GENETIC RESPONSE TO TOXICITY IN *ETROPLUS SURATENSIS* (BLOCH) AND *ETROPLUS MACULATUS* (BLOCH)

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By

PARAMANANDA DAS M.Sc.

POST GRADUATE PROGRAMME IN MARICULTURE
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
INDIAN COUNCIL OF AGRICULTURAL RESEARCH
COCHIN - 682 031, INDIA

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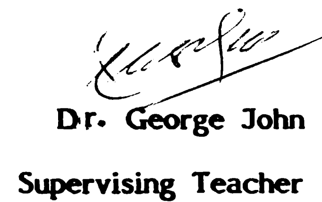
CERTIFICATE

This is to certify that the thesis entitled **'Genetic response to toxicity in Etroplus suratensis(Bloch.) and Etroplus maculatus (Bloch)'** is a bonafide record of the work carried out by Mr. PARAMANANDA DAS under my guidance and supervision and that no part thereof has been presented for the award of any other Degree.



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Dr. George John

Supervising Teacher

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INTRODUCTION

Environmental mutagenesis has emerged as a new field in genetics. The possibility that many man-made chemicals present in the environment could be mutagens has heightened concern for safety. The importance attached to this area is reflected by the fact that during the last 15 years or so about two dozen international journals dealing with environmental problems have been launched.

Studies in this new area also known as genetic toxicology tries to focus not only on the biological effects on individuals but also on their offspring. Man's most precious heritage happens to be his genome and protection of this is an awesome responsibility.

Exploratory research in this new field has identified an important problem for human population all over the world. It has also created a need for development of agressive new research programmes to develop highly efficient assay systems for mutagenic activity to screen untested environmental chemicals. The exploratory experiments started in the sixties to test chemicals in the environment have shown that mutagenic chemicals can be found in all major categories: food additives, drugs, pesticides, cosmetics, air and water pollutants as well as household and industrial chemicals. The challenge presented to geneticists by the recognition of this problem can only be met effectively by the development of new theoretical and applied research programmes (Serres, 1976).

Genetic toxicology identifies and analyses the action of agents whose toxicity is directed towards the hereditary components of living organisms. Although it is true that many substances with broad spectrum toxicity can damage genetic material in a non-specific manner, this discipline focuses only on those agents which are highly specific in their attack on nucleic acids and which are capable of producing deleterious effects at sub-lethal levels (Brusick, 1980).

From the very dawn of human civilization aquatic sources were used as the dumping ground of terrestrial, biological and technological wastes with an idea that the vast column of water through dilution and by action of many detoxyfing agents would make the toxic agents innocuous (Hynes, 1960).

The first major publication, 'Silent Spring' on hazards of pesticides, though an exaggerated account succeded in focusing attention on the problem and the need for intensified research. Most of these problems have been compounded by the phenomenon of biological magnification. This results in a higher concentration at one trophic level than that at the preceding level. It is not comforting to note that man's position is at the higher trophic levels.

Investigations on the various aspects of aquatic pollution have been going on in the country for sometime. The initiation of programmes like

the Ganga Action Plan are acknowledgements of the fact that environmental deterioration is serious. Though a vast amount of information has been generated in the country on different types of aquapollution, the concepts embodied in 'genetic toxicology', 'environmental mutagensis', 'genotoxicity', or 'environmental genetic damage' have not been accorded the due recognition in pollution studies. Conventional pollution studies quite often miss this important aspect.

The most obvious effect of pollution on aquatic organisms is mass mortality. However, even at sub-lethal levels, mutagenic pollutants can cause subtle but enduring genetic damage. It is this aspect that is more ominous and needs attention.

The use of genetic techniques for identification and monitoring environmental mutagens in aquatic systems in now being recognised by the aqua-pollution workers. A number of studies conducted in the country have been based on conventional chromosome aberrations. Screening for chromosome aberrations is no doubt a good approach but sensetivity is not very high. Some of the chemicals tested yield false negatives or false positives. It is the false negatives that are of concern. The chances of false negatives in mutagen screening are somewhat indirectly proportional to the sensitivity of the test adopted. Thus it is precisely here that the need for dependable and sensitive assays for genotoxicity testing is felt.

SCOPE OF THE STUDY

Though a number of investigations have been undertaken by Indian researchers to examine the link between pollution and genetics there is an urgent need to develop more sensitive genetic assays for the purpose. Use of chromosome aberrations and micronuclei incidence have been studied by different workers (Krishnaja and Rege, 1982; Manna, 1982; Manna and Mukherjee, 1984; Mukherjee, 1984; Manna et. al. 1985) and others. Assays based on sister-chromatid exchanges in fish for detecting xenobiotics have not been employed. The problem is compounded by the fact that sister-chromatid differentiation and sister-chromatid exchange studies are more exacting and difficult to carry out. Moreover, the standard protocols largely based on mammalian systems need considerable development at different stages to suit fish species.

Etroplus maculatus and Etroplus suratensis were taken as the two test species in the present study. A major emphasis of the study was on the development of methods for sister-chromatid differentiation (SCD) and and sister-chromatid exchange (SCE) studies. Two known mutagens and two pesticides were selected to study the genetic response as manifested through cytogenetic endpoints like micronuclei, chromosome aberrations and sister-chromatid exchanges. Experiments were designed to test the dose response.

The purpose of selecting two known mutagens was to study the response of the test species to these chemicals whose mutagenic potentials

have been well documented. These results were useful for conducting experiments to screen pesticides for mutagenicity. The two pesticides selected i.e., Methyl parathion and Phosphamidon are in wide use in agriculture and invariably find their way into aquatic systems. Based on the screening of these two pesticides further programmes can be taken up to scrutinise the mutagenic status of others currently in use.

In both the test species chromosome numbers were not found to be very high and chromosome morphology quite suitable for the studies. However E. suratensis was a better model species than E. maculatus due to its chromosome morphology. All chromosomes though more or less uniform did not pose difficulties as in the case of E. maculatus complement where some chromosomes were very small. This necessitated dropping some tests using E. maculatus.

In general it is seen that studies using SCE are based on cytogenetic models with large chromosomes and low diploid number. However such ideal species are not always available. Despite some of these inherent limitations, the present study was undertaken to develop cytogenetic assays using locally available fish as cytogenetic models.

The use of SCE in fish for mutagenicity testing appears to be the first work in the country. It is realised that this attempt may have some lacunae or flaws. However it is hoped that this study would provide the much needed orientation towards testing mutagenicity of aqua-pollutants using sensitive methods like sister-chromatid exchanges in fish.

REVIEW OF LITERATURE

1. FISH CYTOGENETICS

Fish cytogenetics as a separate discipline started taking shape only a couple of decades or so back. Most of the methodologies employed are offshoots of technical developments in the study of mammalian chromosomes. In comparison to mammalian cytogenetics, fish cytogenetics has received less attention. Though about 20000 fish species are known in the world not even 10% have been studied cytogenetically. The vast majority of fish are therefore chromosomally unknown.

Besides aiding routine taxonomy or helping in unraveling the evolutionary sequence of species, fish cytogenetics has also been put to a more direct use i.e, in developing cytogenetic assays for monitoring environmental mutagenesis and in genotoxicity testing. Evaluation of a potential cytogenetic model must necessarily be preceded by a study of its karyotype. Hence the importance of information on chromosomes of as many species as possible cannot be overemphasised.

The review attempted here does not purport to be comprehensive. It only outlines some of the landmark studies in fish cytogenetics and development of methodologies or protocols. The advances in fish cytogenetics in India have been given a wider treatment.

It was probably Schwarz in 1887 who first attempted to count fish chromosomes accurately (Svardson, 1945). According to Makino (1951) the first attempts were made a century back on Agnathans (Retziat, 1890); on shark (Kastschento, 1890), and teleosts (Bohm 1891). The real stride took place after the pioneering contribution of Tjio and Levan (1956) which revolutionised cytogenetic studies. However there was a rather slow development of suitable techniques as regards the chromosome of Osteichthyes, the most dominant group of fishes. Obtaining good quality chromosome plates consistently is the limiting factor in the study of chromosome cytology of fishes. Technical difficulties handicapped early workers, resulting in several reports on chromosome number and morphology now considered incorrect (Chaiarelli and Capanna, 1973; Denton 1973; Ohno 1970). Most of the earlier work made use of colchicine injections and squashes of the testes and haemopoietic tissues (Roberts, 1964; Ohno et.al. 1965); corneal and conjunctival epithelium (Sick et al. 1962; Drewry, 1964); gill epithelium (Mc Phail and Jones 1966; Chen and Ebling, 1968); embryological material (Simon, 1963; Simon and Dollar, 1963; Swarup, 1959), sectioning of testes (Nogusa, 1960); in vitro studies (Roberts 1964, 1966, 1967; Heckman and Brubaker, 1970; Ojima et al., 1970) and the scale epithelium method (Denton and Howell, 1969) which did not require sacrificing the fish.

With the revolution in mammalian cytogenetic techniques, several innovative procedures including pretreatment with mitotic inhibitors (colchicine colcemid etc.) hypotonic treatment of the cells and subsequent fixation and slide preparation by air or flame drying (Ojima, 1967; Manna and Prasad,

1968, 1971, 1973a,b,c, 1974, 1976) rendered simplifications. An easy and rapid process of preparing chromosomes from solid tissues of fishes was developed by Kligerman and Bloom (1977). This was a modification of techniques of Meredith (1969), Evan et. al. (1972) and Stock et. al. (1972). The method is useful for many species, both marine and freshwater and is being widely employed.

Further improvements in protocol were possible by developing methods for in-vitro studies (Labat et al., 1967, Heckman and Brubaker, 1970; Heckman et. al. 1971; Barker, 1972; Etlinger et. al. 1976; Al-Sabti,1985c and others.

Low mitotic index has been a limiting factor and a handicap with in-vivo systems for chromosome analysis. Use of chemicals to enhance divisions have been attempted by some workers with in-vivo systems to overcome this difficulty. A method recently developed by Cucchi and Baruffaldi (1989) used phenylhydrazine administration in-vivo. The haemolytic effect of this chemical was found to induce haemopoeitic tissues to actively proliferate cells, yielding a higher number of metaphases. Another study by the same authors (Cucchi and Baruffaldi,1990) showed that Cobalt Chloride injections produced the desired effect of increasing mitotic index.

Among the early Indian studies have been those of Nayyar (1962, 1964, 1965, 1966) who studied chromosomes of 22 freshwater species. Kaur and Srivastava (1965) reported on the chromosomes of five freshwater

teleosts. Natarajan and Subrahmanyam (1968) studied the chromosomes of _Tilapia mossambica_. Studies by Srivastava and Das (1969), Subrahmanyam (1969, 1970) and Subrahmanyam and Natarajan (1970) are also among the pioneering contributions.

Manna and his collaborators have made noteworthy contributions to cytogenetic studies in Indian fishes. Manna and Prasad (1968) studied chromosomes of _Channa punctatus_ using cells derived from kidney. Manna and Prasad (1973a, 1973b, 1973c) studied chromosomes of _Channa_ spp., _Anabas testudineus_ and _Puntius_ spp. employing both somatic and germinal tissues. Rishi (1973) reported the karyotype of 18 marine fishes. Das (1973) studied chromosomes of some teleosts. Two distinct forms of _Mystus vittatus_ were identified as two species based on chromosomal evidence brought out by Manna and Prasad (1974). Manna and Khuda-Buksh (1974) studied the chromosomes of two carp hybrids. Further studies on more fresh water species were conducted by Manna and Prasad (1974 and 1976). Natarajan and Subrahmanyam (1974) studied the karyotype of _Etroplus suratensis_ (2n = 48) and _E. maculatus_ (2n = 46) in addition to 14 other species collected from Porto Novo area. Rishi (1975) reported on the chromosomes of _Trichogaster fasciatus_ while Khuda-Buksh and Manna (1976a) studied the chromosome complements in two Indian mullets. Indian carp hybrids also were investigated by them (Khuda-Buksh and Manna, 1976b).

Rishi (1976a, 1976b) provided additional information on Indian species including evidence for male heterogamety in _Callichrous bimaculatus_.

Rishi and Gaur (1976) provided chromosomal evidence for female heterogamety in *Molliensia sphenops*.

Manna and Khuda-Buksh (1977a, 1977b) cytologically evaluated cyprinids and drew up a check-list of chromosomes in cyprinid species. Chromosomes of *Barilius bendelensis*, *Rasbora daniconius*, *Aplocheilus panchax*, *Lates calcarifer* and *Gadusia chapra* were studied by Khuda-Buksh (1979a, 1979b). Rishi (1979) studied G-bands of somatic chromosomes of *Colisa fasciatus*. The study also produced evidence for confirmation of female heterogamety in the species. Khuda-Buksh (1980, 1982) reported on chromosomes of *Tor* spp. Rishi and Rishi (1981) examined the utility of G-banding studies in fish. Khuda-Buksh and Nayak (1982) reported on the chromosomes of two hill stream fishes from Kashmir. Rishi and Singh (1982) reported on the chromosome complements in five estuarine fishes including *E. suratensis*. Krishnaja and Rege (1980, 1982) studied the karyotypes of *Gambusia affinis* and *Boleopthalmus dussumieri* for evaluating their potential as cytogenetic models in genotoxicity studies. Das (1983) reviewed the status of cytogenetic studies in marine fishes. Manna (1984) reviewed extensively the progress registered in fish cytogenetics. Out of the 1400 species listed the diploid numbers ranged from 16 to 239. The modal number (2n=48) was observed in 460 species while the diploid number of 46 was next in frequency i.e., in about 225 species. About 140 species had the diploid number of 50. Reddy and John (1986) demonstrated the usefulness of a modified air drying method for study of carp chromosomes. Barat and Khuda-Buksh (1986) studied karyomorphometry in two cobitid species. Khuda-Buksh and Barat (1987) further reported

on the chromosomes of freshwater teleosts. Nayak and Khuda-Buksh (1988) found the diploid number to be 48 in *Platycephalus tuberculatus* with a chromosome configuration of 2 metacentric, 2 sub-metacentric, 3 sub-telocentric, and 17 telocentric pairs. No heteromorphic chromosomes were observed. Chromosomes of *Psilorhynchus succatio* (2n = 50) revealed a configuration of 11 m + 9 sm + 5 t; that of *Labeo dero* (2n = 50) a chromosomal configuration of 13 m + 6 sm + 1 st + 5 t and *Ompok pabo* (2n = 54) showed a chromosome pattern with 18 m + 6 sm + 3 t pairs (Khuda-Buksh and Chanda, 1989). Studies on two marine species *Otolithes cuvieri* and *Nibea diacanthus* revealed a diploid count of 48 acrocentric chromosomes in both species (Chakraborty and Kagwade, 1989).

2. GENOTOXICITY AND GENETIC ASSAYS

Mutagenicity studies perhaps started with the report on gene mutation induced by radiation (Muller, 1927), followed by the report on mutagenicity of chemicals (Auerbach, 1946). The exploratory experiments started only in the late sixties using several mutagenicity testing methodologies on mammalian and other models. Pioneering studies were on somatic and germinal cells (Brookes & Lawley, 1971), transformation in microorganisms (Ames, 1971) induced mutation in yeast (Mortimer and Manney, 1971), micronuclei test (Evans, 1976; Schmid, 1982) SCE analysis (Perry and Wolff 1974; Latt, 1974), dominant lethal test in mice (Rohrborn, 1970) etc.

a) Chromosome aberration:

Among non-mammalian animals it is the aquatic organisms and mainly

fish that have received most attention. Regan et al. (1968) reported chromosome aberration in the marine fish cells in-vitro. Schroder (1969) conducted specific locus test in fish. Tystsugima (1972) found eggs of *Scorpaoma* sp. with higher frequency of chromosome damage on chronic exposure to radiation. The chromosomes of *U. limi* showed typical responses to low levels of X-irradiation (325 R) and about 30% of metaphases had aberrations as against the control rate of 0.03% (Kligerman et al. 1975). Acute irradiation was found cause chromosome damage in cultured cells of the fish *Ameca splendens*, (Woodhead, 1976). Radiation induced chromosome damage was also demonstrated in *U. limi* by Mong and Berra (1976). Fish exposed to 350 R, 660 R and 990 R of X-irradiation showed chromatid breaks, gaps and chromatid exchanges between several chromosomes. Parry et. al. (1976) reported mutagenic activity of tissue extracts of the mussel *M. edulis* collected from polluted sites. Continuous irradiation in fish embryos caused severe chromosome damage as demonstrated by Peckkurenkov (1976). Chromosome mutagenesis was reported in developing mackerel (*Scomber scombrus*) eggs sampled from the New York Bight area (Longwell, 1976). Stich et al. (1976) studied tumours in fish caused due to sublethal effect of pollutants and suggested the feasibility of using the system for early detection of mutagens in the marine environment. Mutagens and toxicants distributed in lake Ontario were detected by microbial procedures, (Dutka and Switzer, 1978). Trenimon, a chemical and polluted river water were found to induce chromosome damage in *U. pygmia* in-vivo, (Sugatt, 1978). Prein et. al. (1978) studied cytogenetic changes in fish exposed to polluted water of Rhine River. Kligerman (1979) and Schroder (1979) also demonstrated the utility of fish as a model to detect damage caused by

irradiation. Preliminary investigations on the effect of some pesticides and detergents revealed chromosome damage in fish (Sofradzija et. al. 1980). Newsome (1980) conducted multigeneration fish toxicity tests to assess the effects of potential aquatic pollutants using Cichlasoma nigrofasciatum.

Berry (1980) and Beardmore et. al. (1980) reviewed the various genetical aspects of pollution monitoring and opined the need to study distribution of genotoxic substances, their effect on gene pools and genetic damage caused. Kocan et. al. (1981) studied in-vitro effects of mutagens and carcinogens on fish cells. Hooftman (1981) reported chromosome aberrations like breaks, interchanges, dicentrics, gaps and fragmentations in Nothobranchius rachowi induced by MMS and benzo (a) pyrene. Wardhaugh (1981) reported dominant lethal mutations induced in tilapia by an anti-lukaemic drug myleran administered at a dose of 15 mg/kg body weight.

