicative of long-term illness as demonstrated in individual reports. Presence of self-replicating minutes detected after 1114 days indicated persistence of rearrangements in the exposed individuals. Among the systemic complications, respiratory, ophthalmic and reproductive systems were significantly affected then and now. Some of the females with elevated chromosome abnormalities had history of fetal loss and high incidence of perinatal and neonatal mortality. Several *in vivo* and *in vitro* experiments concluded that MIC may exert geno-toxicity by binding of carbamoylating agents to nuclear proteins. The cancer-incidence among the MIC-exposed survivors is calculated from hospital records. Moreover, continuous soil contamination by multiple of chemical wastes in the site might have augmented the genetic changes through interaction with other biologic and a-biologic factors. Owing to variable latency period of chemicals, and also unavailability of genetic information measured in stratified cohorts immediately after the disaster, it is worthy of screening genetic condition in the exposed survivors and their progenies, though 32 years have gone. This review further pays importance to compounding effects of multiple confounding variables on the exposed individuals. Nevertheless, comparison of a current genetic screening with the previous genetic condition would actually discuss about the long-term genotoxic effect of MIC on Bhopal population; however, such exercise would not be a straightforward approach due to interaction of several confounders.

Keywords: Methyl isocyanate (MIC) disaster in Bhopal; Systemic complications; Genetic alterations; Cancer incidence; Future perspectives

Introduction

"Who is interested about the old disasters? Anyone who works in the process safety field" answered by Ronald J Willey [1]. He stated "as if time has stood still since 1984... all of the safety systems are not effective management is not alert enough… prevention of victims of course, but also focus on victims after the event. That is an important responsibility that must be taken seriously". Methyl isocyanate (MIC) gas-leak from Union Carbide India Limited (UCIL) factory on 2-3 December 1984 in Bhopal, India has claimed to be the world's worst industrial disaster for killing over 10000 people in its immediate aftermath and causing multi-systemic injuries to over 500000 people for decades [2]. Introduction of 500 liter water into MIC storage tank no. E610 resulted in uncontrolled exothermic reaction with release of >41 ton of MIC into the atmospheric environment in the midnight of December 2, 1984. The safety system was on stand-by (a caustic scrubbing tower) or out-of-service (a flare tower). Thus the mitigation of external release of MIC failed allowing expulsion of MIC 35 meters from ground level for ~15-30 minutes. In that winter night the atmospheric temperature was ~8-10°C with the thick winter-fog settled at ground level. The gas cloud infiltrated the surrounding densely populated residential areas of poor Shanties and flew towards the city center located to the south of UCIL at a wind-speed of 10-12km/hr. The direction of gas-spread was largely followed the direction of atmospheric wind in south west direction. People were asleep, wrapped in quilts in closed rooms; however, woke up with severe breathlessness and coughing, and acute burning of eyes, and tumbled out of their houses where a dense cloud of more gas killed many people instantly by blocking the airway passage. The survivors suffered with eye opacity, burning and watering; bleeding through mouth, eyes and ears; chronic breathlessness and coughing; and multiple systemic and psychiatric complications [2]. In fact 'lucky were those who died immediately', and thus that night were 'fateful' for the deceased. A cohort of 82,021 individuals in 36 municipal wards were classified with severe, moderate and mild exposure and another cohort of 15,931 people from unexposed areas were registered for conducting survey of health effects followed by MIC exposure. Much of the discussion on the disaster has been reported elsewhere [3-7]. Most of the information on the medical consequences of the Union Carbide disaster in Bhopal has been generated by the Indian Council of Medical Research (ICMR) [2].

Several safety flaws were demonstrated to be the cause of the accident. The management decisions on saving operating expenses had led the scrubber system on stand-by, flare system defunct and removal of coolant from the refrigeration of MIC storage tank, besides inadequate emergency management due mainly to lack of knowledge on potential toxic impact of MIC on the human health [8]. MIC was used as an ingredient for manufacturing pesticides in this Bhopal-based plant. There was no data available on the extent of its toxicity except a publication of 1964, and no antidote provided by the agency for accident management and/or occupational safety measure [9]. In fact the gas

***Corresponding author:** Bani Bandana Ganguly, MGM Center for Genetic Research & Diagnosis, MGM New Bombay Hospital, Vashi Sector 3, Navi Mumbai 400703, India, **Tel:** 91 22 61526527; **E-mail:** mgmgeneticlab@yahoo.com, bani.b.ganguly@gmail.com

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released was not just MIC alone, but a number of by-products generated such as hydrogen cyanide, nitrogen oxides, carbon monoxide, phosgene, mono-methylamine and many other contaminants through exothermic reaction with water and atmospheric air and moisture. Exothermic reaction of MIC leads to its degradation and conversion to hydrogen cyanide (HCN) at ~200°C, which was evidenced in cherry red color in blood and in the viscera of some of the victims indicating acute cyanide poisoning [2].

Bhopal Gas Disaster was totally a new experience for the entire medical community. As no specific antidote to the toxic gases was known, the management was totally on empirical basis, though many survivors responded well to sodium thiosulphate treatment, an effective antagonist for cyanide poisoning but not for MIC [10], which was initially recommended by Union Carbide Corporation (UCC) but denied later. As 62.6% of the total population was affected, it was expected that a large number of survivors would suffer from multisystem morbidity. Total lack of knowledge regarding the biological effects of the offending chemical raised several pertinent questions concerning long term effects and genetic defects in children born to exposed mother. Ironically, there was no prior awareness or information regarding MIC toxicity neither with the public nor with the authorities who should have known about this. The preliminary experimental toxicity study carried out by Kimmerle and Eben [11] on 4 human volunteers exposed for 1-5 min revealed: 0.02 ppm–no symptoms; 2-4 ppm–increased irritation of eyes, lacrimation, cough, chest pain, dyspnea; and 21 ppm–unbearable symptoms. A very similar pattern of symptoms was seen in the exposed population [11].