Krishnaja and Rege (1982) studied the effects of in-vivo exposure of mitomycin-C and heavy metal compounds on the chromosomes of Boleopthalmus dussumieri. Intramascular injections of mitomycin-C, @ 0.5-2.0 mg/kg body weight showed a dose response increase in aberrations. Phenyl mercuric acetate, sodium dioxide and sodium dichromate also induced a marked enhancement in aberration frequency at most of the dose levels tested. Dixon (1982) observed aneuploidy in mussel embryos collected from polluted dock areas. Kocan et. al. (1982) reported significant increase in chromosome damage demonstrated through anaphase test of cultured rainbow trout gonadal cells treated with N-methyl N-nitro N-nitrosoguanidine, benzo (a) pyrene, 9-amino acridine, 3-methyl colanthene, anthracene, 1-napthol

and mitomycin. Burke (1982) scored chromosome aberrations like fragments, deletion, complex rearrangements, rings, translocations etc. in Liposetta putnami subjected to acute and chronic exposure to (2%, 4% and 7%) water soluble fractions of crude oil. Manna (1982) reviewed mutagenesis and emphasised the necessity to study mutagensis caused by living mutagens. Protic and Kurelec (1983) showed the high mutagenecity of several polyaromatic hydrocarbons based on studies on liver post-mitochondrial fractions from treated carps. Lethal and mutagenic effects of radiation and chemicals on cultured Carassius auratus cells was studied by Mitami (1983). Overipening of eggs and irradiation were found to induce chromosomal changes in salmonids (Yamazaki, 1983). Landolt and Kocan (1983) in their review on fish cell cytogenetics described and evaluated some of the methods like Ames test, fish cell mutations, micronuclei formations and anaphase aberrations for measuring genotoxicity in fish.

Som and Manna (1984) studied somatic chromosome aberrations in X-irradiated tilapia and their F 1 offspring. Cytogenetic effects of selected environmental chemical pollutants on Tilapia were studied in detail by Mukherjee (1984). The experimental treatments induced chromosome aberrations like breaks, fragments, pycnosis, stickiness etc. Zajiceck and Phillips (1984) reported the mitotic inhibition and anaphase aberrations in rainbow trout embryos treated with MMNG and gamma radiations. Manna and Mukherjee (1984) reported the genotoxic effect of organophosphorous pesticide malathion as revealed by increased chromosome aberrations in tilapia. Studies carried out by Al-Sabti et.al. (1984) on the effect of detergent compounds and benzene in Salmo gairdneri showed frequent occurrence

of chromosome aberrations. Landolt and Kocan (1984) reported the lethal and sub-lethal effects of marine sediment extracts on fish cells and chromosomes. Al-Sabti (1985b) studied chromosome aberrations in S. gairdneri exposed to phenol, decamethrine, malathion, neguvon and crude oil. All were shown to increase the rates of chromosome aberrations. Al-Sabti (1985a) also studied chromosome aberrations induced by carcinogenic-mutagenic chemicals in kidney cells of cyprinids. Kocan et. al. (1985) demonstrated visible and heritable chromosome damage in trout cells and embryos. Among the compounds tested benzo (a) pyrene and the nitrosamide MNNG produced significant increase in aberrations. Kocan et al. (1985) demonstrated the use of fish cell cultures for measuring genotoxicity of marine sediment pollutants. In another study Kocan and Powell .(1985) showed anaphase aberrations to be an effective in-vitro test for genotoxicity of individual compounds and complex environmental mixtures. Manna (1986) reviewed genotoxicity studies in fish and opined tilapia to be a good cytogenetic model for testing genotoxic agents.

Carcinogenic-mutagenic chemicals aflatoxin, aroclor, benzidine, benzo(a) pyrene and methylchloranthrene were found to induce dose dependent chromosome aberrations in the cells of common carp, C. carpio (Al-Sabti, 1986b). Induction of dominant lethal mutations were demonstrated in O. niloticus treated with the alkylating agent methyl methane sulphonate (Shah and Beardmore, 1987). Goodier et. al. (1987) reported chromosome fragmentation and loss in two salmon hybrids. Walton et. al. (1988) observed increase of aberrations in cultured U. limi cells exposed to S9 activated benzopyrene. Manna (1989) reviewed the various protocols for genotoxicity

testing using fish species.

b) Sister-chromatid exchanges (SCE):

SCE analysis is a very sensitive cytogenetic assay in comparison to the routine techniques employing chromosome aberrations and micro-nuclei for genotoxicity testing. SCE analysis using the DNA analog bromo-deoxyuridine (BrdU) has been popular as a new cytogenetic method for determining the potential genetic hazards of chemicals in the environment (Latt, 1974a, 1974b; Latt et. al. 1975; Kato and Shimada, 1975; Perry and Evans, 1975; Bloom and Hsu, 1975) and others. The development of FPG technique (Perry and Wolff, 1974) was a step forward in this respect. Significant increases in the incidence of SCE have been demonstrated both in-vivo (Allen and Latt, 1976; Vogel and Bauknecht, 1976; Tice et. al. 1976; Pera and Mattias, 1976; Nakanishi and Scheider, 1979; Sutou, 1981) and in-vitro (Latt, 1974a, 1975; Perry and Evans, 1975; Carrano et. al., 1978; Chaganti et. al., 1974; Ishi and Bender, 1978; Wolff, 1977) etc., using mammalian systems.

Kligerman and Bloom (1976) developed an in-vivo system for detection of SCE using the fish Umbra limi. SCE analysis of intestinal cells showed a value of 2.64 per metaphase and 2.42 per metaphase from gill cells. Their study showed the usefulness of U. limi in measuring the mutagenicity of water borne chemicals. Kligerman (1977) further explored the possibilities of using U. limi as an aquatic in-vivo model for SCD and SCE studies for mutagen testing. Barker and Rackham (1979) studied SCE in cell cultures of Ameca splendens on exposure to carcinogenic

mutagens ethyl methane sulphonate, mitocyin C, methyl methane sulphonate and MNNG. The incidence of SCE showed a tendency to increase with mutagen dose. Kligerman (1979c) and Kligerman et.al. (1981) demonstrated the usefulness of central mud-minnow as a model in-vivo system for detection of active substances through SCE analysis. Their results showed significant increases in the rate of SCE in the animals exposed to microgram quantities of cyclophosphamide, neutral red and methyl methane sulphonate. In another study (Alink et.al. 1980) the gill and testis tissues of U. pygmaea showed manyfold increases in the SCE rates when exposed to polluted Rhine water, compared to fish exposed to unpolluted ground water. This study also demonstrated the usefulness of using fish species as models to detect environmental genotoxicity. Maddock and Kelly (1980) studied sister-chromatid exchanges in a marine fish, Opsanus tau and demonstrated the utility of the technique for detecting genetic damage caused by environmental mutagens. Stromberg et. al. (1981) extensively udied the alterations in the frequency of SCE in Leptocottus armatus, a flatfish exposed to benzo (a) pyrene or sampled from polluted areas. Pesch and Pesch (1980) and Pesch et.al. (1981) used a marine polychaete, Neanthes arenaceodentata as a cytogenetic model to assess the effect of genetic toxicants. An assay system based on sister-chromatid exchanges showed an increase from the base-line value of 0.14 exchanges per chromosome to 0.5 exchanges per chromosome on exposure to mitomycin C. Positive responses were demonstrated on exposure to other mutagens like methyl methane sulphonate (MMS), benzo (a) pyrene, dimethyl nitrosamine (DMN) and cyclophosphamide (CP). In another study ethyl methane sulphonate and cyclophosphamide induced significant increases in SCE in Nothobranchius rachowi administered through

the water medium (Hoeven et.al., 1981). Exposure to ethyl methane sulphonate @ 120 mg per litre induced 0.66 SCEs per chromosome as against 0.10 of control while 0.35 SCEs per chromosome were detected on exposure to 50 mg per litre of cyclophosphamide. Bishop and Valentine (1982) evaluated the potential of a few directly and indirectly acting mutagens to induce SCE in the mudminnow U. limi. They observed dose dependent responses. The use of non-mammalian i.e. avian and aquatic cytogenetic systems for mutagenicity testing was reviewed thoroughly by Bloom (1982).

A note on SCE in Indian live fish Channa punctatus by Mohanty and Prasad (1982) seems to be the only publication in India on SCE in fish. The spontaneous exchange rate was estimated to be 0.17 per cell. Dixon and Clarke (1982) studied MMC induced SCE in the mussel Mytilus edulis. They reported a dose dependent increase in SCE on exposure to mitomycin C, Park and Grimm (1982) reported a correlation between genetic damage and pathological condition as revealed by elevated SCE rates in lymphocytes of the European eel Anguilla anguilla affected with 'cauliflower tumour'. Harrison and Jones (1982) conducted experiments to study in-vivo sister chromatid exchanges in the larvae of mussel Mytilus edulis exposed to 3 mutagens. Gaag et.al. (1983) studied the effect of polluted water from river Rhine on SCE in N. rachowi. The SCE frequency doubled to 0.104 per chromosome as against the control of 0.055 indicating a mutagenic load in pollution.

The response of cultured fish cells to chemical mutagens have been studied with reference to DNA damage and repair synthesis mechanisms

by Walton et. al. (1983). U. limi exposed to insecticides like endrin, chlorodane, diazinon and guthion at concentrations ranging from 5.4×10^{-12} M to 5.4×10^{-9} M showed significant increases in the frequency of sister-chromatid exchanges (Vigfusson et. al., 1983). They suggested use of the system for detecting water borne mutagens. Zakour et. al. (1984) studied sister-chromatid exchanges in cultured peripheral blood cells of a marine fish, Leptocottus armatus for detecting mutagenecity. A more than four fold increase in SCE rates were observed on exposure to 5 μg/ml of N-methyl-N-nitro N-nitrosoquanidine (MNNG) a direct mutagen. Walton (1984) studied DNA breakage and repair mechanisms in fish cells as measure of detecting genotoxic activity of chemicals. Kerkhoff and Gaag (1985) reviewed the various methods adopted for SCD and SCE studies. They suggested improvement for in-vivo studies using Nothobranchius rachowi. Gaag and Kerkhoff (1985) opined SCE to be a reliable assay in mutagenicity testing of water samples using fish species. Another study conducted on larvae and adults of M. edulis (Dixon, 1985) showed that cyclophosphamide (CP) increased SCE frequencies. The presence of phenobarbital (PB) an inducer of microsomal detoxification was found to enhance the activity of CP. On an average, PB treated individuals displayed a two fold increase in SCE levels when compared to animals exposed only to CP. Maddock (1986) observed significant increases in SCE and chromosome aberrations in the haemopoetic tissues of marine fish in-vivo exposed to genotoxic carcinogens. Dixon and Prosser (1986) measured the cytogenetic endpoints of chromosome aberrations and sister-chromatid exchanges in M. edulis larvae to assess the genotoxicity of tributylin anti-fouling paint residues. Their investigations however did not indicate any genotoxicity. Variations

in the frequency of sister-chromatid in the larvae of different groups of _M. edulis_ were reported by Jones and Harrisson (1987). They suggested that direct analysis of transgenerational transfer of genotoxic agents could explain the high incidence of SCE.

Harrison 1987 reported enhancement of SCE and chromosome aberrations in the marine polychaete _N. arenaceodentata_ as an outcome of irradiation. Studies on in-vivo SCD and SCE frequencies of renal cells in grass carp _Ctenopharyngodon idella_ were carried out by Wei (1987).

c) Micronuclei study:

Micronucleus is a chromosome fragment that lags behind during anaphase and passes into one daughter cell. It may fuse with the principal nucleus or remain as a separate secondary nucleus. Their sizes vary depending on the size of the fragment.

For many years biologists have been aware of the existence of micronuclei. Haematologists referred to them as Howell-Jolly bodies. However, the use of micronuclei study for an assay is a relatively recent development (Schmid, 1976). Ever since Schroeder (1966) discovered micronuclei in bone marrow after treatment with enzymes ıe method has been developed and expanded for a wide range of genotoxicity testing. The micronuclei test (MNT) has been widely used as a fast method of testing genotoxic agents in various mammalian models (Evans, 1976; Schmid, 1975; Schroeder, 1970; Heddle, et. al. 1983; Sutou 1981 and Sutou, 1986 and others. The accent has been rather poor on fish and other non-mammalian

vertebrates. Clastogen induced micronuclei in peripheral blood erythrocytes of fish was reported by Mc Gregor et. al. (1980). The study reported radiation induced micronuclei in the species. Hooftman and Roat (1982) reported micronuclei formation in the peripheral blood of U. pygmaea exposed to ethyl methyl suphonate for 3-6 weeks. 3 weeks exposure to 200 mg/litre resulted in micronuclei incidence of 1.6%. With exposure to 0.8 and 40 mg/litre of EMS the micronuclei incidence were 0.07, 0.4 and 3.7% respectively. A study on the erythrocyte nuclear measurements of diploid channel catfish, Ictalurus punctatus carried out by Wolters et al. (1982) indicated the loss of chromatid fragments during anaphase. Longwell et. al. (1983) used flounders and Atlantic mackerel to study the frequency of micronuclei in mature and immature lymphocytes for measuring chromosome mutations. Manna et. al. (1985) reported significant increase in the rates of micronuclei in peripheral erythrocytes of Sarotherodon mossambicus exposed to different genotoxicants. Micronuclei occurrence was found to be 0.4, 0.7, 0.7 and 0.8% with treatment of aldrin (0.3%), cadmium chloride (0.1%), D-glucosamine hydrochloride (0.1%) and X-irradiation (400 r) respectively. In another study Manna and Sadhukhan (1986) reported micronuclei formation in the gill and kidney cells of S. mossambicus exposed to X-radiation and chemicals, cadmium chloride, aldrin and D-glucosamine hydrochloride. Micronuclei incidence varied from 0.38 to 2.05% in response to the different treatments. Micronuclei formation have also been reported in cyprinid fishes exposed to carcinogenic and mutagenic chemicals (Al-Sabti, 1986a). Das and Nanda (1986) reported induction of micronuclei in the peripheral erythrocytes of Heteropneustes fossilis. Grinfield et al.(1986)

studied micronuclei in the red blood cells of newtt Pleurodeles wattl after treatment with benzo (a) pyrene. Jaylet et. al. (1986) examined erythrocytes of axolotl larvae for micronuclei incidence after exposure to mutagenic agents. Elevated rates of micronuclei in the circulating erythrocytes of fish from contaminated sites off southern California was reported by Hose et. al. (1987). Majone et. al. (1987) reported increase of micronuclei in the marine mussel M. galloprovincialis after treatment with mitomycin C. Chlorothalonile induced increases of micronuclei in erythrocytes of eel were studied by Mingde et. al. (1987). Manna (1989) reviewed the different studies for assessing genotoxicity using fish and stressed the relevance of cytogenetic endpoints including micronuclei.

MATERIALS AND METHODS

1. TEST ANIMALS

Two fish species viz., Etroplus suratensis and Etroplus maculatus were chosen as the test animals. These two species inhabit the brackish-waters and estuaries of India, Sri Lanka and Pakistan. In India, the major area of distribution lie between Karnataka coast on the west and upto Chilka on the east. E. suratensis feeds on detritus, plankton and small aquatic insects whereas E. maculatus is carnivorous in nature, feeding on a wide range of food items. Both are available almost throughout the year, but the peak season is January to April and September to November. E. suratensis mature when they attain a size of 10-12 cm in length. They grow to a maximum length of about 25 cm. They contribute a significant percentage of the landings in Chilka lake, Pulicat lake and Kerala backwaters. It is one of the prized species in Kerala. E. maculatus is smaller in size. They mature at a length of about 5-6 cm. Both species are not very difficult to maintain in the laboratory as they accept artificial feeds.

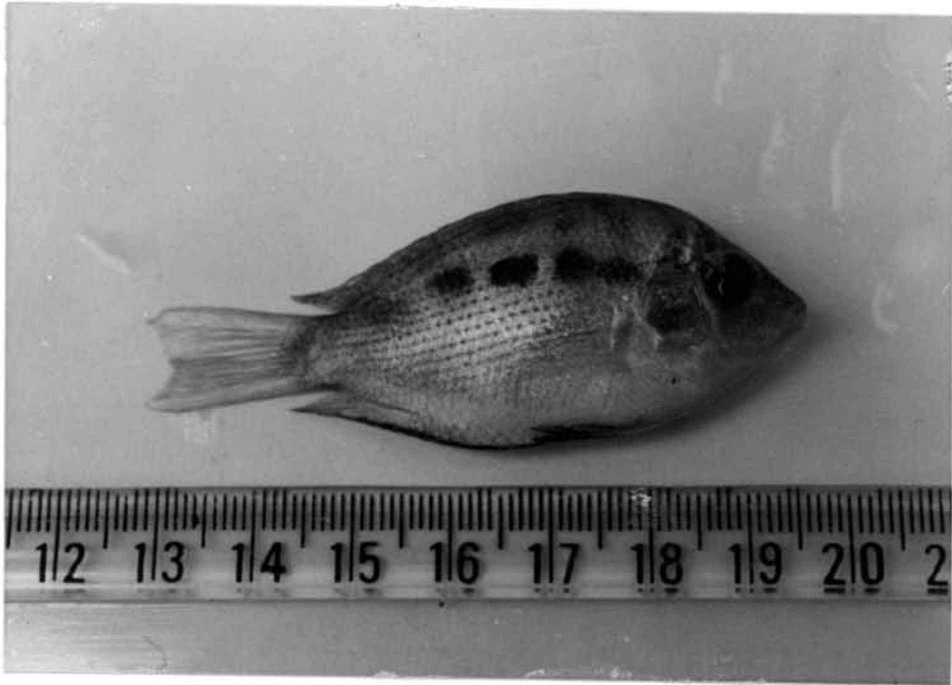
Collection of test animals:

Young specimens of E. suratensis and E. maculatus were collected from the Narakkal field station of CMFRI, Malipuram and Fisheries College, Panangad. All the collection spots were not very far off from the laboratory .

Plate 1. *Etroplus maculatus* (test species)

Plate 2. *Etroplus suratensis* (test species)

1

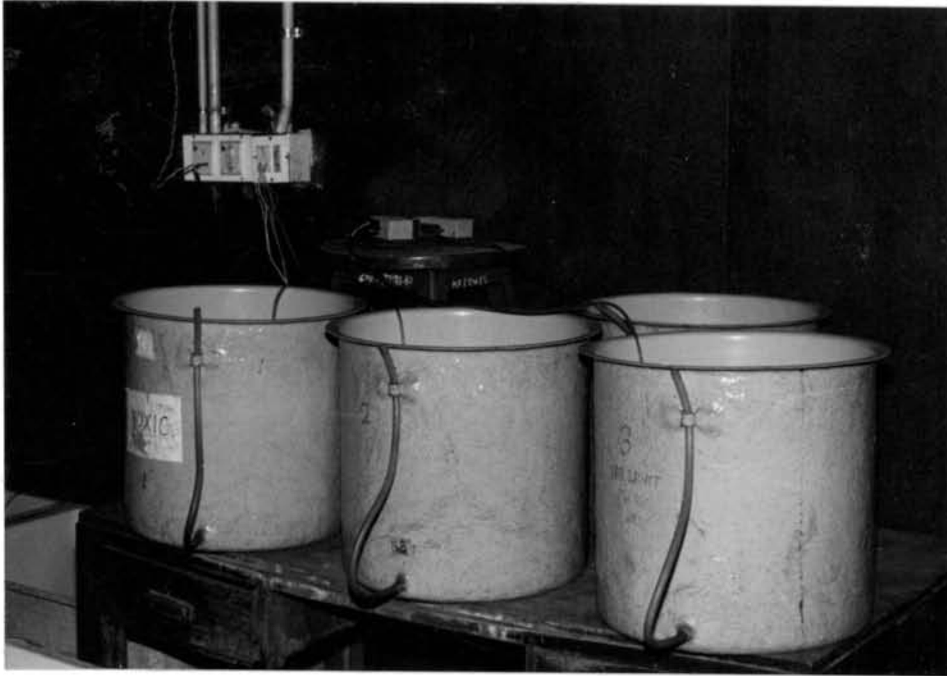


2

Plate 3. Experimental set-up for treatments

Plate 4. U.V. irradiation system.