Initial autopsy revealed a characteristic cherry red discoloration of lungs alongside massive pulmonary edema, emphysema, hemorrhages, visceral congestion, cerebral edema and anoxic brain damage as acute effect of gas exposure [2]. The sub-acute phase was characterized by persistent morbidities caused by inhalation of gas amongst survivors of acute phase. The chronic effect was documented by a large number of study groups initiated by government and non-government organizations *in vivo* and in *vitro* in different organisms across the world [9]. Survivors continue to experience high incidence of reported health problems including febrile illnesses, respiratory, neurologic, psychiatric and ophthalmic symptoms. Clinical studies have shown chronic illness including breathlessness, pulmonary fibrosis, bronchial asthma, chronic obstructive pulmonary disease, recurrent chest infections, keratopathy and corneal opacities in exposed cohorts. Loss of appetite, menstrual irregularities, recurrent fever, persistent cough, neurological disorders, fatigue, weakness, anxiety and depression persisted among the most common symptoms. The systemic complications in acute and chronic phase among MIC-exposed population and in experimental animals have been summarized in Table 1.

In a preliminary epidemiological survey of the effect of the Bhopal accident on pregnant women living adjacent to the facility, Varma [12,13] reported a very high rate of unsuccessful pregnancies and a higher than normal infant mortality during the first 30 days of life. The data collected on 2566 pregnant and exposed women revealed a significant amount of pregnancy loss (18%), including 373 (15%) abortions and 82 (3%) stillbirths. The mothers (no.30) who were exposed in the first trimester of their pregnancy delivered with congenital malformations [2,14]. A larger study involving 18,978 exposed and 13,539 unexposed households presented a manifold increase in perinatal and neonatal mortality rates in women who had last menstrual period (LMP) before November 18, 1984 [3]. *In vivo* animal experiments also showed similar pregnancy outcome with fetal loss and intrauterine growth retardation (IUGR) [15-17]. Short exposure of pregnant mice to near let concentrations caused the loss of total litters, and longer exposures lower concentrations of MIC resulted in decreased litter size at bir and a higher rate of neonatal mortality [15]. MIC-exposure relat maternal hormonal changes and hypoxia, and teratogenic and fetoto effects of MIC were the causes of fetal and neonatal malformatic [18]. However, more detailed investigation was recommended on t population as well as *in vitro* to conclude whether the effect was due selective toxic effect of MIC on the fetus, or a manifestation of materi toxicity since evidence of significant teratogenicity was not found, n did there appear to be effects on fertility of exposed mice.

Following MIC-accident, extensive investigation was carried o in different living systems on different parameters and published Environmental Health Perspectives [19]. The reactivity of isocyana with specific functional groups on proteins was extensively examin by Brown et al. [20]. The reactions of MIC with non-essential functio groups and the highly exothermic hydrolysis reaction would both act compete with an interaction with specific enzymes or proteins duri inhalation exposures. However, the modification of functional grou can apparently lead to antibody-formation as shown by Karol and c workers in experimental animals and in survivors of the Bhopal accide [21]. However, the significance of these findings in relation to the over health effects of MIC exposure will require further study.

In summary, the principal findings of the ICMR was document as the toxins from UC factory have crossed into the blood stream those exposed and have caused damage to the lungs, brain, kidne muscles as well as gastro-intestinal, reproductive and immune syste [2]. The toxins have also crossed the placental barrier leading to fe poisoning [22]. Of the patients treated at the Hamidia hospital, Bho immediately after the disaster, 98.9% suffered breathlessness, 85. had eye problems, 91% had gastro-intestinal problems, and in varyi degrees, there were at least 16 other complications involving ot organs. More than ten of these symptoms persisted among the survi till as late as 1992 (when the last ICMR report was published) w the addition of menstrual irregularities, spontaneous abortions a neurological and mental health problems [2,23]. Six-monthly morbid surveys from 1987 to 1991 demonstrated an increasing trend of number of people with exposure-related symptoms. According to study there were three times more people with respiratory symptoms 1991 as compared to 1987 [2].

Genetic Damage in The Gas-Victims

Following MIC-exposure, genetic damage in Bhopal populati was a serious concern. Genetic alteration could increase cancer-risk a multi-factorial morbidities, disturb immune system, and physical a mental retardation in offspring of the next generation. A multicen program was conducted by Indian Council of Medical Resea (ICMR) for screening of genetic damage in MIC-exposed populati which unfortunately has not been reported yet to the public but lik to be available soon. All genetic studies were carried out in periphe lymphocytes with a view to evaluating MIC-induced chromosor alterations as guided by EHC [24,25]. The author had studied over 1 individuals from different distances from UCIL plant from which excerpt of the data on 129 individuals was reported [26].

Chromosome aberrations (CA)

Genetic damage in human lymphocytes was measured on M exposed population immediately after the accident by Goswami [Deo et al. [28], Saxena et al. [29] and later by Ghosh et al. [26]. Goswa

Table 1: Systemic complications in MIC-exposed Bhopal population: Acute and chronic phases.