3



4

Specimens were transported live in oxygen bags or buckets to the laboratory, acclimatised and maintained for 20-30 days in a salinity range of 5-10 ppt. They were fed regularly with artificial pellet feed and prawns. Water was changed once or twice a week. Fibre glass tanks (40 L capacity) with outlets on the base were used for the experiments.

2. TEST CHEMICALS

a) Methyl methane sulphonate (MMS):

This known mutagen was procured from Ms John Baker Inc. of USA. It is an organic sulphate compound (liquid) easily miscible in water. This mutagen is known to directly affect the target molecule(DNA).

b) Cyclophosphamide (Endoxan):

Cyclophosphamide (CP) is also a known mutagen and is used as an anticancer drug. The chemical as such is not active but is metabolised in the body to give mutagenic metabolites while injected to any organism (Perry and Evans, 1975). Such chemicals are referred to as indirectly acting mutagens. This chemical was procured from Ms. Khandelwal, Bombay. It is soluble in water.

c) Methyl Parathion (Metacid-50)

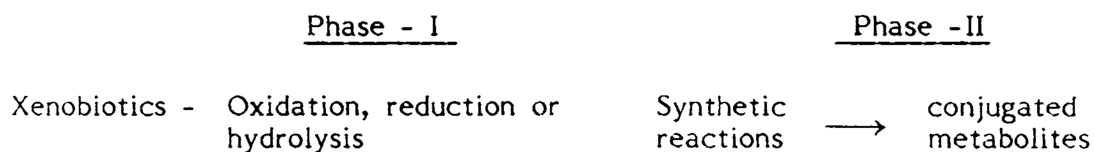
Methyl Parathion (MP) is an organophosphorus insecticide mostly used in agricultural fields against insect pests. This compound is a contact poison and soluble in water. It was procured from Bayer (India) Ltd., Bombay. As regards the chemical nature, it is a phosphorothioate compound i.e., O, O-dimethyl O-(p-nitrophenyl) phosphorothioate.

d) Phosphamidon (Dimecron 85% SL)

Phosphamidon (PM) is also an organophosphorus compound acting as a systemic insecticide cum acaricide and is used in agricultural fields. The chemical is soluble in water (Manufacturers: Hindustan Ciba Geigy Limited).

The chemical is a vinyl phosphate compound, i.e., 2-chloro-2-diethylcarbonyl - 1 - methylvinyl - dimethyl phosphate.

The above two pesticides i.e., methyl parathion and phosphamidon are organophosphorous compounds inhibiting the action of the enzyme cholinesterase which normally hydrolyse acetylcholine at nerve junctions. If not hydrolysed, acetyl choline accumulate at nerve junctions as a result of the passage of the nerve impulses, and eventually blocks the transmission of these impulses to gonads and muscles. Symptoms of poisoning in animals included lacrimation, vertigo, muscular weakness, tremors and laboured respiration (Rude, 1964). Animals actually die of suffocation. There is very little residual accumulation of organophosphorous compounds in tissues although additive effects are possible if exposure is repeated before cholinesterase levels have returned to normal. The general pattern of metabolism of xenobiotics for all animals including fish is as follows:



The reaction of both phases is catalysed by enzymes, either through microsomal or non-microsomal mechanisms. The metabolic steps in fish include glucoronic acid conjugation, glycine conjugation thyocyanate synthesis, hydrolysis, reduction of azo and nitrogroups, N-oxidation, hydroxylation, demethylation and dealkylation. Other termination mechanisms in fishes include bilary excretion and gill excretion (Adamson and Sieber, 1981).

3. CHROMOSOME PREPARATION

Standardization of methods for chromosome preparation for genotoxicity studies is quite a challenging job. Most of the species have generally very small chromosomes and secondly, getting consistently good number of metaphase plates in in-vivo is not easy. Species to species variations are experienced quite often. Standardisation of procedures may vary even from laboratory to laboratory.

The various methods tried, with modifications to evolve a suitable technique were as follows:

1. Ford and Hammerton (1956)
2. Mc Phail & Jones (1966)
3. Denton and Howell (1969)
4. Reddy and John (1986)
5. Kligerman and Bloom (1977)

1. Ford & Hammerton (1956):

The method originally developed for mammalian systems was tried with modifications. Specimens injected intramuscularly with 0.1% colchicine

@ 1 ml/100 gm body weight were dissected after 3 hours, gills intestine and kidney tissues collected. Tissues were homogenised in a water bath at 37°C for 30 minutes. The material was centrifuged for 5 minutes at 1200 rpm twice at an interval of 15 minutes with addition of fixation (3:1) methanol-acetic acid in each step after decanting the supernatant. The cell suspension was dropped on alcohol chilled slides, flame dried and stained in Giemsa.

2. Mc Phail & Jones (1966):

Posterior gill arches of specimen were dissected out 2-4 hours after colchicine injection (0.01%) and hypotonised in 0.4% KCl for 30 minutes at room temperature. The material was fixed in acetic-alcohol (1:3) followed by staining in 2% Giemsa for 20 minutes. The stained gill arches were shaken lightly on a clean slide until a slurry of cells were deposited. The slurry was squashed using a cover glass.

3. Denton & Howell (1969):

Small sized animals were allowed to swim in well aerated colchine solution (0.01%) in a container for 4-5 hours. Gills were dissected and hypotonised with 0.4% KCl for 20 minutes. Tissues were minced and the suspension was centrifuged at 1000 rpm for 5 minutes. The supernatant was discarded and fixative added. Slides were prepared by dropping the suspension on alcohol chilled slides, airdried and stained in Giemsa.

4. Reddy & John (1986):

Kidney tissue was dissected out from fishes, 3-4 hours after injection with 0.05% colchicine (0.5 ml/100g) and hypotonised in 1% sodium citrate for 30 minutes. The material was then gently agitated in glass homogeniser and the suspension centrifuged, fixed in methonol-acetic acid (3:1). Slides were prepared on alcohol chilled slides, air dried and stained in Giemsa.

5. Kligerman and Bloom (1977):

Tissues like gill, intestine and kidney were collected from fish 3 hours after an intramuscular injection of 0.01% colchicine (1 ml/100 g), hypotonised with 0.04% KCl for 20-30 minutes followed by fixation in methanol acetic acid (3:1). A cell suspension was prepared using 50% acetic acid and slides prepared by dropping on warm (40-50°C) slides. The drop was immediately drawn back with a pipette, forming a ring of cells on the slide.

This method gave better results in comparison to the others. However improvements were found necessary. Trials were conducted on the following aspects to evolve a suitable method.

a) Colchicine administration: Intramuscular injections were preferred since the animals were small. Colchicine concentrations (0.1, 0.05, 0.01, 0.005, 0.001%) @ 1 ml/100 g body weight were tried with different exposures ranging from 2 to 4 hrs. 1, 0.1 and 0.05% colchicine gave sufficient number of contracted metaphase plates after 3 hours of treat-

ment. Colchicine (0.001%) gave good results with respect to total number of spreads. However most of the spreads were in the premetaphase stage with the same hours of treatment. 0.01 and 0.005% of colchicine (3 hours) gave moderate number of metaphase spreads. The best results were obtained with 0.005% colchicine and an exposure of 2-2½ hours.

b) Tissues: Gill, kidney and intestine tissues were taken for the study. Gill tissues were found to be the best since they gave the best spreads, followed by intestine and kidney. Hence gill tissue was chosen for the study.

c) Hypotonic treatment: Sodium chloride (1%) sodium citrate (1, 0.5%) KCl (0.4, 0.04%) and double distilled water were tried as hypotonic solutions. The duration ranged from 15 - 60 minutes both at room temperatures and in cold. Best results were obtained with 1% cold sodium citrate for 30 minutes. In most of the samples swelling was excellent in cold conditions i.e, in a refrigerator (6-8°C).

d) Fixation: Fixation was done in methanol acetic acid (3:1) with three changes at intervals of 15 minutes, 30 minutes and 1 hour. Good results were obtained by fixing the material at lower temperatures. Methanol storage was tried to improve chromosome morphology as shown by Bantock and Cockayne (1975). It was not very useful.

e) Preparation of cell suspensions: Quite often *E. maculatus* gave chromosome spreads with poor morphology i.e, fuzzy appearance of

chromosome margins. This was suspected to be caused by acetic acid used for suspension preparation. Hence acetic acid was diluted upto 20% but the problem was not entirely solved, though there were improvements.

4. METHOD DEVELOPED FOR CHROMOSOME PREPARATIONS

Based on the above trials on the various steps, the following technique was developed for chromosome preparation of the two test species in the present study:

i) An intramuscular injection of colchicine solution (0.005%) @ 1 ml/100 g body weight on the base of the fin and an exposure of 2 - 2½ hours.

ii) Hypotonic treatment in cold trisodium citrate (0.8 - 1%) for 30 minutes.

iii) Fixation by methanol acetic acid (3:1) with 2-3 changes (First after 15 minutes; second after 30 minutes; third after 1 hour). The total duration of fixation was around two hours.

iv) Cell suspension using 40 - 50% acetic acid (3-5 minutes).

v) Dropping suspension on warm slides (40-50°C) using small Pasteur pipette and withdrawing the drop with the pipette, leaving a ring

of cells. 3-4 rings were deposited on each slide.

vi) Staining of slides with 4% Giemsa solution for 15-20 minutes in a phosphate buffer (pH 6.8).

This standardised method was used for all the experiments during the study.

5. DIPLOID CHROMOSOME NUMBER

For determining the diploid chromosome number, 15 animals each were studied from both species. Only well acclimatised untreated animals were used for the purpose. About 20-30 metaphases per animal were screened. The diploid number was determined on the basis of the largest number of cells showing a particular count i.e., the modal number. A total number of more than 300 metaphases were studied from each species.

6. KARYOTYPE ANALYSIS

4 good metaphase plates approximately of similar chromosome lengths from each species were photomicrographed and prints made of the same enlargement along with the scale. The individual chromosomes from each plate were cut out, visually paired and pasted on a white board paper. The arrangement was in the descending order of chromosome length in each group. In the case of *E. suratensis*

it was arranged according to size only since all chromosomes belonged to one group.

Chromosome lengths, relative lengths (%) and arm ratios were measured from the metaphase plates separately. Chromosomes were classified using the methodology of Levan et. al. (1964). The best among the plates considered in each species was used for karyotype display. Generalised idiogram and histograms were also made.

7. DIFFERENTIAL STAINING OF SISTER CHROMATIDS

Induction of sister-chromatid differentiation (SCD) is a prerequisite for observing sister chromatid exchanges (SCE) i.e., exchanges between two sister-chromatids in a chromosome. The chromosomes must be treated so that sister-chromatids are chemically different from one another. This is accomplished by labelling DNA during its synthesis, either by using tritiated thymdine followed by autoradiography or by using the nucleotide (thymidine) analog Bromodeoxyuridine (BrdU). Autoradiography is not required with BrdU labelling. A schematic representation of steps in DNA labelling using BrdU is given in Figs. 1 and 2.

a) Labelling with tritiated thymidine:

This was originally accomplished by Taylor et. al. (1957). Cells are allowed to replicate their DNA for one cell cycle in the presence of tritiated thymidine followed by another cycle in the presence of non-radioactive thymidine. Because of the semi-conservative replication

Fig. 1. Formation of differentially labelled chromatids

G_1	-	Pre-synthesis
S	-	Synthesis
G_2	-	Growth
- - - -	-	Labelled DNA strand (BrdU incorporated)
——	-	Unlabelled DNA strand.

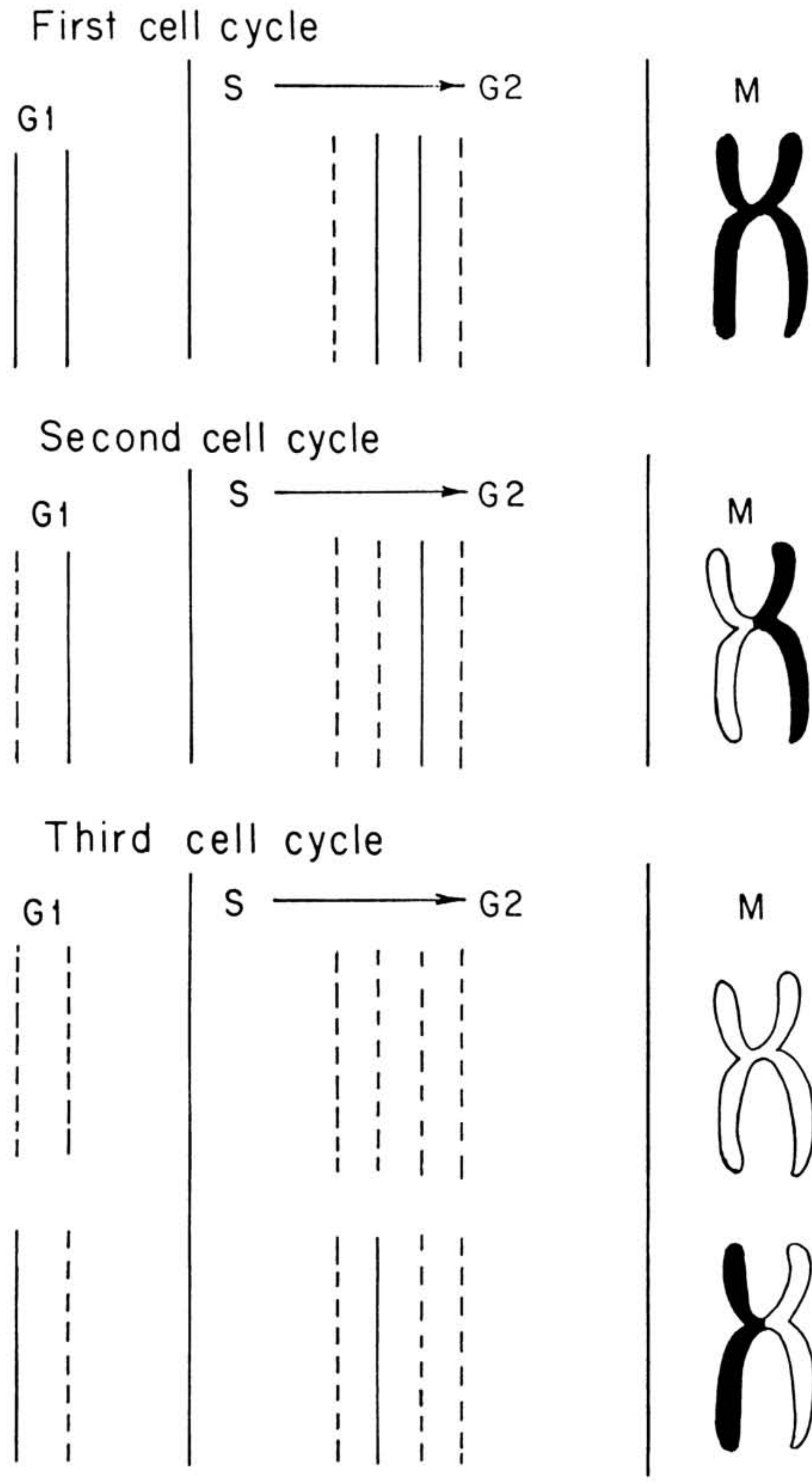


FIG.1. Formation of differentially labelled chromatids

Fig. 2. Formation of SCE

G_1	-	pre-synthesis
S	-	Synthesis
G_2	-	Growth
----	-	Labelled DNA strand(BrdU incorporated)
——	-	Unlabelled DNA strand.

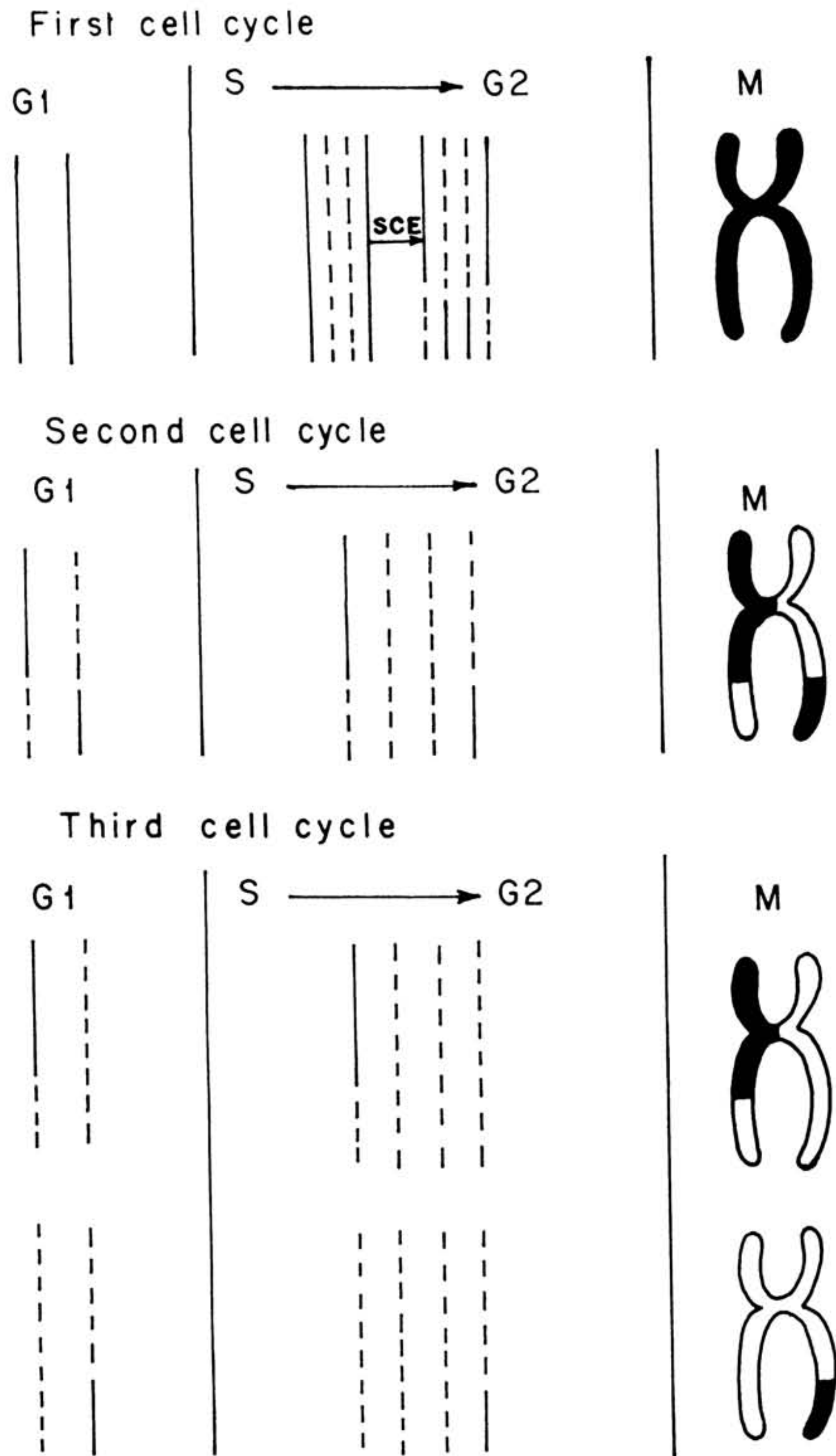


FIG.2. Formation of sister-chromatid exchanges (SCE)

this treatment results in the formation of chromosomes containing one chromatid with one polynucleotide strand of its DNA labelled and its sister-chromatid not labelled. Thus the two chromatids are chemically different and this can be visualised with autoradiography techniques.

b) Labelling with bromodeoxyuridine:

This is a newer method of DNA labelling using chemical analogs of thymidine (nucleotide) that become incorporated into the chromosomes during DNA synthesis. After one round of replication with BrdU, chromosomes contain chromatids that are unifilarly substituted. After a second round of replication in the presence of BrdU the resultant chromosomes have one chromatid unifilarly substituted (one nucleotide strand labelled) while its sister is bifilarly substituted (both nucleotide strands labelled). Other analogs like bromouracil or fluorouracil can also be used.

BrdU administration is generally done through in-vivo methods like injections or administration through the medium. It is also possible to administer BrdU during in-vitro procedures (cell cultures). The labelled chromosomes are stained with fluorescent dyes like Hoechst 33258 acridine orange, DAPI and exposed to light for pre-fading. Hoechst dye provides an intense localised source of light after it is excited. (Wolf and Bodycote, 1977). Light treatment (pre-fading) results in lesser staining intensity with Giemsa in those chromatids bifilarly substituted.

This newer method is relatively better since it does not involve the use of autoradiography and good quality preparations are possible with precise treatments. Hence this basic method was adopted after modifications of the various steps.

c) Protocols evaluated:

Except for one note (Mohanty & Prasad, 1982) there is no information on the SCD/SCE on any Indian fish species. Hence it took a considerable time to evolve a suitable method for our laboratory conditions. The various protocols tried during standardisation were as follows:

i) Perry and Wolff (1974): Slides were stained in 0.5 μg/ml Hoechst 33258 in Sorensen's Buffer at pH 6.5-7.0 for atleast 20 minutes. The slides were rinsed in running distilled water, dried, mounted in buffer with a coverslip and exposed to fluorescent light for 30-60 minutes. Slides were then treated with 2 X SSC (0.3 M NaCl + 0.03 M trisodium citrate) at 60°C in a waterbath. After 1 hour, slides were rinsed thoroughly in distilled water stained in Giemsa and mounted with DPX.

ii) Kligerman and Bloom (1976): Slides were treated with 0.5 μg/ml Hoechst in deionised water for about 10 minutes, rinsed in deionised water and mounted in Sorensen's buffer at pH 7.0. Treatment with light for pre-fading was done as described in the previous method.

iii) Stromberg et. al. (1981) (Modified): Slides were treated with 0.5 μg/ Hoechst in PBS solution at pH 7.0 for 5 minutes. Exposure to fluorescent

light was for 30-60 minutes. Staining was done in 4% giemsa in Sorensen's buffer at pH 6.8.

iv) Bloom (1982) (Modified): Slides were treated with 0.5 μg/ml of Hoechst in Sorensen's buffer at pH 7.0 for 10-15 minutes. Exposure to fluorescent light was for 30 minutes. Slides rinsed thoroughly in distilled water were airdried and stained in 4% Giemsa in phosphate buffer at pH 7.0.

v) Kerkhoff and Gaag (1985): Slides were treated with 5 μg/ml Hoechst in PBS (8 g NaCl; 0.2 g KCl; 1.44 g $Na_2HPO_4 \cdot 2H_2O$ and 0.2 g KH_2PO_4 per litre distilled water) solution for 15 minutes. Slides were mounted in the same solution with coverslip and exposed to black light bulb (8W) for 10 minutes at room temperature. Slides rinsed in distilled water were treated with 5 N HCl solution for 15 minutes at room temperature, rinsed in distilled water, stained in 4% Giemsa in phosphate buffer at pH 6.8 for 15-20 minutes.

As described above a spectrum of treatment protocols were tried for developing a suitable method for the test species. On the basis of these, protocol variations tried with the different steps were as follows:

BrdU administration: Bromodeoxyuridine administrations tried were as given below. BrdU solution was prepared in double distilled water.

Total dose (mg/g body weight)	Injection (Nos)	Duration (hours)
0.5 mg	1	24, 48
1 mg	1	24, 48
1 mg	2	24, 48
2 mg	4	24

Hoechst treatment variations: Hoechst concentrations were 0.5, 1, 5, 50 μg/ml. Buffer used was Sorensen's buffer (pH 6.5-7.0) and PBS. Exposure durations were 10, 15, 20 and 30 minutes.

Light treatments and sources:

Sunlight	:	30-60 minutes
Flourescent light (40W)	:	30-60 minutes
U.V.germicidal tube (30w)	:	15-30 minutes
U.V. black bulb (125 w)	:	5-15 minutes

Post-treatment:

2X SSC :	:	60 minutes at 60°C
5N HCl	:	10-30 minutes

Staining: Giemsa 1-4% (pH 6.5-7.5)

Mounting:

In most cases slides faded a few days after mounting with DPX. Hence slides were screened without mounting.

8. METHOD DEVELOPED FOR SCD/SCE IN THE PRESENT STUDY

On the basis of the above protocols and trial experiments with the different steps described, the following fluorescence Plus giemsa (FPG) method was developed by incorporating the best results obtained in each step.

BrdU administration: 0.5 mg/g body weight (one injection) and an exposure of 24 hours.

Hoechst treatment: Hoechst (0.5 μg/ml) prepared in PBS solution (8g NaCl; 0.2g KCl; 1.44g $Na_2 HPO_4$, 0.2 KH_2PO_4 per litre) was used. Slides were treated for 15-20 minutes.

Light treatment: Slides mounted in the above Hoechst solution and with coverslips were exposed to U.V. black bulb (125W) for 5-8 minutes at a vertical distance of about 8 cms.

Post treatment: Slides were rinsed in DDW, dried and treated with 5N HCl for 10-15 minutes.

Staining: Slides thoroughly rinsed in distilled water dried and stained in 4% Giemsa in phosphate buffer at pH ranging from 6.8-7.0.

Slides were processed on the same day the labelled chromosome spreads were prepared. Good results were obtained in 0-day old slides. All SCD/SCE experiments were conducted in subdued light.

9. CYTOGENETIC ENDPOINTS USED

The endpoints used in this study were chromosome aberrations (CA) micronuclei test (MN) and sister chromatid exchanges (SCE).

a) Chromosome aberrations

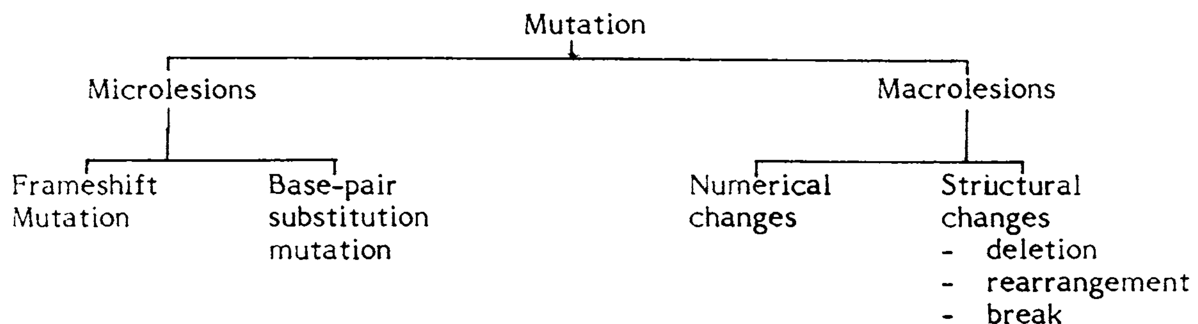
DNA damage consists of two broad catagories like macrolesion and

microlesions. Macrolesions are the visible effects detectable through cytological analysis of chromosomes and microlesions refer to the invisible changes occurring at the nucleotide level.

Microlesions consist primarily of base pair substitutions or base pair addition/deletion changes. Base pair substitution mutations result from quantitative changes in nucleotide composition of a codon whereas base pair addition/deletion mutations result from the addition or deletion of one or a few nucleotide pairs from the nucleotide complement in a gene (Ames and Whitfield, 1966).

macrolesions can be subdivided into changes in chromosome number (gain or loss of single chromosomes or sets of chromosomes) and changes in chromostructure (breaks, deletions, rearrangements etc.).

A schematic representation of DNA damage is given below:



(after Brusick, 1978)

Classically CA involves structural changes classified according to their time of formation relative to the cell cycles. According to this the lesions induced in G phase of the cell cycle gives rise to chromosome type aberrations (in two chromatids at identical regions) and lesions induced in G_2 give rise to chromatid type aberrations (one chromatid only) when they are scored in M_1 (Brusick, 1978).

b) Micronuclei formation:

The micronuclei test is an in-vivo cytogenetic screening procedure for the detection of freshly induced structural chromosome aberrations and for revealing chromosome loss due to partial impairment of the spindle apparatus (Schmid, 1975). Micronuclei are small incomplete nuclei originated fom chromatin material which lag during anaphase. In the course of subsequent division, this material is included in the cytoplasm of one of the daughter cells where it can either fuse with the main nucleus or form one or several secondary nuclei. These micronuclei represent acentric chromosome fragments or multicentrics connected by bridges. In case of spindle malformations they may consist of entire chromosomes.

c) Sister-chromatid exchanges:

Reciprocal exchange of parts between two sister-chromatids is called a sister-chromatid exchange (SCE). It is the number of exchanges between the differentially stained chromatids of a chromosome. The fluorescence plus Giemsa (FPG) technique as described earlier was

used for studying sister-chromatid exchanges. Figs. 1 and 2 show a schematic representation of BrdU incorporation and differential staining for visualising SCE.

10. BASELINE VALUE ESTIMATION

The spontaneous occurrence of CA, MN and SCE were studied using animals not deliberately exposed to any chemical. For chromosome aberrations, data was collected from 11 animals of *E. suratensis* and 8 animals of *E. maculatus.* For micronuclei studies erythrocytes from 10 specimens were screened. SCE baseline data was collected from 10 animals of *E. suratensis* and 8 animals of *E. maculatus.* The units of expression in the case of CA and SCE were chromosome aberrations or sister-chromatid exchanges per metaphase. In case of micronuclei the values were expressed as micronuclei per 1000 cells. Data from the cell populations of different animals in a particular treatment were pooled for assessing genotoxicity.

11. TREATMENTS

For treatments, sublethal doses were selected on the basis of tolerance limits of each chemical used. The maximum tolerated dose for 96 hours was considered as the highest dose in each case. Subsequent doses were fractions of the maximum dose. In each case 3 doses (lowest middle and highest) were tried. For both known mutagens viz., MMS and CP the doses were 50, 100, 150 μg/g; 25, 50, 100 μg/g body respectively. In the case of the two pesticides MP and PM, the doses were 0.05, 0.1,

0.2 ppm and 0.5, 0.1, 0.2 ppm respectively. The route of exposure was through intramuscular injection for the two known mutagens tested due to the risk factor involved in exposure through the medium, besides the prohibitive cost of large amounts of mutagens. The pesticides were administered through the water medium. In all cases 96 hours was the exposure duration.

The effects of the 3 different doses of the two mutagens and two pesticides in the present study were evaluated on the basis of data from different cell populations derived from each test animal in a particular treatment. 3-4 animals were used in each dose of treatment.

The exposure of the two test species and end points studied in both were as below:

E. maculatus

1. Methyl methane sulphonate (MMS)
   - a) Chromosome aberrations (CA)
   - b) Micronuclei (MN)
2. Cyclophosphamide (CP)
   - a) Chromosome aberrations (CA)
   - b) Micronuclei (MN)

E. suratensis

1. Methyl methane sulphonate (MMS)
   - a) Chromosome aberrations (CA)
   - b) Micronuclei (MN)

c) Sister-chromatid Exchange (SCE)

2. Cyclophosphamide (CP)

   a) Chromosome aberrations (CA)

   b) Micronuclei (MN)

   c) Sister-chromatid Exchange (SCE)

3. Methyl parathion (MP)

   a) Chromosome aberrations (CA)

   b) Micronuclei (MN)

   c) Sister-chromatid Exchange (SCE)

4. Cyclophosphamide (CP)

   a) Chromosome aberrations (CA)

   b) Micronuclei (MN)

   c) Sister-chromatid Exchange (SCE)

In the case of E. suratensis all three cytogenetic endpoints CA, MN and SCE were studied after exposure to all the four chemicals. In the case of E. maculatus, SCE studies were confined only to the base-line values. Cytogenetic endpoints, CA and MN only were studied in E. maculatus, that too in response to MMS and CP only. Some experiments were conducted on endpoints with MP and PM also, but the chromosomes of E. maculatus appeared rather unsuitable for the studies. The chromosome complement of E. maculatus consists of even very small pairs thereby making it difficult to estimate aberrations, especially exchanges. This was noticeably so with MP and PM. Hence screening was confined to

MMS and CP only. In *E. maculatus* only the largest 4 pairs of chromosomes were considered.

The control animals in the case of the two known mutagens received intramuscular injections of the solvent (distilled water). In the case of two pesticides, animals maintained in well aerated water were used as control since the exposure was through the water medium.

a) Chromosome aberration:

For study of chromosome aberrations the animals received an intramuscular injection of 0.005% colchicine @ 1ml/100 g body weight during the last 2-2½ hours of exposure. The colchicine treatment was therefore for 2-2½ hours. Gills were removed, processed according to the standardised protocol and slides prepared. About 6 slides per animals were considered. A minimum of 40 well spread metaphases were evaluated. Coded slides were screened and data collected on chromosome aberrations like gaps, breaks, fragments, rings, exchanges, centromeric fusion, minute complex rearrangements, ploidy etc. The data from the cell population of each animal were pooled for each dose of the chemical and values expressed: aberrations/metaphase.

b) Micronuclei:

Blood was collected from the caudal fin region by a neat amputation and smeared on clean slides. 4-5 slides/animal were prepared and immediately fixed in absolute methanol for 10 minutes. Staining was

in May-Grunwald solution followed by Giemsa for 10 minutes. Slides were screened without mounting. 5000 cells per animal were screened for estimating the rate of MN formation which was expressed as MN per 1000 cells.

c) Sister-chromatid exchanges:

The animals were given an intramuscular injection of BrdU @ 5mg/g body weight in the last 24 hours, i.e., at 72 hours of exposure to the test chemical and colchicine injection about 2-2½ hours before their sacrifice at 96 hours. Chromosome preparations were as described earlier. All experiments involving SCD/SCE were conducted in subdued light. Only the second cells (SCD_2) were considered for screening and analysis. 10 to 30 SCE_2 plates were examined per animal wherever possible. The unit of expression was SCE per metaphase.

12. FIELD STUDY

Live specimens of E. suratensis were collected from known and suspected polluted spots like Thaneermukham,Thevara Ferry and Integrated Fisheries Project Jetty near Cochin. Blood samples collected from animals from Thaneermukham and Thevara Ferry were screened for micronuclei occurrence. The sensitive experiments for SCE studies could not be conducted in the field as subdued light conditions were not easily simulated. These technical problems placed restrictions on large scale field studies which were confined to one area (IFP Jetty) suspected to be polluted with oil.

13. DATA ANALYSIS:

Data from cell populations of different animals in a particular treatment were pooled for analysis. An equality of proportion test (Z-test) was used for statistical analysis of the results.

RESULTS

1. DIPLOID NUMBER

(i) *E. maculatus*:

The diploid number was found to be 46. From a total of more than 300 metaphases studied from atleast 15 animals the diploid number (2n = 46) was observed in the maximum number of cases.

(ii) *E. suratensis*:

The diploid (2n) number of chromosomes in this species was found to be 48. This was observed to occur in the maximum number of cases out of a total of more than 300 metaphases screened from atleast 15 animals.

2. KARYOTYPE ANALYSIS

(i) *E. maculatus*:

The chromosome complement was found to comprise of a heterogenous group i.e, metacentrics, submetacentrics, subtelocentrics or acrocentrics. Chromosome sizes ranged from 0.456 to 5.988 μ. Relative lengths varied from 1.275 - 16.737%. No heteromorphic sex chromosomes were observed (Table 1, Plate 9).

(ii) *E. suratensis:*

Karyotype analysis showed all chromosomes to be of the acrocentric type. In a few cases some of the elements showed small telomeric ends. Most of the chromosomes were more or less of equal sizes. The size of chromosomes ranged from 2.669-4.401 μ. No heteromorphic sex chromosomes were identified. Relative lengths varied from 3.172-5.230% (Table 2, Plate 14).

3. BASE-LINE VALUES

(i) *E. maculatus:*

(a) Chromosome aberrations: In this species the base-line value was determined to be 0.003 per metaphase. The type of aberrations observed were only chromosome gaps (Table 3).

(b) Micronuclei: No micronuclei were observed in the base-line studies on the species.

(c) Sister-chromatid exchanges: the rate of sister-chromatid exchange per metaphase was 0.67 (Table 5).