	Respiratory	Ophthalmic	Skin	GI	Neuro/psycho	Reproductive
Immediate: Acute	+++ (>98%) Acute breathlessness/cough/chest pain/ chocking. Immediate chest radiographs highlighted alveolar lesions. Obstructive airway passage, progression of lesions in lungs, necrotizing lesions affecting bronchioles/alveoli, capillaries with excessive fluid flow into alveoli. Interstitial fibrosis and bronchiolitis obliterans, necrotic epithelial debris in alveoli and bronchioles were predominant in autopsies. 55% elevated levels of Carboxyhaemoglobin, and Methaemoglobin.	+++ (>98%) Lacrimation, photophobia, profuse lid oedema, corneal ulcerations, foreign body sensation, chilli burning, and blurring of the vision	+ Dermal sensitization	++ 92% loss of appetite, 52% nausea, 82% vomiting, 80% techypnoea, 54% tachycardia, 2% had fever.	+++ (>98%) Weakness, tremors, tetany, hypersomnolence, neurotic depression, anxiety. Children born to exposed parents appeared disobedient/stubborn (4.36%), tremor tentrum (4.36%), mental retardation (2.3%) and delayed/poor development of speech, lower intellectual levels; exposed children suffered from mental health	Irregular menstrual cycle, leucorrhoea and dysmenorrhoea. ~44-48% did not deliver a live child, miscarriage at first (58.8%), second (42.1%) or third (40.1%) trimester
Long term: Chronic	++ 3-year post disaster lung biopsy revealed bronchiolitis obliterans in fairly large sized terminal bronchioles, subpleural/septal fibrosis, interstitial aggregates, compensatory emphysema, bronchiolitis and peribronchial and perivascular fibrosis. Broncho-alveolar lavage study revealed alveolar macrophages and neutrophils in lower respiratory tract (highly prevalent among smokers) along with elevated levels of fibronectin indicating fibrosis of lung parenchyma and bronchial asthma, and hypersensitivity to pneumonitis.	++ Photophobia, conjunctival and circumcorneal congestion with relatively little oedema, corneal ulcer, cataract (polychromatic lusture), burning and watering sensation, sign of red eye, superficial interpalpabral erosions, corneal opacity, discharge and fundal changes	+ Not detailed	+ Renal failure/ Chronic kidney disease increased significantly (? excessive use of drugs by gas exposed population for their morbidities)	++ 75% psychiatric morbidities: muscle weakness, poor memory, neurotic depression, emotional disturbance visual perceptual and attention/response speed along with attention/vigilance were severely affected; cognitive impairment; traumatic stress disorder, pathological grief reaction, emotional reaction to physical problems; depressive neurosis. In 1994, epidemiological and clinical studies conducted by International Medical Commission reported: 7.27% suffered with current post traumatic stress disorder and 15.19% suffered with life time post traumatic stress disorder.	Fetal loss 26.3% compared to 7.8% in control; 5-6 times higher infant mortality within 30 days of birth; increased perinatal and neonatal mortality studied in 18, 978 households : 12.1% died within 30 days; 10% died at <5yrs with high morbodity of 200 children evaluated in 1990 of 273 surviving children born to exposed parents.
Experimental animals	F334/N rats and B6C3F1 mice exposed to 30 ppm of MIC developed obstructive lung disease, peribronchial and intra luminal fibrosis, fibrosis of major bronchi, acute to chronic bronchitis, chronic alveolitis, atelectasis and obstructive airway lesion	Cataract formation in young rat lenses incubated in 50 mmol/L MIC; no irreversible damage in Fischer rats exposed to 3-30 ppm for 2 hrs	Not detailed	Not detailed	Ischemia of rat brain leading to cerebral hypoxia; growth of muscle fibers and death of fibroblast and myoblasts in rats; higher doses caused muscle aches and repeated episodes of extreme muscular weakness; persistent muscle weakness, lasting from 1-60 months following exposure to CO	2-5 ppm exposure reduced foetal and placental weight; complete loss of all fetuses in mice exposed to 9 and 15 ppm for 3 h; 0, 1, 3 ppm/6 hrs/day during 14-17d gestation lowered survival in Swiss (CD-1) mice; inhalation of 1.3 ppm/6 hrs/day for 4 days to mice of both sexes revealed no effect on mating/fertility after 1/ 8/17 weeks post exposure

ported a higher frequency of chromosome aberrations (CA) and sister hromatid exchanges (SCEs) in MIC-exposed individuals compared to ontrols [27]. SCE frequencies were tripled in MIC-exposed persons n that study. The result was compared to two groups of controls (one roup comprising persons present in the same house; the second group persons were chosen from distant places, 20-50 km away from the cident-site. Chromosomal breaks have been observed in 10 out of 14 IC-affected people (71.4%) studied while only 6 out of 28 (21.4%) ntrols had chromosomal aberrations. Some MIC-exposed persons d chromatin bodies in addition to the normal 46 chromosomes. ese observations suggest that chromosomal DNA has been damaged. gnificant degree of chromosomal aberrations was reported two d half months post disaster, however, no increase in chromosome errations was reported by other studies [27].