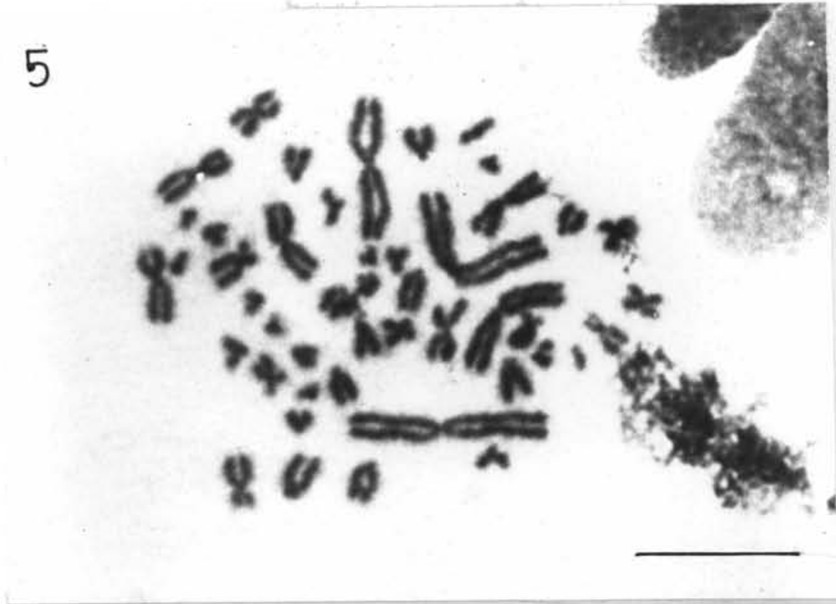
(ii) *E. suratensis:*

(a) Chromosome aberrations: the value of aberrations in the species was 0.006 per metaphase (Table 4).

(b) Micronuclei: No micronuclei were seen in the normal population of the species.

Plate 5. Metaphase spread of *E. maculatus* (bar = 10 μ)

Plate 6. Metaphase spread of *E. maculatus* (bar = 10 μ)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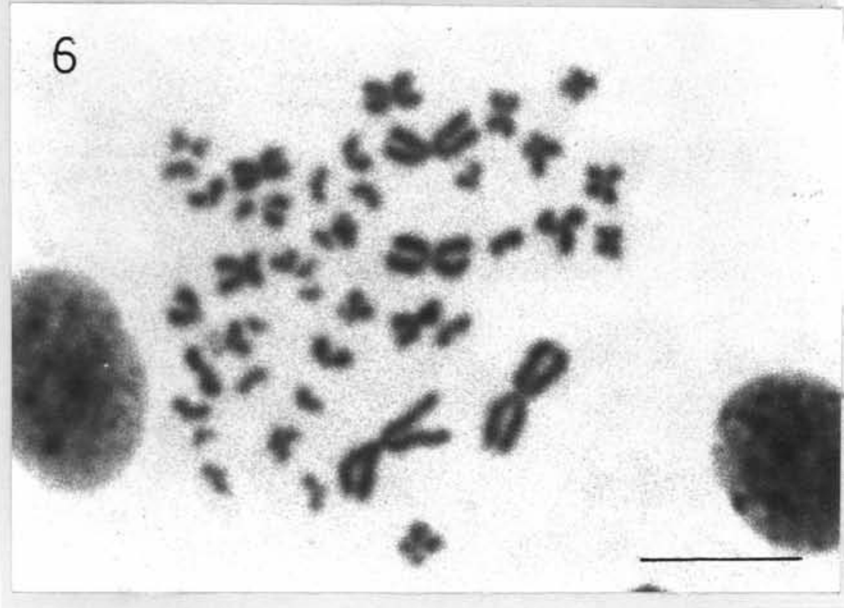
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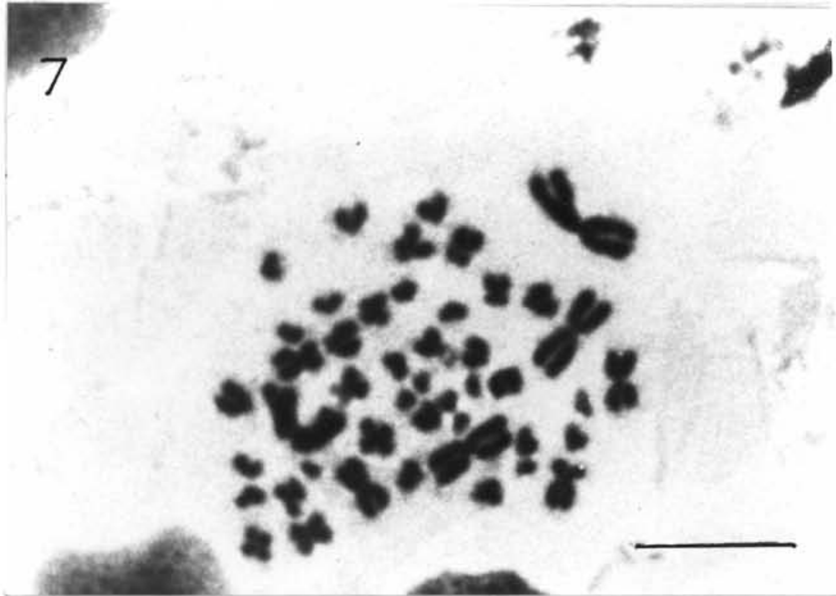
Plate 7. Metaphase spread of *E. maculatus* (bar = 10 μ).

Plate 8. Metaphase spread of *E. maculatus* (bar = 10 μ).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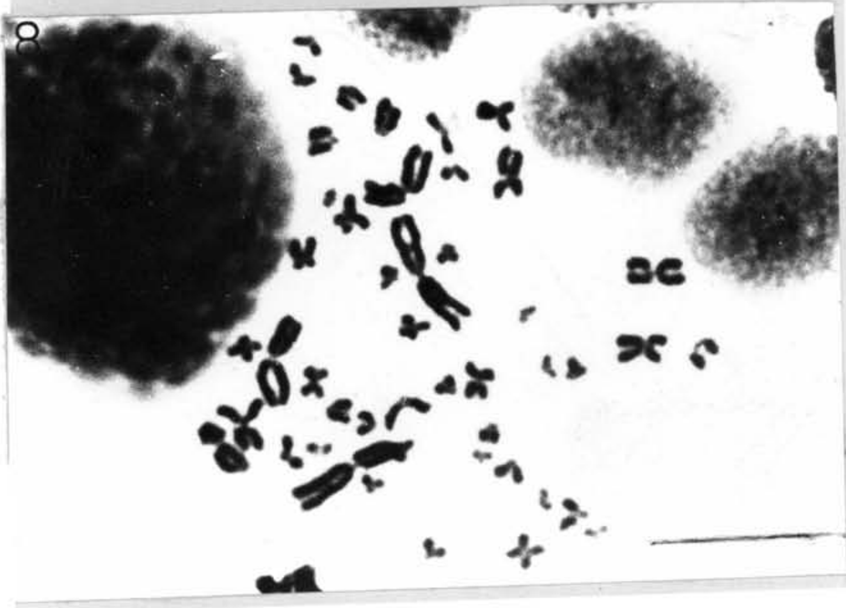
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Plate 9. Karyotype of *E. maculatus.*

M 1 2 3 4 5

M 7 9

10 μ

SM, ST 6 14 15

A 8 10 11 12 13

A 16 17 18 19 20

A 21 22 23

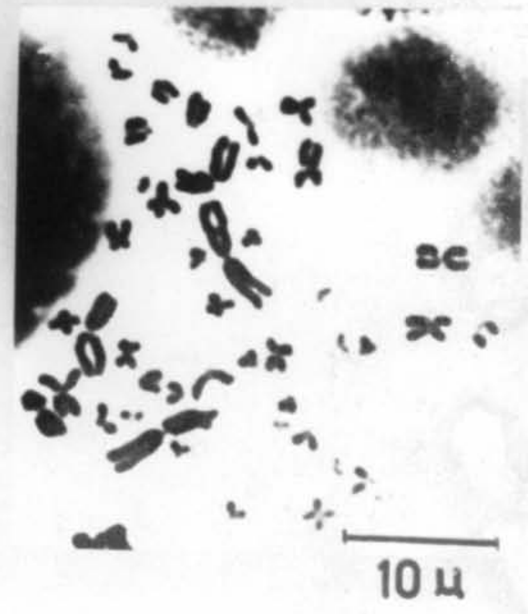
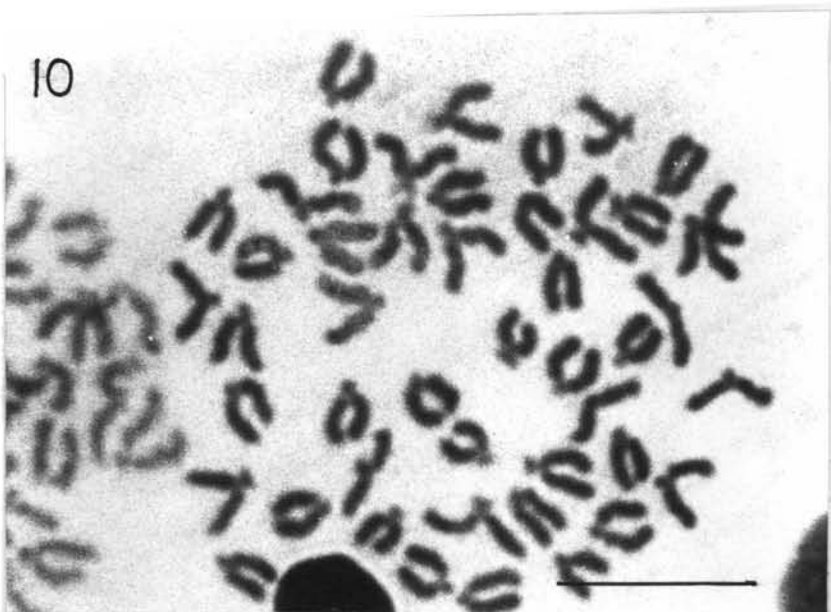


Plate – 9

Karyotype of *E. maculatus*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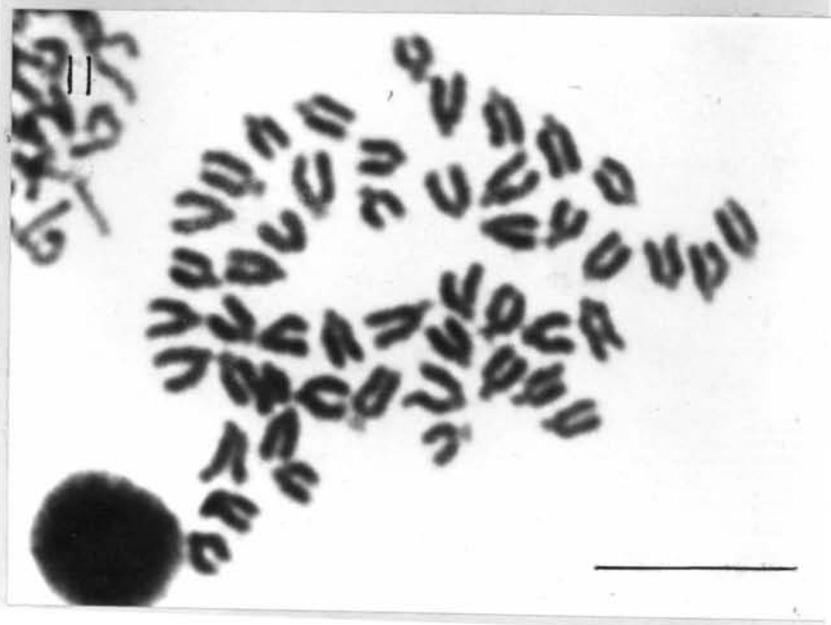
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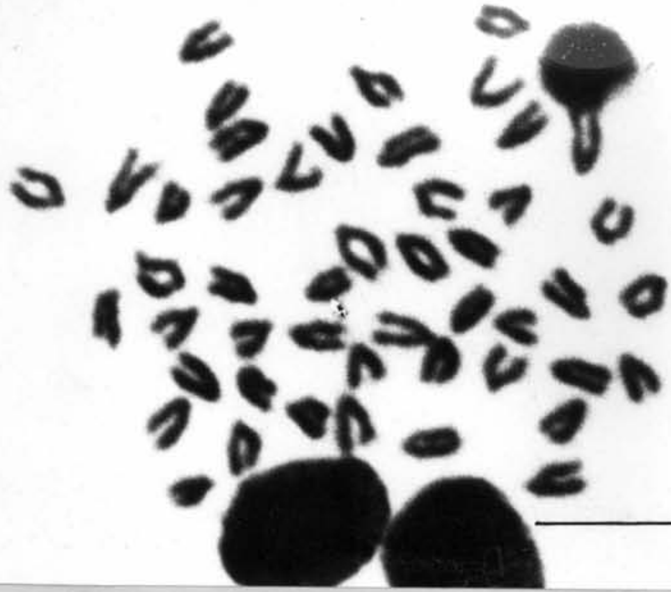
Plate 12. Metaphase spread of *E. suratensis* (bar = 10 μ).

Plate 13. Metaphase spread of *E. suratensis* (bar = 10 μ)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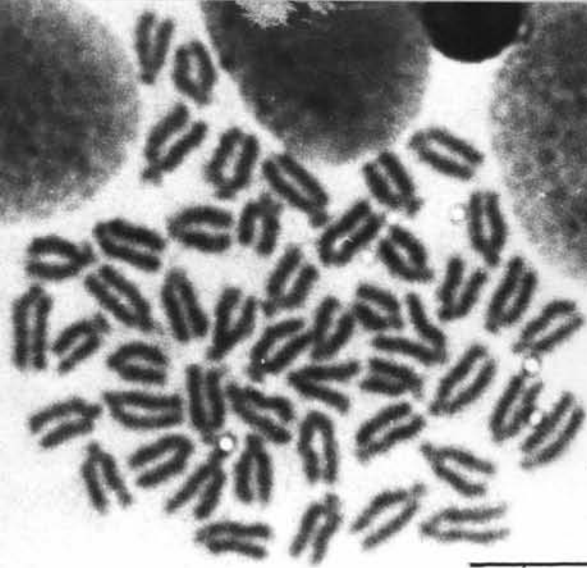
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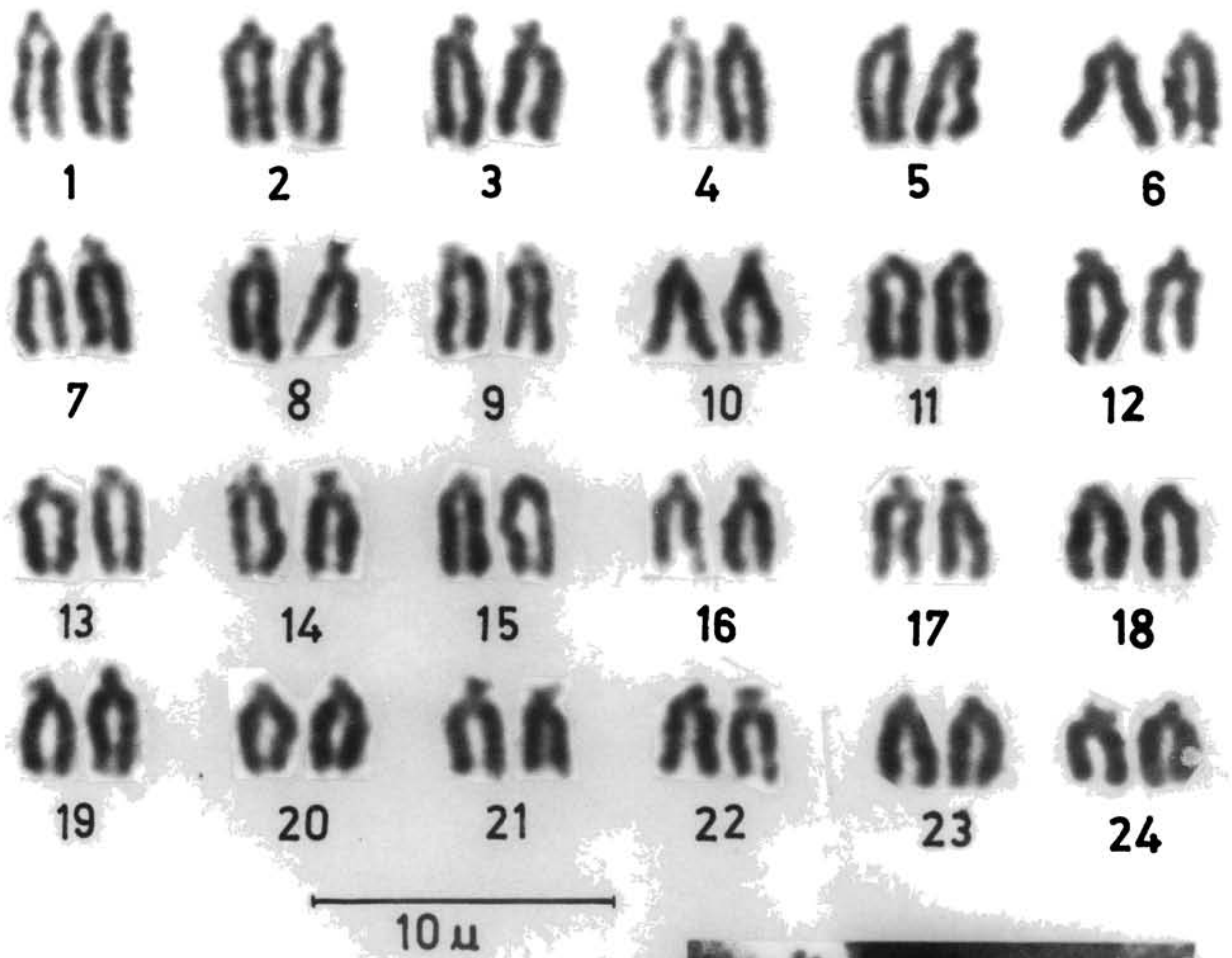
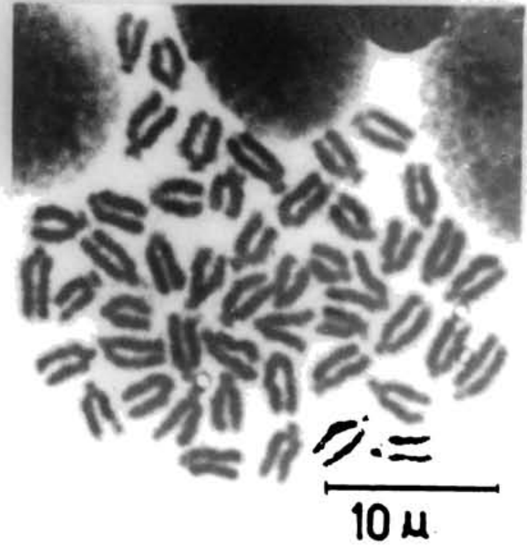
Plate 14. Karyotype of *E. suratensis.*



late – 14.

ıryotype of E. suratensis



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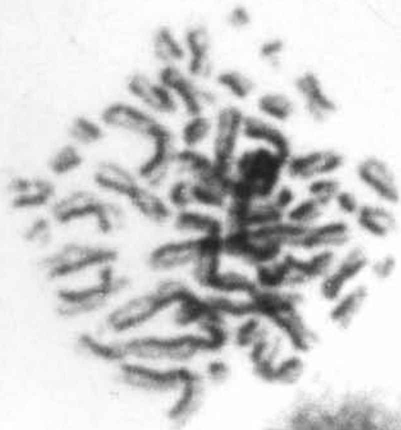
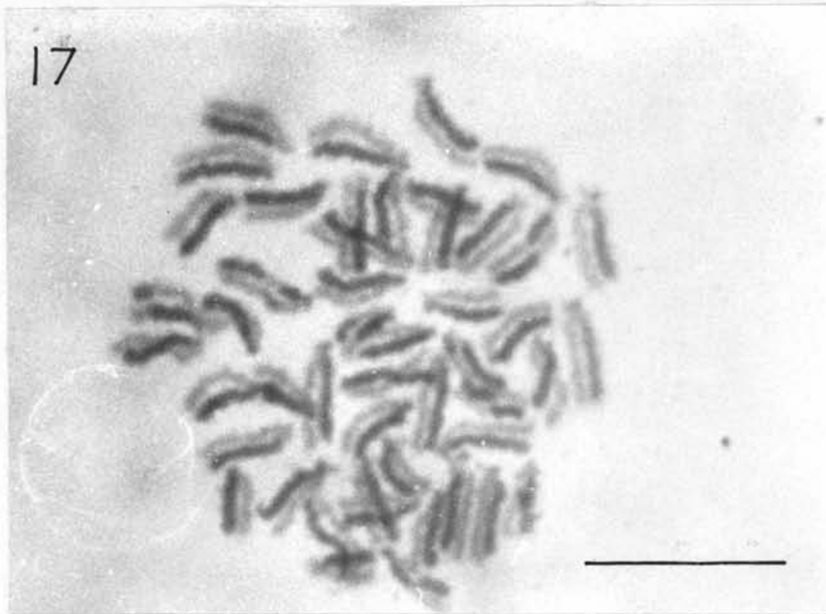


Plate 17. Differential staining of chromatids in _E. suratensis_ (bar = μ). Lightly stained chromatids indicate BrdU incorporation in both DNA strands (bifilarly substituted). Darkly stained chromatids indicate incorporation in one strand only ie., unifilarly substituted.

Plate 18. Differential staining of chromatids in _E. suratensis_ (bar = 10 μ).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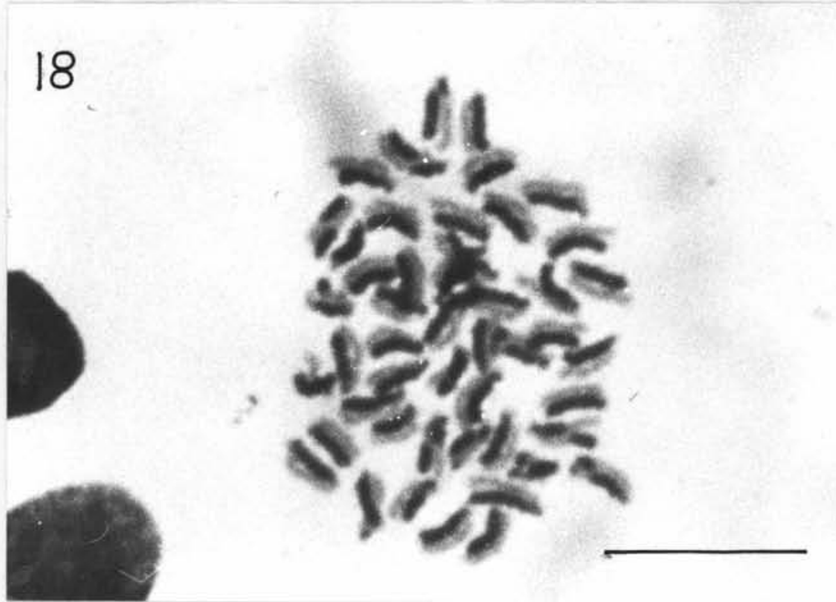
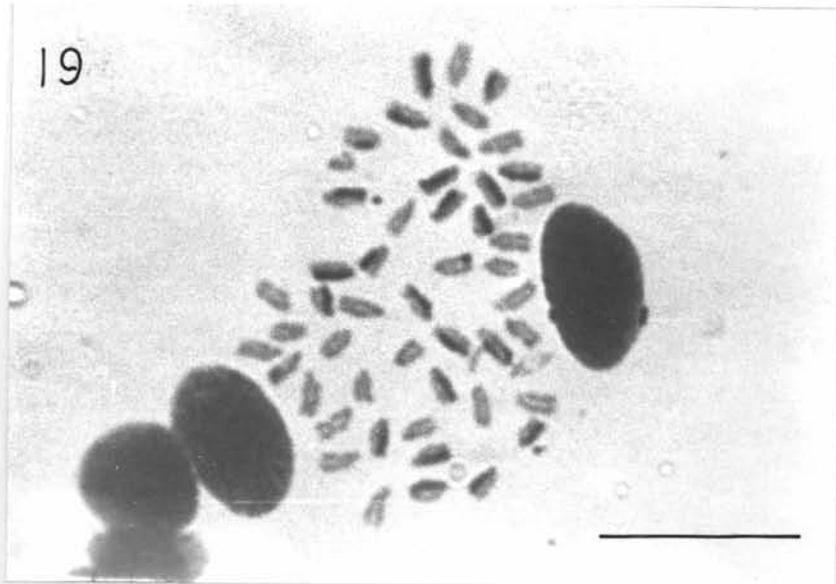

18

Plate 19. Third division cells of *E. suratensis* in the presence of Bromodeoxy-uridine showing total substitution in both DNA strands in most chromatids. (bar = 10 μ).

Plate 20. Third division cells of *E. suratensis* in the presence of Bromodeoxy-uridine showing total substitution in both DNA strands in most chromatids (bar = 10 μ).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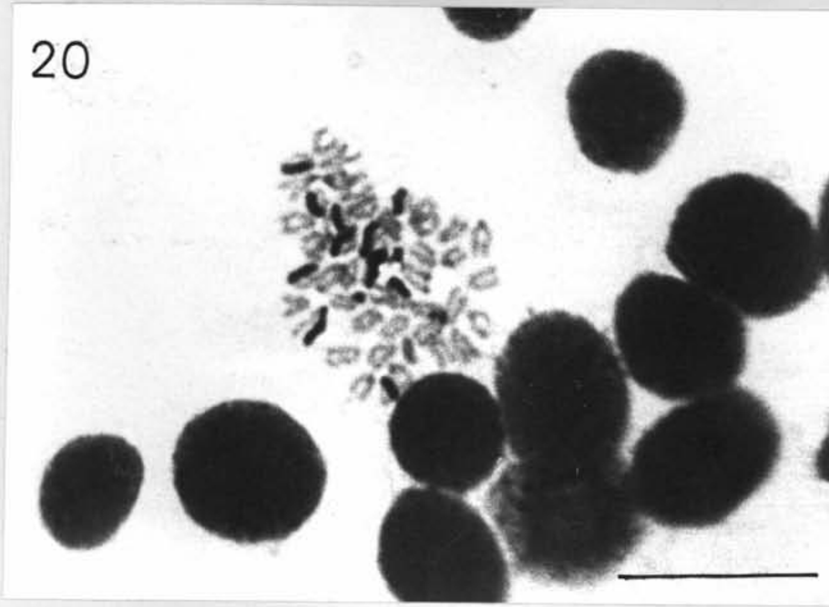

20

C Sister-chromatid exchanges: Base-line value of SCE was found to be 1.79 per metaphse (Table 6).

4. TREATMENTS

The results of various treatments are presented as follows:

(a) Methyl methane sulphonate (MMS):

(i) *E. maculatus:*

(a) Chromosome aberrations: The rate of chromosome aberrations per metaphase induced by three doses (lowest, middle and highest) were 0.029, 0.038 and 0.035 respectively against a control value of 0.007/metaphase. The middle and highest doses induced significant increases in chromosome aberrations. The aberrations scored were gaps, breaks and polyploids. The response trend was lowest $<$ highest $<$ middle (

(b) Micronuclei: No micronuclei incidence was observed in any of the doses tested.

(ii) *E. suratensis:*

(a) Chromosome aberrations: The chromosome aberrations induced by 3 different doses (50, 100, 150 μg/g) of MMS were 0.024, 0.059 and 0.040 per metaphase against a control value of 0.006 per metaphase. The middle and highest doses showed significant increases in the rate

of chromosome aberrations. The different types of aberrations scored were gaps, breaks fragments, rings etc. The results are presented in Table 9 and Fig. 5.

(b) Micronuclei: No micronuclei were observed in any of the three doses tested.

(c) Sister chromatid exchanges: The rates of SCE per metaphase induced by the lowest, middle and highest doses were 4.05, 9.0 and 12.0 respectively against the control value of 1.82 exchanges per metaphase. The results showed a dose dependent trend. Metaphase with long chromosomes showed a comparatively higher rate of exchange per chromosome. The results are presented in Table 13and Fig. 9.

(b) Cyclophosphamid (CP)

(i) E. maculatus:

(a) Chromosome aberrations: The rates of CA/metaphase were 0.035, 0.063 and 0.17, induced by the doses 25, 50 and 100 μg/g respectively as against the control rate of 0.007/metaphase. The several types of aberrations were gaps, breaks, exchanges, fragments, polyploids etc. The result showed a dose dependent trend and significance in all the three dose levels. Occurrence of telomeric end in one chromosome was observed in plates from one

of the animals exposed to a dose of 100 μg/g. All metaphases of this animal showed this condition in one chromosome of the second pair. The results are summarised in Table 8 and Fig. 4.

(b) Micronuclei: There was no occurrence of MN in any of the treatments in the species.

(ii) E. suratensis:

(a) Chromosome aberrations: The chromosome aberrations per metaphase induced by the 3 doses (25, 50 & 100 μg/g) of CP in this species were 0.014, 0.06 and 0.129. The control rate of CA was 0.006/metaphase. Significant results were obtained with the middle and highest doses whereas the lowest dose (25 μg/g) could not induce significant increase in CA. Aberrations were gaps, breaks, fragments, rings, centromeric separation etc. A dose dependent trend was evident. The results are given in Table 10 and Fig .6).

(b) Micronuclei: In this test, none of the doses could induce micronuclei formations. The control value of MN/1000 cells was zero.

(c) Sister-chromatid exchanges: Significant induction of SCE was observed in all the three doses. The SCE rates per metaphase were 3.12, 4.14 and 3.69 induced by 25,

50 and 100 μg/g body weight of CP respectively. The results though significant did not show a typical dose dependent trend. The control value was 1.82/metaphase. The response trend was lowest dose $<$ highest dose $<$ middle dose. Results are shown in Table 14 and Fig. 10.

(c) Methyl parathion (Metacid-50) MP:

(i) *E. suratensis*:

(a) Chromosome aberrations: This organophosphorus pesticide also induced chromosome aberrations which were significant at all dose levels tested. The rates of aberrations per metaphase were 0.025, 0.058 and 0.056 in the three dose levels i.e., 0.05, 0.1 and 0.2 ppm respectively. The control value was 0.006/metaphase. The aberrations scored were gaps, breaks, rings, centromeric separation etc. The middle and highest doses showed similar rates of CA. The results are shown in Table 11 and Fig. 7.

(b) Micronuclei: There was no occurrence of micronuclei in all the 3 doses of methyl parathion.

(c) Sister chromatid exchanges: The rates of SCE/metaphase induced by this pesticide were 3.17, 4.23 and 4.88 against the doses of 0.05, 0.1 and 0.2 ppm respectively. A clear dose dependent trend was indicated in SCE incidence. The control was 1.79 SCE per metaphase. Metaphase plates were low in frequency and differentiated plates

were somewhat sticky. Third division cells were most frequently observed and occasionally the number of third division cells were more than the second round cells. Results are presented in Table 15 and Fig. 11

(d) Phosphamidon (PM). Dimecron SL-85.

(i) *E. suratensis:*

(a) Chromosome aberrations: The values of chromosome aberrations per metaphase with 3 doses i.e., 0.5, 1 and 2 ppm were 0.01, 0.031 and 0.029 against a control rate of 0.006/metaphase. Except in the case of the lowest dose the values in the two higher doses were significant and more or less equal. The types of aberrations observed were gaps, breaks and fragments. Centromeric separations were frequently observed. In some cases stickiness and some non-specified aberrations were also seen. The results are given in Table 12 and Fig. 8.

(b) Micronuclei: As in all previous test chemicals, PM also could not induce MN in peripheral blood cells of the species with any of the doses tested.

(c) Sister-chromatid exchanges: The SCE rates induced by the pesticide did not show a typical dose dependent trend. The rates of SCE/metaphase were 3.22, 6.46 and 5.89, induced by 0.5, 1 and 2 ppm of PM respectively against

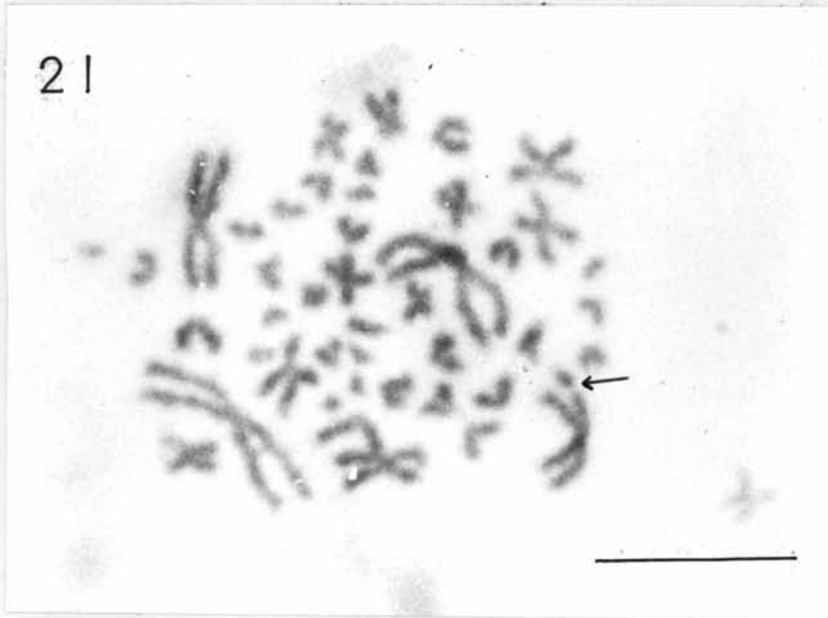
a control rate of 1.79 exchanges per metaphase. In this case the frequency of differentiated metaphase (SCD) cells was low. The results are summarised in Table 16 and Fig. 12.

5. FIELD STUDY

5000 erythrocytes from each of the 10 specimens studied showed no micronuclei. The SCE rates/metaphase in specimens collected from IFP Jetty were significantly higher than the control. The rate was 2.69 exchanges per metaphase against the control rate of 1.79. The results are summarised in Table 17.

Plate 21. Chromatid break in one of the chromosomes of the second pair (bar = 10 μ)
Species : *E. maculatus*.

Plate 22. Chromatid break in one chromosome of the first pair (bar = 10 μ).
species : *E. maculatus*.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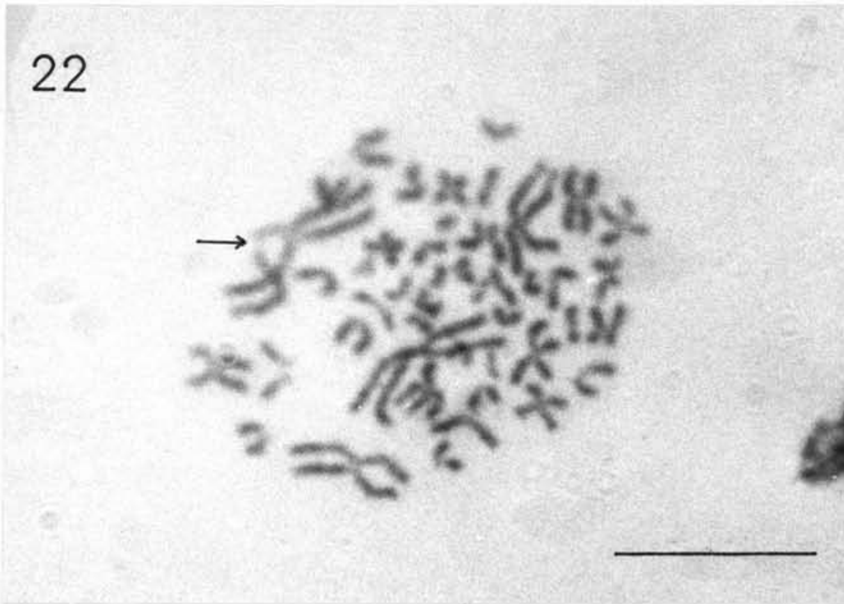

22

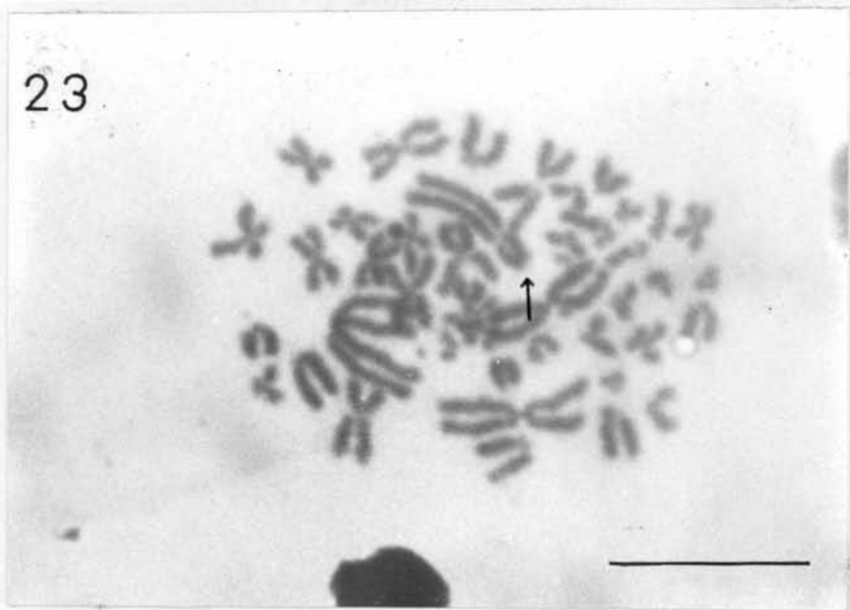
Plate 23. Deletion of distal part of a chromosome in the first pair (bar = 10 μ).