Deo et al. also reported increased chromosomal aberrations MIC-exposed cases when compared to controls obtained from umbai, a different socio-economic stratum [28]. Ghosh et al. [26] assessed frequencies of CA, SCE, and replicative index following peripheral blood lymphocyte culture in 129 individuals (83 exposed: 40 male and 43 female, and 46 unexposed from Bhopal) from Bhopal, 1114 days after the disaster. CA was recorded in first cycle metaphases (M1) and SCEs at second mitotic cycle (M2), following standard phytohemagglutinin (PHA)-stimulated blood culture protocol [30]. The frequency of CA, in general, was higher in individuals from the exposed populations, and that was predominant in females. Non-disjunction of chromosomes or laggard was rare resulting in absence of aneuploidy. The persistence of CA in the form of replicating double minutes and exchange configurations, even 1114 days post-exposure to MIC, indicated a residual effect on T-cell precursors [26]. None of them had been exposed to any other known clastogenic agents except a single chest X-ray. Majority of the recruited females were house-wives and the males were day-labors. Because the lesions induced by chemicals are mostly S-dependent for expression in subsequent divisional cycle, the damaged T-lymphocytes may remain circulating

for long periods, and these aberrations can be observed only if the cells are stimulated to divide *in vitro* [25]. Such findings on CA suggest that the future generations of the survivors might possibly carry the effects of the leftovers of the industrial toxins. Therefore, it is evidenced that MIC can establish some genetic effect on T-cell precursors; however, the knowledge about the extent of genetic alterations and their clinical impact is very meager. The spectrum of SCEs in Ghosh et al. [26] study could not be correlated to exposure status or sex, which was in agreement with others [28]. However, elevated SCEs were reported by Goswami [27] and Mason et al. [31]. Depression of mitotic (MI) and replicative indices (RI) in Ghosh et al. [26] study was not consistent with MIC-exposure in Bhopal population, which was consistent with Conner et al. [32] in murine alveolar macrophages. However, delayed cell cycle was reported by Deo et al. [28] in peripheral lymphocytes of the MIC-exposed people, and also in experimental animals [33].

An extremely important concern was the possibility that MIC exposure might lead to genetic damage in the MIC-exposed Bhopal population. MIC was tested for genotoxic potential in a variety of *in vitro* and *in vivo* assays by Shelby and other groups [31, 34, 35]. They have reported negative results in Salmonella/mammalian microsome assay in five bacterial strains in a pre-incubation protocol, and Drosophila sex linked recessive lethal test. Mutagenic expression was also tested in urine of the exposed individuals [28], which did not reveal any mutation. Reproducible dose related increase in both SCE and chromosome aberrations were reported in Chinese Hamster Ovary (CHO) cells with or without metabolic activation *in vitro* [35]. In mice, a single 2 h-exposure to concentrations upto 30 ppm by inhalation didn't induce chromosome aberration in bone marrow; however, cell cycle was delayed significantly. In other experiments involving exposure on 4 consecutive days to a maximum dose of 6 ppm resulted in significant delay in cell cycle and increased SCE and chromosome aberration in bone marrow cells, and a dose-dependent increase in SCE in cultured lung cells from mice exposed to 1, 3, or 6 ppm MIC but not in peripheral blood lymphocytes of mice exposed to concentrations as high as 6 ppm [34]. When micronuclei (MN) was considered as indicator of genotoxic potential of MIC, a significant dose-dependent depression of polychromatic erythrocytes was demonstrated but no increase in frequencies of micronucleated polychromatic erythrocytes and micronucleated normochromatic erythrocytes were reported in bone marrow and peripheral blood samples of mice [36]. No micronucleus was observed in 7 and 14 days post-exposure in bone marrow of rats exposed to MIC by inhalation. However, a significant increase in micronucleated polychromatic erythrocytes was observed in peripheral blood of male mice in one experiment. Genotoxic/cytotoxic effect was evidenced through depression in bone marrow-cellularity in animals exposed by inhalation [33]. Genotoxic response of MIC-modified DNA described decrease in plaque formation in *E. coli*. [37]. Collectively, *in vivo* and *in vitro* geno-toxicity studies concluded that MIC has clastogenic potential.

The studies concluded that MIC may exert genetic toxicity by binding of carbamoylating agents to nuclear proteins. From these results and from considerations of the chemistry of isocyanate-DNA and isocyanate-protein reactions, it was speculated that MIC may exert its genotoxic activity through interactions with proteins affecting chromosomal structure, rather than through direct genetic mutations.

Plausible artifacts were indicated as MIC-induced CA: undue overestimation: Genetic damage in Bhopal-exposed population at chromosomal level was first published by Goswami [27]; however, the figures indicated as aberrations were purely artifacts. In fact, the metaphases were scratched due to unsafe handling of glass sli Deo et al. [28] conducted chromosomal analysis on people admit in Hamidia Hospital in Bhopal and from Railway colony within weeks after exposure where controls (very few) were selected mo from Mumbai, a different socioeconomic group. The data documen was not uniformly collected with number of cells ranging from 6-2 which indicated inconsistency in culture outcome. Mean data poorly documented with high standard deviation. The paper has addressed the possible consequences at cellular or genetic level. I later publication by Goswami et al. [38], the result was highly critici by scientific fraternity and recommended withdrawal from the jour [39]. The figures showing Robertsonian translocations are sim association of acrocentric chromosomes, and the metaphase w arrowed chromosomes are not showing any deletion. Chromoso identification was not correct as one chromosome #11 was labeled chromosome #5. Giemsa banding was of substandard quality and appreciable for chromosome-classification. It is surprising that Human Genetics journal had reviewed and published the articles Goswami [27,38]. Another publication by Ghosh et al. [26] documen chromosomal aberrations including self-replicable double minu breaks, gaps, dicentrics, rings and tri- and quadriradial chroma exchanges etc., and SCEs on 129 MIC-exposed individuals 1114 d post exposure, which were more pronounced in females. Mishra al. [40] reported genomic instability in human colon epithelial-F cells exposed to MIC *in vitro*. However, G-banded karyotypic analy appears to be very poor with a possibility of false positive results chromosome with overlapped chromatids was described as a dicent chromosome by Mishra and his group [40].

In all, the data on abnormalities was collected following sol staining, wherein G-banding would have facilitated recognition translocations and other stable rearrangements (inversions, deletio duplications, etc). Nevertheless, presence of self-replicating minu after 1114 days of the incident in Ghosh et al. report may indicate of transmissible and heritable rearrangements in the population [26

To address the long-term genotoxic effects among the survivo Malla et al. [41] reported the frequency and pattern of chromoso instability through conventional chromosome aberration as in the peripheral blood of exposed individuals to unveil the lo term genotoxicity of the exposure. Considering fragments sol as chromosome type and terminal deletions as chromatid t aberrations raise question on the accuracy of the data collected a interpretation drawn on the result, which could have been suppor partly by figures or images of aberrant cells; however, no figures h been included for understanding the classification. Discussion preponderance of aberrations in males and the result on pedigrees also missing completely. However, their result collected during 20 2011 has indicated long-term genetic effects induced by MIC-expos in 1984 in 100% of exposed cases studied. The prevalence of gen abnormalities in 100% cases after 27 years from the disaster has not b discussed with sufficient information. The same group, after report chromosome data, has reported micronuclei frequency in MIC expo population as an indicator of cancer incidence; however, the report not stated the incidence of cancer in their hospital registry [42].

There was a great deal of variation in study design, sample selecti selection of assay systems and parameters, and most importantly of validated protocol and interpretative comments of the result. MIC disaster had actually sensitized the global scientific fraternity estimation of MIC-toxicity and associated health-risk. The data collec on exposed individuals did not follow any harmonized protocol

study design and assay system, and also for eliminating the impact of the confounders. Although, a multi-center genetic investigation was carried out by ICMR following unified protocol, the result has not been made public till date. However, after three decades, some of the records have been retrieved by the author, which are presently under analysis for reporting, and will be published shortly. It is noteworthy that the author was one of the participants of that genetic survey program.

Carcinogenic potential of MIC

Carcinogenic potential of MIC in exposed individuals were documented by a number of institutions [43,44]. Analysis of a population-based cancer registry in Bhopal revealed a relative cancer risk of 1.4, 1.3 and 0.7 for lung, oropharynx and buccal cavity respectively, after adjustment with age and tobacco consumption. ICMR's cancer registry reported higher incidence of tongue, mouth, hypopharynx, oesophagus and lung cancer in affected areas compared to unaffected ones; however, adjustment of tobacco consumption neutralized the cancer incidence between the exposed and unexposed groups. ICMR's conclusion could not resolve the issue of tobacco consumption in unexposed areas. Several broadcasting media stated that cancer cases in Bhopal have more than tripled among men and more than doubled among women between 1988 and 2007. A study conducted at Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal also demonstrated an increase in the incidence of cancer in the exposed population. However, correlation with the life-style and other biological factors may reduce the risk of malignancy induced solely by MIC-exposure decades after the exposure. Nevertheless, MIC has not been classified as carcinogen by International Agency for Research in Cancer (IARC) and US-Environment Protection Agency (US-EPA) [45-47].

Cancer is a multi-step process of acquisition of somatic mutations in stem cells, and that may not be caused solely by one mutation. Rather interaction of several co-operating mutations may facilitate onset to progression of a cancer, which may happen over a period of time through clonal development and expansion [48-50]. To discuss about the carcinogenic incidence in MIC-exposed population, well defined/designed survey is essential for the affected cases to capture the interactive effects of their demographic variation, life-style, nutrition, living and work-environment, source of drinking water, and so on. Also, lack of information on baseline pre-MIC-disaster cancer prevalence in the marked municipal wards or Bhopal as a whole does not allow talking about MIC-related cancer prevalence without discussing their clinical and laboratory records. Investigation on founder and driver mutations for the most prevalent cancer in MIC-exposed Bhopal population and its correlation with other published reports could describe the MIC-related changes. However, time-gap after the disaster would certainly introduce some error due to interaction of multiple confounding factors. The reported cancer incidence did not charter about the age of onset and presence of co-morbidities since the data has mostly been retrieved from hospital records [44]. Thus, targeted screening of precancerous lesions would be meaningful for the common organs affected such as head & neck cancer as projected in consumers of tobacco. Personal interaction with affected cases could extract some information about the preponderance of cancer in MIC-exposed population of Bhopal.

Immunotoxicity of MIC

A particular concern immediately following the accident in Bhopal was the fear that the exposed population would be unduly susceptible to disease and would suffer disproportionate morbidity from a variety of infectious agents. The immunotoxicity studies of Tucker and co-workers reported minor deficits in T-cell lymphoproliferative response and more susceptibility to influenza challenge in MIC-exposed mice [51]. Evaluation of the cellular and humoral immunity in MIC-exposed individuals had presented increased T cells and T_H cell population and normal range of B cells and CD8, 4-8 weeks post exposure [28]. Significant depression in phagocytosis and T-cell rosettes were reported after 10 weeks, which indicated suppressed cell-mediated immune response among exposed individuals [29]. Reduced response to T and B cell mitogens was reported in 71% of the exposed population. Low titers of IgG, IgM and IgE class of antibodies were noticed in 11% of the exposed cases [52]. MIC-specific antibodies were correlated with severity of lung damage [53]; though MIC didn't reveal any synergistic effect on humoral immunity in MIC-exposed protein deficient people [54]. In animal models, increased weight of liver and thymus, and impaired alveolar and peritoneal macrophage functions were described to be associated with suppression of phagocytosis of sheep erythrocytes resulting in susceptibility to bacterial *E. coli* endotoxin [55]. Resistance to other infections such as influenza virus, *Listeria monocytogenes* and mouse malaria parasite was not compromised in sheep [51]. MIC was negative in the male mouse dominant lethal assay [19]. Altogether, low-titer and transient antibody response was noticed in exposed human and animals [21].