Species : _E. maculatus._

Plate 24. Multiple aberrations like gaps, breaks, fragments, complex rearrangements etc. None of the aberrations are specifically marked.

(bar = 10 μ)

Species: _E. maculatus_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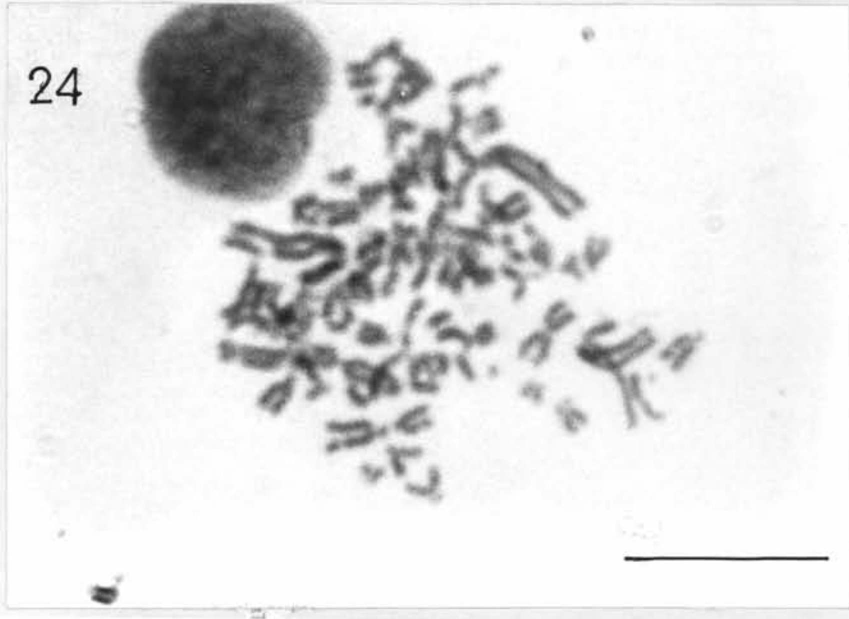
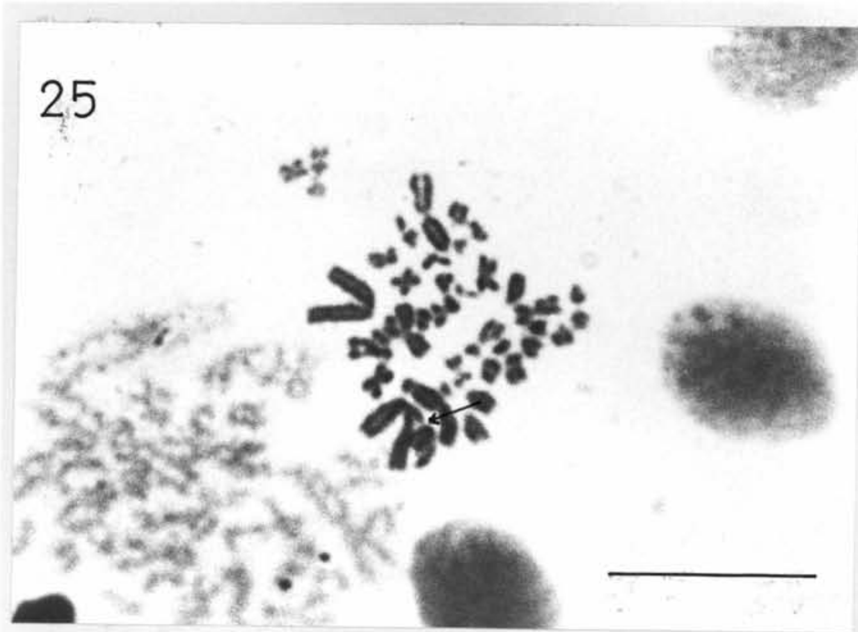

24

Plate 25. Chromatid break and probable exchange (bar = 10 μ).

Species : _E._ _maculatus_.

Plate 26. Chromatid gap (distal) in one of the chromosomes (bar = 10 μ).

Species : _E._ _suratensis_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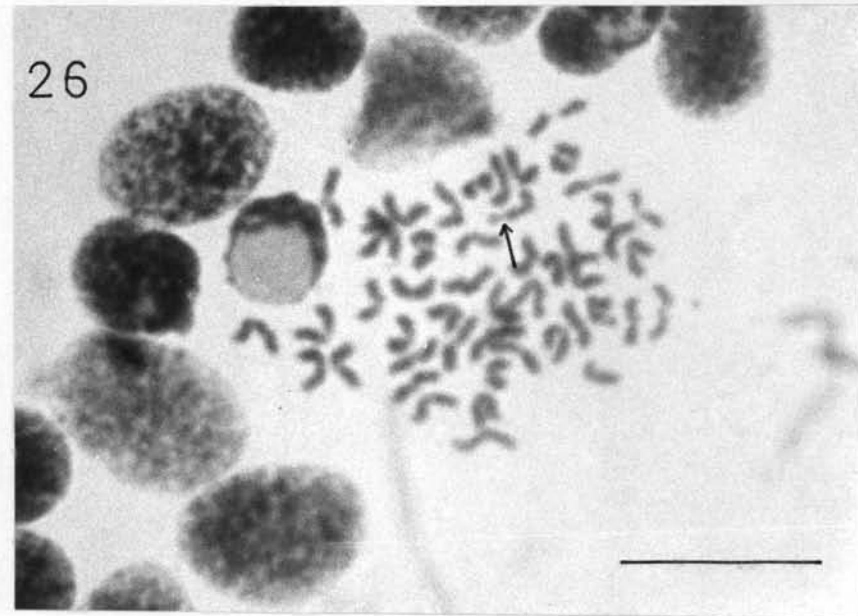
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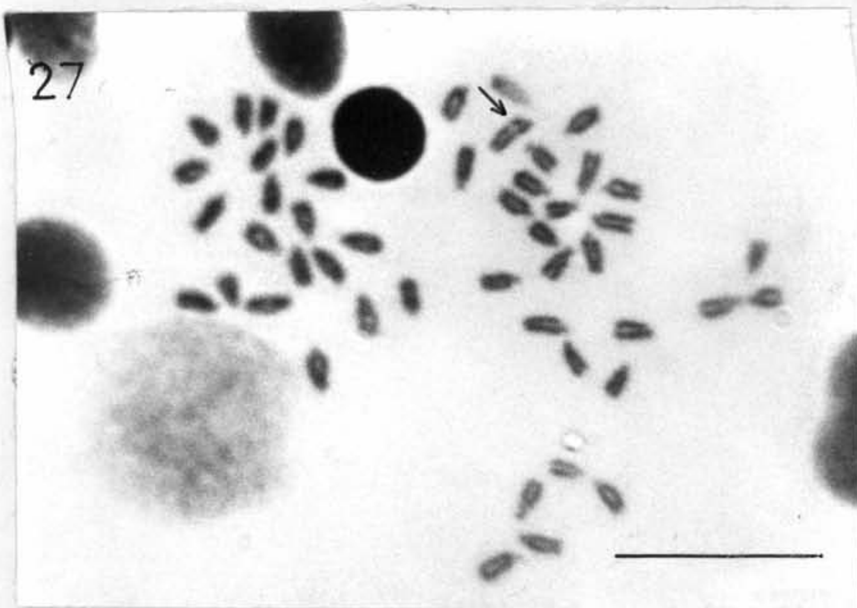
Plate 27. Iso-chromatid gap in one of the Chromosomes (bar = 10 μ). Species: *E. suratensis*

Plate 28. Chromosome fragmentation (bar = 10 μ). Species : *E. suratensis*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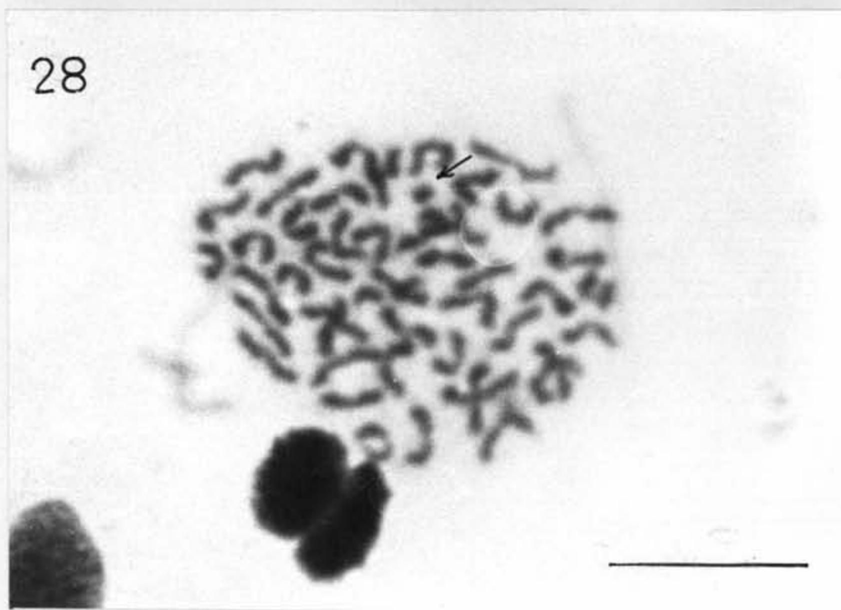
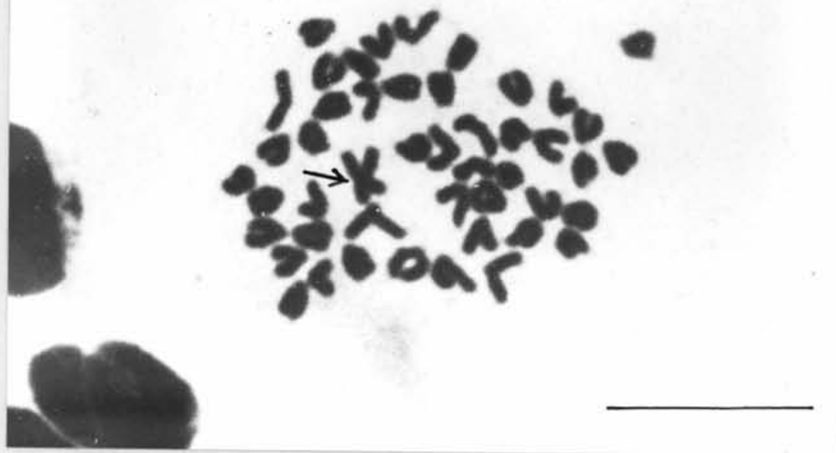

28

Plate 29. Centromeric fusion (bar = 10 μ)

Species : _E_. _suratensis_

Plate 30. Centromeric separations (fissions)
Only some indicated.
(bar = 10 μ).

Species : _E_. _suratensis_.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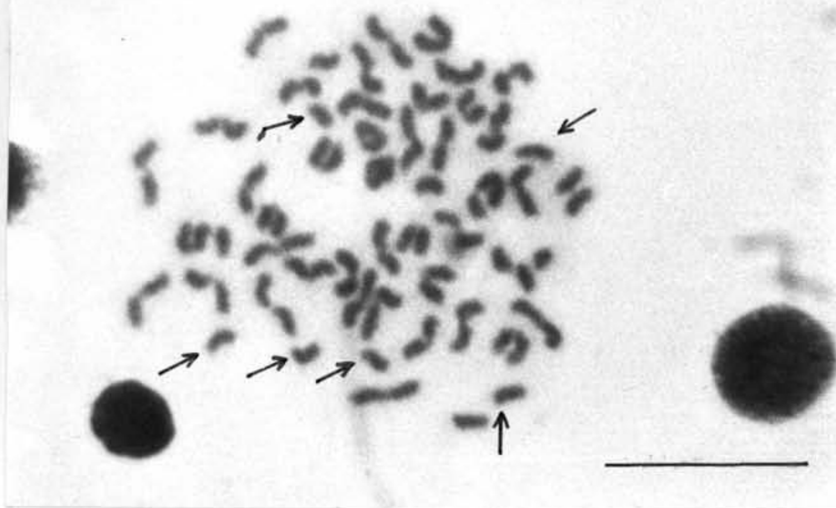
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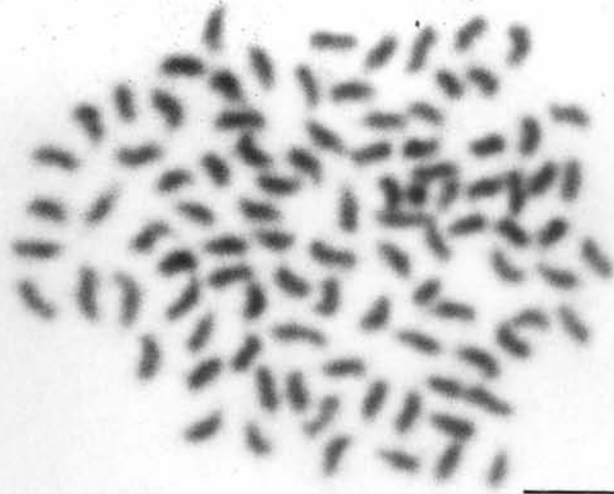
Plate 31. Polyploid cell (bar = 10 μ)

Species : *E. suratensis*

Plate 32. Micronucleus formation (bar = 10 μ).

Species : *E. suratensis*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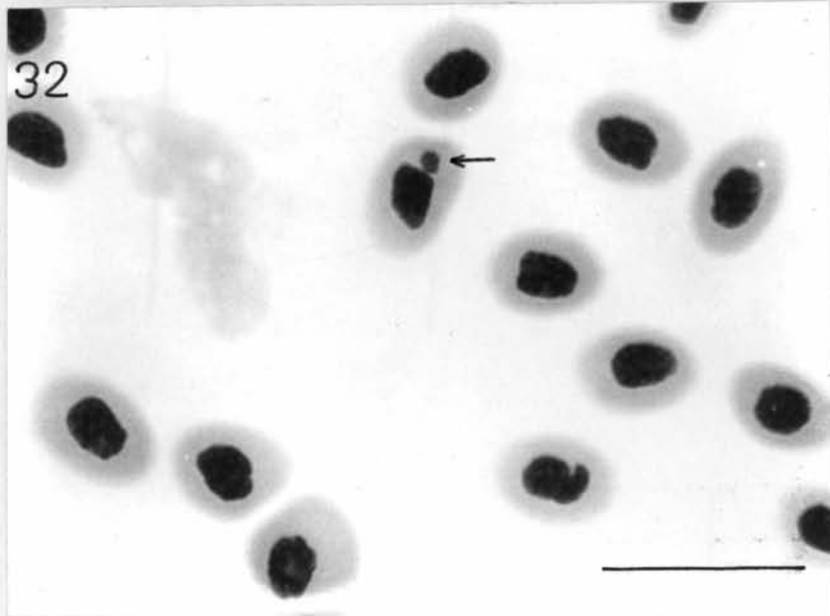
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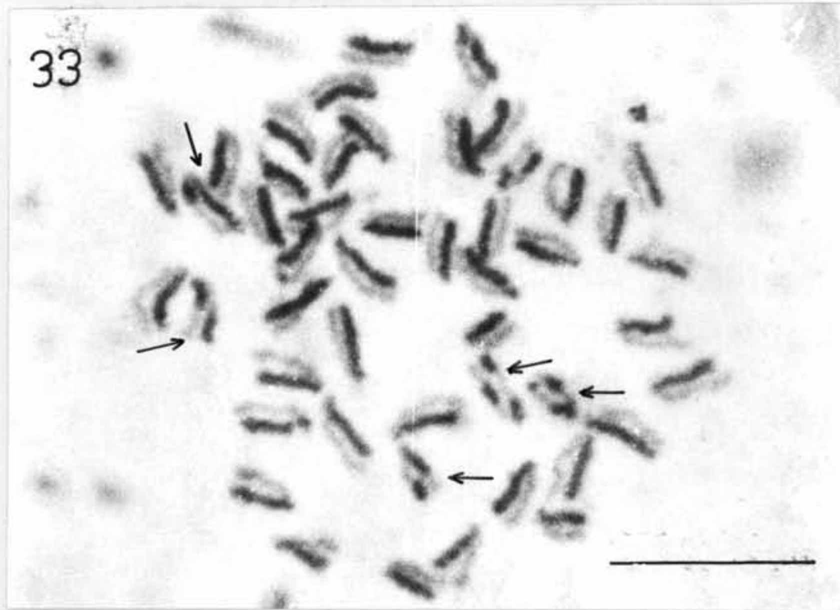
Plate 33. Multiple sister chromatid exchanges
Only some indicated.
(bar = 10 μ)
Species : *E. suratensis*