In vitro and *in vivo* tests provide convincing evidence that MIC is capable of inducing chromosomal damage and that this genetic toxicity is not strongly expressed *in vivo*, perhaps because of the selective reactivity of MIC with proteins [19]. Experimental studies have provided mechanistic understanding of MIC-exposure at a molecular level. Immunotoxic implications, toxico-genomic effect, inflammatory response, elicitation of mitochondrial oxidative stress, chromosomal and microsatellite instability have been studied comprehensively in cultured mammalian cells [35]. Isocyanates are able to modulate bio-molecules, resulting in a series of bio-transformations [37,56,57], which in turn may affect health adversely, yet they have a wide array of industrial applications. MIC, a reactive byproduct, is detrimental to numerous organ and its functional systems. It forms DNA cross links/adducts by reacting with exocyclic amino group of dNTPs, in turn contributing to cytotoxicity [58,59]. MIC intermediates (N-methylcarbamate) are also toxic to cultured mammalian cells [53,60,61].

Srivastava et al. [22] stated that the long term genotoxic effects of MIC need further in-depth studies to design newer and more effective diagnostic and therapeutic strategies for helping the survivors. The paucity of data on the toxic effects of MIC led to contradictory pronouncement ranging from "no long term effects" to "effects that may last for generations to come". There is almost no current data on the prevalence of tuberculosis, cancers, infertility and birth defects among the exposed population, all of which are reported by doctors to be on the rise involved in treatment of the survivors. However, to review the long-term MIC-effect 32 years after disaster it is mandatory to consider the environment, source of drinking water, socio-economic changes, life-style, history of morbidity, demographic changes, etc. and correlation with genetic instability for extraction of the residual effect and its transmission through generations.

Long-Term Health Effects and Future Perspectives

Lesson learned from the past mistake witnessed in catastrophic Bhopal accident has cautioned the developing countries for implementation of safety regulations alongside concurrent industrial developments. The disaster in UCC's pesticide manufacturing unit in Bhopal demonstrated local problem of industrial pollution and hazards

are also linked to global market matrix. Three decades post-disaster, understanding the key issues of health-determinants in a complex scenario of living environment and life-style will direct sketching up policies and programs for strengthening and uplifting the children of the present and future generation [62,63]. The story of MIC-disaster and the related health index needs to be resolved. Apart from fragmented and unplanned studies, which failed to generate convincing report, lack of consolidated health reports on different key parameters, especially genetic alteration, incidence of cancer and cancer-related mortality, and child health and morbidity puts a responsibility to conclude the long-term effect of MIC on exposed population and their offspring. However, the issue is highly critical due to unavailability of pre-MIC baseline information on the population, and also complex due to interaction of life-style, exposure, nutrition, and ageing over a period of three decades. Nevertheless, individual genetic make-up and variable susceptibility and morbidity may contribute to a great degree of variation in health outcome. Moreover, there could be bias in recalling health status and reproductive outcome, especially miscarriages, over such a long period. Thus, for charting the present health condition of the victims and their descendants, some of the key issues must be considered. Collectively, health survey, including genetic changes, birth defects and cancer-related mortality shall be measured at least annually as implemented for Hiroshima-Nagasaki survivors [64].

Deficiencies in earlier reports and future direction

Approximately 600,000 persons were exposed to MIC in Bhopal, of which 30% are estimated to be suffering from long-term health effects. Despite inflammatory damage to the eyes and lungs contributing to chronic morbidity resulting in opacity and stressful respiration, pregnancy loss, birth defects and genetic damage reported in victims were of serious concern. For a disaster of this magnitude, there is a relative paucity of medical information, as very little information has been published on the late recovery period, a phase in which the detection of chronic and long-term effects would be vital.

During the early phase following the accident, the major priority was to provide medical management to the victims over planning of epidemiological studies. The studies conducted by ICMR or other institutions had failed to establish causal relationship owing to defects in study-design including validity and precision of exposure and outcome variables, selection of study and control groups, etc., resulting in a significant bias (case selection, recall, small sample size, etc). If exposure was classified based on location of residence, some degree of misclassification was expected because it was not known that the person was actually exposed or not. Address and coding errors would have increased such misclassification. Socioeconomic differences between areas might also have affected the cancer incidence. Mehta et al. [65] rightly mentioned that 'bias was pervasive and there was insufficient information to allow careful operational definition of crucial matters, such as criteria for inclusion and exclusion of controls, and effects of independent and dependent variables on study outcome'. Koplan et al. [66] suggested that epidemiologic studies following disasters should accurately establish dose-response relationship which would be useful in identifying exposed and ill persons and determining long-term effects. Selection of cohort was recommended by Betrazii [67] for epidemiological studies rather than use of a population registry to avoid two major biases: (1) dilution of exposure prevalence and (2) selective migration of people from the disaster area. Some of the Bhopal population might have left Bhopal forever immediately after the disaster, thus resulting in dilution of exposure prevalence in the selected cohort.

The issues of study design and analysis by stratification of st population, random selection of subjects, blinding of investiga to exposure status and use of personal exposure measure would h increased the accuracy of exposure estimation [68, 69]. Self repo data was expected to be more reliable to reconstruct the indivi exposure level. The authors concluded that despite the time elap since accident and the potential for recall bias; it might be poss to estimate individual exposure in the survivors with some degre accuracy. Use of exposure strata would be meaningful for samp in future epidemiologic investigation for MIC-community of Bho Exposure-stratified sampling techniques might provide valid estim of exposure-response without including the total exposed commu [6]. ICMR surveys indicated that the exposed population for most part was residing in their homes where they lived in at the t of exposure in 1984. However, this condition needs verification confirmation from the ration card, voter card, ICMR card num and personal-exposure history since in- and out-migration has crea mixing of population after the disaster. Exposure-based strati random sampling would also minimize bias that results from s selection and exposure misclassification, and it would enable d response and interaction relationships to be understood.

Passive attack from more chemicals dumped at UCIL-Bho site

Pre-disaster, several tons of obsolete pesticides and process wa of UCIL were contaminating the surroundings of the factory and str into open drains, which rendered Bhopal city a dangerous place to l Immediately after MIC-disaster, the Union Carbide factory was clo and pipes, drums and tanks were cleaned and sold, except the M Sevin plants and storages of different residues. The leftover load of M and other hazardous chemicals was dumped in the factory site, wh is contaminating soil and underground water till date. Chemic including naphthol, naphthalene, Sevin, alpha naphthol, tarry resi mercury, volatile organo-chlorine compounds, chromium, cop nickel, lead, chloroform, hexachloroethane, hexachlorobutadiene, the pesticide HCH (BHC) and volatile halo-organics are left in UCIL leading to continuous leakage resulting in pollution of the groundw through percolation. Studies made by Greenpeace and others f soil, groundwater, well-water and vegetables from the residential a around UCIL and from the factory site show contamination wi higher range of toxic heavy metals, solvents and a wide range of o chemical compounds. Among the hazardous waste in the plant soil, heavy metals such as mercury, chromium, copper, nickel, lea well as organochlorine compounds were persistent (Figure 1). M of these chemicals have been demonstrated as potent cancinog Also trichloroethylene is known to impair fetal development a times above safety limits specified by the United States Environme Protection Agency (EPA) [47]. Several broadcasting media repo that the site is contaminated with 'thousands' of metric tons of chemicals, held in open containers or loose on the ground.

Elevated levels of volatile chloroform, carbon tetrachloride chlorobenzenes were also demonstrated in drinking (undergro well) water [17]. UCC's laboratory tests in 1989 revealed that soil water samples collected from near the factory and inside the plant toxic to fish. The poor socio-economic group residing in the vici of the UCIL factory was dependent on underground water both drinking and other domestic uses. In 1991, the municipal author declared water from over 100 tube wells to be unsafe for drinking were abandoned. Well and groundwater in the surrounding areas te in 1999 showed mercury levels to be at "20,000 and 6 million ti

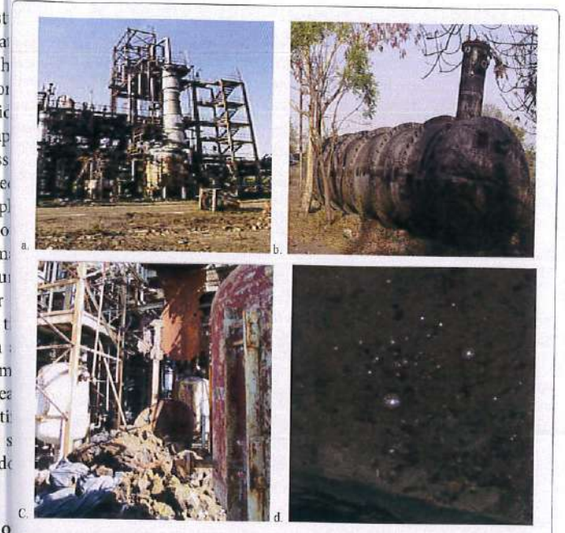

Figure 1: Post-disaster Union Carbide India Limited at Bhopal: a. UCIL factory; b. MIC-storage tank 610; c. left-over of unused chemicals; d. mercury droplets.

igher than expected levels. A sample of drinking water from a well ear the UCIL site had 500 times higher levels of contamination than he maximum limits recommended by the World Health Organization WHO). Many of these chemical contaminants were also found in reast milk. Underground water was the major source of irrigation or agriculture in the vicinity. Therefore, consumption of agricultural roduces in these affected areas might have doubled the burden f chemical consumption by the residents. In addition, use of and sposure to pesticides may interact with other toxic substances *in vivo* hysiological system among the gas-victims.

ssessment of present genetic condition

The interaction of biological and non-biological factors are of major ncern for adjustment of MIC effect and tracking the cause of present alth status 32 years post MIC-disaster. There are changes in the vironmental admixture and population composition due to in and out igration. At this moment it is difficult to ascertain the health effects to posure level of MIC in different individuals. Both in the environment well as in human body, it is apparent that MIC-effect has been diluted hrough natural and physiological reactions. Thus, it would be justified to nsider a second control (migrated) group who were not exposed to MIC-cident in 1984 but living in the same affected areas for 20-25 years, to timate the effects of MIC and other chemicals through soil and ground d well-water. Screening of this migrated control group will extract the fect of soil and water contaminations with leftover chemicals in the UCIL e and accurately adjust the long-term effect of MIC. Therefore, detail sidential and occupational history will be crucial for both MIC-exposed, exposed and migrated controls. Explicit information on residence nvironmental condition), health status, life-style and occupational story, including changes in work place, type of work, and occupational d environmental exposure to hazardous chemicals, gas or radiation shall recorded in time-scale of five-ten years interval.

A detailed health and outcome information could be collected from ree generations. Since 1984, there are two different types of families, such as

i. In 1984, if men of 25-30 years and women of 20-25 years (child bearing age) were exposed and survived, they are 55-60 years old and 50-55 years old respectively after 30 years, and they can have male and female children of 20-25 years and grandchildren of 1-5 years old (at least one).

ii. Individuals with exposure at pre-adolescent age (10-12 years) have become parents of 10-20 years old children after 30 years.

Pedigree chart will record whether both partners were exposed or one was from unexposed control zone or from different town, and if consanguinity was there. It is expected that there will be minimum of five members in each family, including parents of first generation (FI) who were exposed at child-bearing age, parents of second generation (FII) who were exposed in childhood, *in utero* or conceived after disaster-exposure and offspring of the third generation (FIII), at least one. However, all the parents of the two generations may not be alive, and there can be more than one member in FIII generation.

Potential confounders

Study of long term effect of MIC after 32 years of the disaster would encounter with a number of confounding factors, more prominently recall of health history since 1984, maintenance and retrieval of health records, history on life-style (tobacco consumption in smoking, chewing and other form), change of residence, change of job, socio-economic condition (especially nutrition status), source of water for domestic use, soil and water contamination with chemical wastes from Union Carbide factory, etc. The issue of socioeconomic condition on health status following MIC exposure would be a major confounder. Other obvious factors such as ambient air pollution, immune status, and psychological fear would also have some interaction on genetic consequences. Although, the local residents are currently living in concrete houses, there is hardly any window to allow fresh air for breathing inside (Figure 2). The open drains in the residential area are carrying the sewage of domestic excrete and small-scale cottage production. There is ample opportunity of contaminating the water pipes if fitted through and submerged in the drains. Recall of occupational history and occupational exposure to genotoxic agents would also be confounding significantly. Recall of pubertal development and reproductive outcome would be difficult for the FI parents. Age of the FI parents, maternal age at conception, number of conception, use of medicines since 1984, etc. would be potential confounders for health assessment of MIC-exposed population. Control of confounders shall consider the study design phase (randomization and sufficient sample size, uniform criteria/questionnaire for selection of study subjects and technical design for estimation of long-term genetic effects (dependent and independent variables). Further, stratification of data shall depend on aging, morbidity and their mutual interaction for leading to age-related disease-onset. Aging is a significant biological confounder 32 years post disaster for the MIC-exposed population. Aging is associated with increase in CA, SCE, MN and delayed cell cycle [70-73].

To some extent, the variables can be controlled on comparison of then genetic alterations with the current state. It is noteworthy that information of the multi-center genetic survey following the accident in 1984 has not been published. Retrieval of the records from the archive and analysis will definitely present scenario of genetic damage immediately post-exposure from different degrees of exposure. It is also worthy to mention that a similar exercise on conventional cytogenetic investigation on exposed and unexposed individuals is underway.

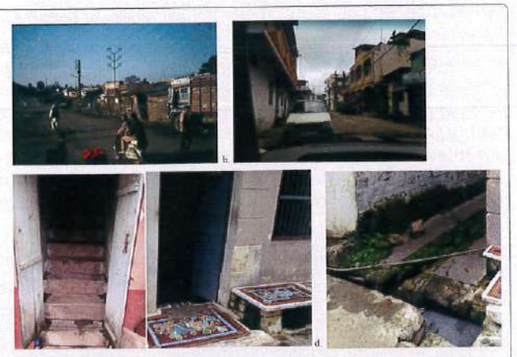

Figure 2: Pre- and post-MIC habitats around UCIL: a. Thatched huts of 1984; b. Present-day residences; c. dark interior of houses with no free flow of air or light; d. drains and water connection.

Briefly, Giemsa (G)-banding karyotyping has identified constitutive abnormalities, and also translocations and hyperdiploid conditions specific to hematopoietic malignancies such as del(5q), trisomy 8, etc. in some individuals (unpublished data). However, generation of a comparative result may take some time to talk on the long-term effects of MIC on MIC-victims.

Conclusion

The immediate screening of genetic damage in the MIC-exposed survivors was carried out by six institutions under the supervision of ICMR; however, the result was made unavailable to public till date. Individual reports have projected overestimation of genetic aberrations as indicated in aberrant images. After three decades, the issue of long-term effect of MIC has been raised, and thus, an approach has been undertaken for screening of genetic condition of Bhopal population, which is under progress. With a view to rationalizing the concept of genetic screening, this review has been raised on the spectrum of health-condition over the three decades post-exposure amidst complex biological and environmental consequences. Incidences of the cancer-burden and chronic kidney disease have been demonstrated increased; however, it cannot directly be linked to MIC-exposure since multiples of compounding factors such as life-style, living environment, nutritional factor, occupational exposure, and inherent genetic condition are interacting. Nevertheless, delayed effect cannot be ruled out since latency period of chemical exposure and perturbation of biological and immune system may contribute to delayed expression. The confounding factors and its control shall be paid importance while screening long-term effects of chemical exposure in any population. Nevertheless, biological aging will render significant health-effect in elderly population.

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Conflict of Interest

Nothing to declare.

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