Plate 34. Multiple sister chromatid exchanges. Only some indicated.
(bar = 10 μ).
Species : *E. suratensis.*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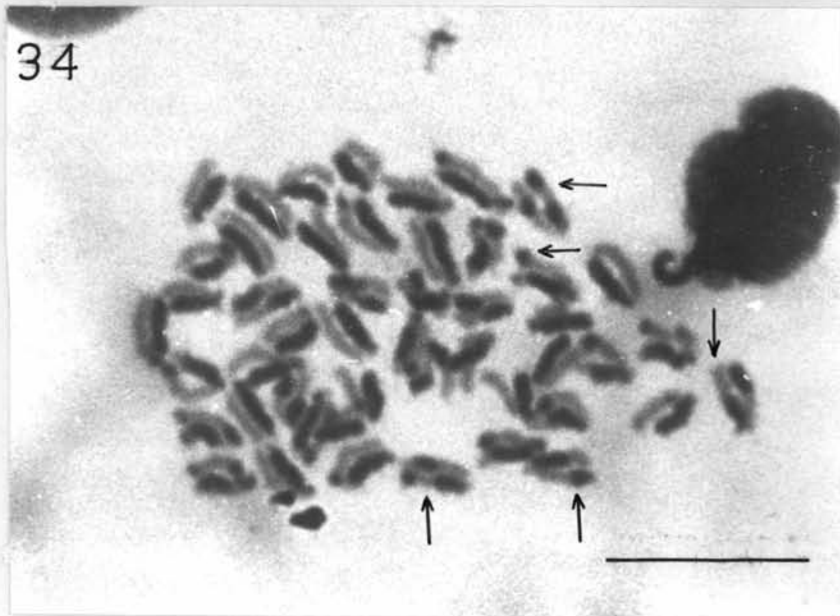
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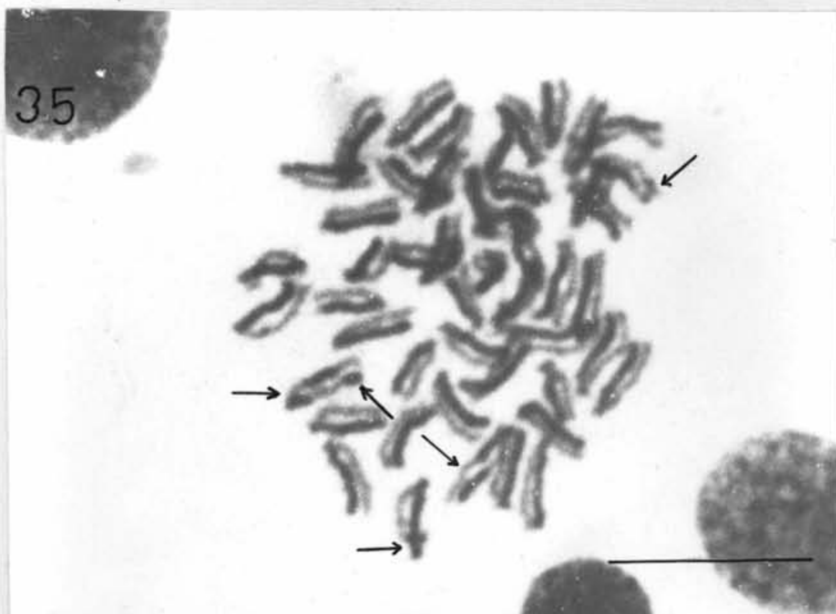
Plate 35. Multiple sister chromatid exchanges. Only some indicated.
(bar = 10 μ).
Species : _E._ _suratensis_

Plate 36. Multiple sister chromatid exchanges. Only some indicated.
(bar = 10 μ).
Species : _E._ _suratensis_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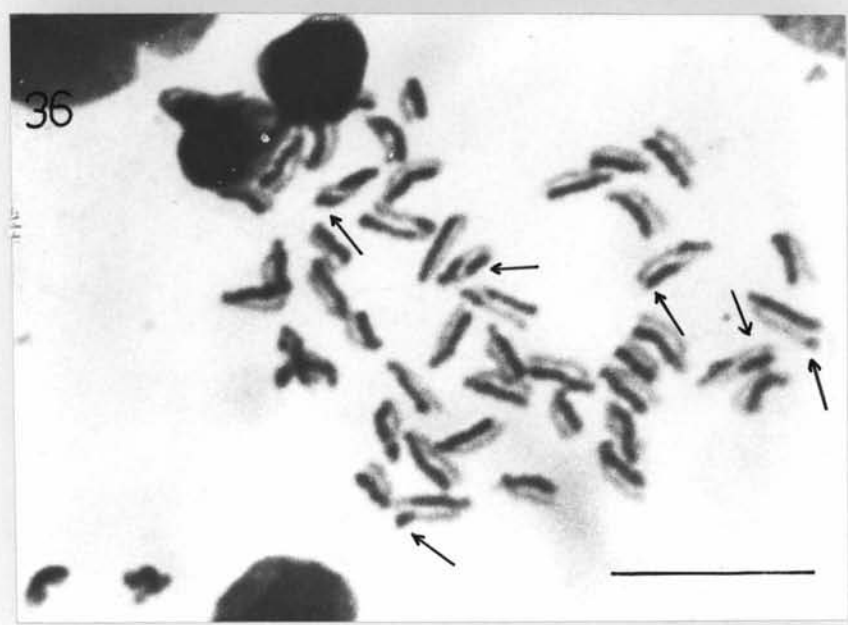
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Table-1. Chromosome lengths, relative lengths and arm ratios of *E. maculatus*

Chromosome pair No.	Chromosome length (μ) ($\bar{X}$ ± S.D.)	Relative lengths (%)	Arm Ratio	Chromosome Type
1.	7.350 ± 0.239	16.741	1.112	M
2.	5.057 ± 0.398	11.518	1.095	M
3.	3.563 ± 0.114	8.115	1.066	M
4.	3.065 ± 0.265	6.981	1.105	M
5.	2.474 ± 0.234	5.635	1.259	M
6.	2.130 ± 0.057	4.851	1.838	SM
7.	1.915 ± 0.132	4.361	1.174	M
8.	1.800 ± 0.178	4.099	-	A
9.	1.739 ± 0.187	3.961	1.141	M
10.	1.532 ± 0.265	3.489	-	A
11.	1.494 ± 0.199	3.402	-	A
12.	1.417 ± 0.175	3.227	-	A
13.	1.364 ± 0.231	3.106	-	A
14.	1.282 ± 0.284	2.920	2.946	SM
15.	1.089 ± 0.251	2.480	3.187	ST
16.	0.996 ± 0.132	2.268	-	A
17.	0.996 ± 0.132	2.268	-	A
18.	0.965 ± 0.165	2.198	-	A
19.	0.888 ± 0.052	2.022	-	A
20.	0.850 ± 0.060	1.936	-	A
21.	0.689 ± 0.229	1.569	-	A
22.	0.689 ± 0.229	1.569	-	A
23.	0.559 ± 0.278	1.273	-	A

Total chromosome length (n) = 43.903 μ.

Table-2. Chromosome lengths and relative lengths. *E. suratensis.*

Chromo-some Pair No.	Chromosome length (μ) ($\bar{X}$ ± S.D.)	Relative length (%)	Chromosome Type
1.	4.401 ± 0.108	5.230	A
2.	4.354 ± 0.094	5.174	A
3.	4.073 ± 0.054	4.840	A
4.	3.956 ± 0.047	4.701	A
5.	3.862 ± 0.090	4.589	A
6.	3.839 ± 0.108	4.562	A
7.	3.745 ± 0.152	4.450	A
8.	3.652 ± 0.180	4.339	A
9.	3.652 ± 0.180	4.339	A
10.	3.511 ± 0.179	4.172	A
11.	3.511 ± 0.179	4.172	A
12.	3.464 ± 0.108	4.117	A
13.	3.464 ± 0.108	4.117	A
14.	3.464 ± 0.108	4.117	A
15.	3.438 ± 0.090	4.086	A
16.	3.418 ± 0.054	4.061	A
17.	3.301 ± 0.090	3.922	A
18.	3.230 ± 0.094	3.838	A
19.	3.137 ± 0.179	3.728	A
20.	3.043 ± 0.094	3.616	A
21.	3.043 ± 0.094	3.616	A
22.	3.043 ± 0.094	3.616	A
23.	2.879 ± 0.090	3.421	A
24.	2.669 ± 0.094	3.172	A

Total chromosome length (n) = 84.149 μ.

Table-3. Analysis of base-line chromosome aberrations in *E. maculatus*

Animal code	No. of metaphases examined	No. of aberrations*	Aberration per metaphase
A	71	0	0.000
B	51	1	0.019
C	76	0	0.000
D	43	0	0.000
E	43	0	0.000
F	49	1	0.020
G	58	0	0.000
H	52	0	0.000
I	52	0	0.000
J	49	0	0.000
Total 10	544	2	0.003 ± 0.007($\bar{X}$ ± S E)

* gap, break.

Table-4. Analysis of base-line chromosome aberrations in *E. suratensis.*

Animal Code	No. of metaphases examined	No. of aberrations*	Aberration per metaphase
A	86	2	0.023
B	100	1	0.010
C	100	0	0.000
D	100	0	0.000
E	55	1	0.018
F	52	0	0.000
G	60	1	0.016
H	60	0	0.000
I	45	0	0.000
J	50	0	0.000
K	64	0	0.000
Total 11	772	5	0.006 ± 0.0027 ($\bar{X}$ ± SE)

* gap, break.

Table-5. Analysis of base-line SCE in E. maculatus

Animal code	No. of metaphases (SCD) examined	No. of SCE	No. of SCE per metaphase
A	22	18	0.81
B	32	21	0.65
C	15	10	0.66
D	20	15	0.75
E	18	12	0.66
F	40	34	0.85
G	17	10	0.58
H	18	7	0.38
Total 8	182	127	0.67 ± 0.051 ($\bar{X}$ ± SE)

Table-6. Analysis of base-line SCE in *E. suratensis*

Animal Code	No. of metaphases (SCD) examined	No. of SCE	No. of SCE per metaphase
A	22	30	1.36
B	29	52	1.79
C	16	28	1.75
D	15	31	2.06
E	17	35	2.05
F	25	52	2.08
G	20	30	1.50
H	20	36	1.80
I	35	57	1.62
J	40	76	1.90
Total 10	239	427	1.79 ± 0.077 ($\bar{X}$ ± SE)

Table-7. Analysis of chromosome aberrations induced by Methyl methane sulphonate in *E. maculatus*

Dose (μg/g)	No. of Animals	No. of metaphases examined	Total no. of aberrations*	Aberration per metaphase ± SE
50	4	167	5	0.029 ± 0.012
100	4	233	9	0.038 ± 0.012**
150	4	168	6	0.035 ± 0.014**
Control	5	269	2	0.007 ± 0.005

* gap, break, polyploidy

** significant ($Z \geq 1.96$)

Table-8. Analysis of chromosome aberrations induced by Cyclophosphamide in E. maculatus

Dose (µg/g)	No. of animals	No. of metaphases examined	Total no. of aberrations*	Aberration per metaphase ± SE
25	4	170	6	0.035 ± 0.014**
50	4	157	10	0.063 ± 0.018**
100	4	170	29	0.170 ± 0.028**
Control	5	269	2	0.007 ± 0.005

* gap, break, fragment, multiple aberration, complex rearrangement

** significant (Z ⩾ 1.96)

Table- 9. Analysis of chromosome aberrations induced by Methyl methane sulphate in <u>E. suratensis.</u>

Dose (µg/g)	No. of animals	No. of metaphases examined	Total no. of aberrations*	Aberration per metaphase ± S E
50	4	165	4	0.024 ± 0.011
100	4	237	14	0.059 ± 0.015**
150	4	171	7	0.040 0.015**
Control	6	307	2	0.006 ± 0.004

* gap, break, fragment, ring, chromatid deletion

** significant (Z ⩾ 1.96).

Table-10. Analysis of chromosome aberrations induced by Cyclophosphamide in *E. suratensis.*

Dose ($\mu g/g$)	No. of Animals	No. of metaphases examined	Total no. of aberrations*	Aberration per metaphase ± SE.
25	4	211	3	0.014 ± 0.008
50	4	198	12	0.060 ± 0.016**
100	4	154	20	0.129 ± 0.029**
Control	6	307	2	0.006 ± 0.004

* gap, break, fragment, centromeric fusion

** significant ($Z \geq 1.96$)

Table-11. Analysis of chromosome aberrations induced by Methyl parathion in E. suratensis.

Dose (ppm)	No. of Animals	No. of metaphases examined	Total no. of aberrations*	Aberration per metaphase ± SE.
0.05	4	198	5	0.025 ± 0.011**
0.1	4	205	12	0.058 ± 0.0.16**
0.2	4	194	11	0.056 ± 0.016**
Control	11	772	5	0.006 ± 0.002

* gap, break, fragment, ring centromeric separation

** significant ($Z \geqslant 1.96$)

Table-12. Analysis of chromosome aberrations induced by Phosphamidon in *E. suratensis.*

Dose (ppm)	No. of Animals	No. of metaphases examined	Total No of aberrations*	Aberration per metaphase ± SE
0.5	4	199	2	0.010 ± 0.007
1	4	315	10	0.031 ± 0.009**
2	4	206	6	0.029 ± 0.011**
Control	11	772	5	0.006 ± 0.002

* gap, break, fragment, minute, centromeric separation.

** significant (Z ⩾ 1.96)

Table-13. Analysis of SCE induced by Methyl methane sulphonate in *E. suratensis*

Dose (μg/g)	No. of Animals	No. of metaphases (SCD) examined	Total no. of SCE	No. of SCE per metaphase ± SE
50	4	95	385	4.05 ± 0.360*
100	4	111	999	9.00 ± 0.805*
150	4	61	732	12.00 ± 1.471*
Control	6	104	190	1.82 ± 0.120

* significant (Z ⩾ 1.96)

Table-14. Analysis of SCE induced by Cyclophosphamide in *E. suratensis.*

Dose (μg/g)	No. of Animals	No. of metaphases (SCD) examined	Total no. of SCE	No. of SCE per metaphase ± SE.
25	4	47	147	3.12 ± 0.376*
50	4	47	195	4.14 ± 0.527*
100	4	65	240	3.69 ± 0.391*
Control	6	104	190	1.82 ± 0.120

* significant ($Z \geqslant 1.96$)

Table-15. Analysis of SCE induced by Methyl parathion in *E. suratensis.*

Dose (ppm)	No. of Animals	No. of metaphases (SCD) examined	Total no. of SCE	No. of SCE per metaphase ± SE
0.05	4	41	130	3.17 ± 0.409*
0.1	4	46	195	4.23 ± 0.546*
0.2	4	43	210	4.88 ± 0.664*
Control	10	239	426	1.79 ± 0.077

* significant ($Z \geq 1.96$)

Table-16. Analysis of SCE induced by Phosphamidon in *E. suratensis*

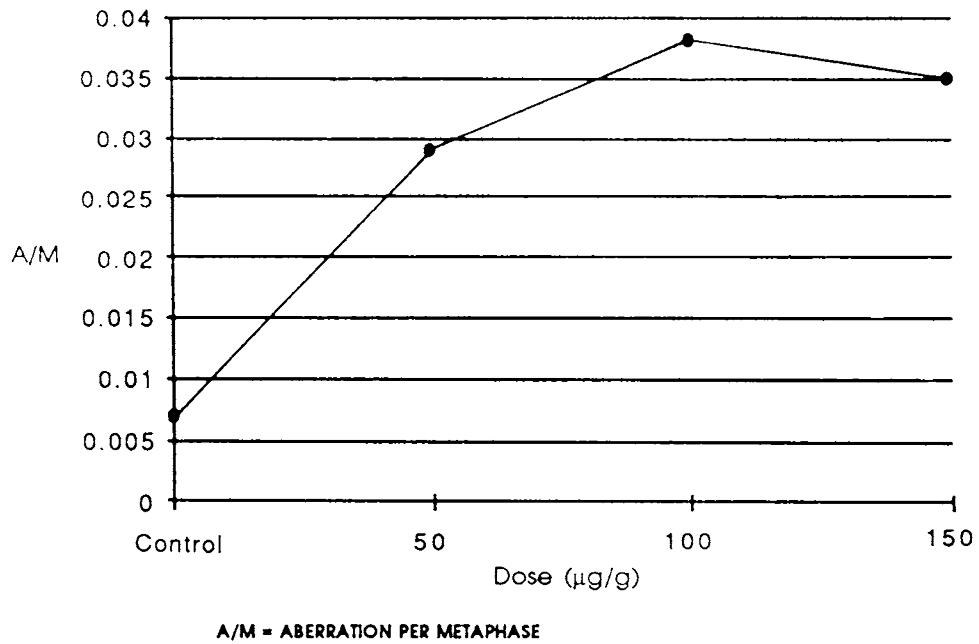
Dose (ppm)	No. of animals	No. of metaphases (SCD) examined	Total No. of SCE	No. of SCE per metaphase ± SE
0.5	4	41	132	3.22 ± 0.418*
1	4	52	336	6.46 ± 0.823*
2	4	49	289	5.89 ± 0.316*
Control	10	239	426	1.79 ± 0.077

* significant ($Z \geq 1.96$)

Table-17. Analysis of SCE in *E. suratensis* collected from polluted area (I.F.P. Boat Jetty area)

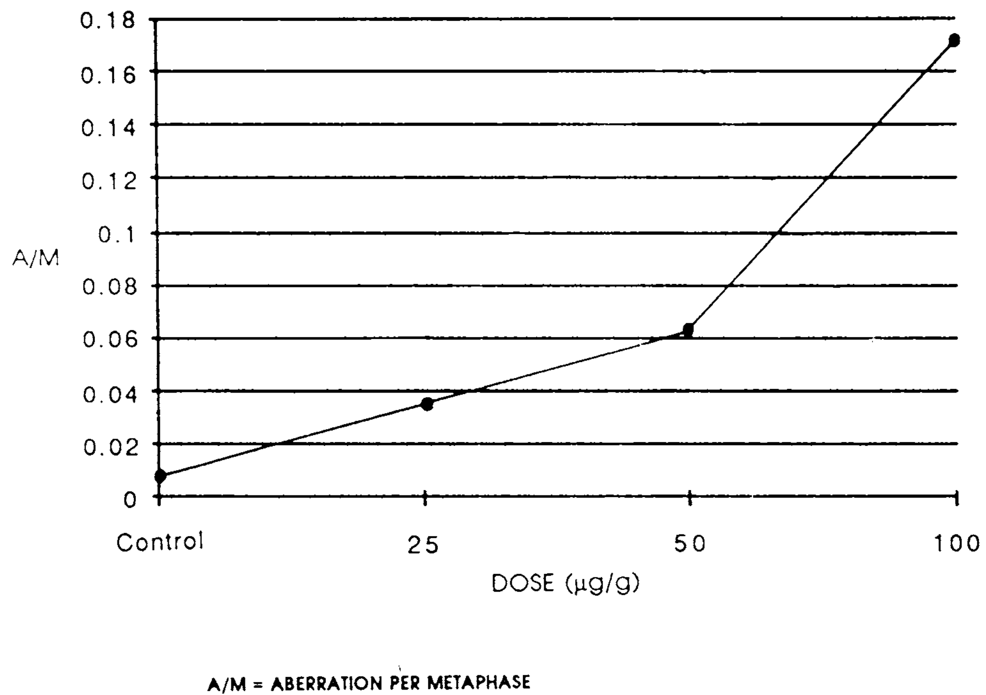
Animal Code	No. of metaphases (SCD) examined	No. of SCE	No. of SCE per metaphase
A	9	24	2.66
B	19	48	2.52
C	10	31	3.10
D	16	37	2.31
E	11	25	2.27
F	23	59	2.56
G	7	22	3.14
H	18	51	2.83
I	6	19	3.16
J	8	19	2.37
TOTAL 10	127	335	2.69 ± 0.110 ($\bar{X}$ ± SE)

FIG.3. ANALYSIS OF CHROMOSOME ABERRATIONS INDUCED BY METHYL METHANE SULPHONATE IN *E. MACULATUS*



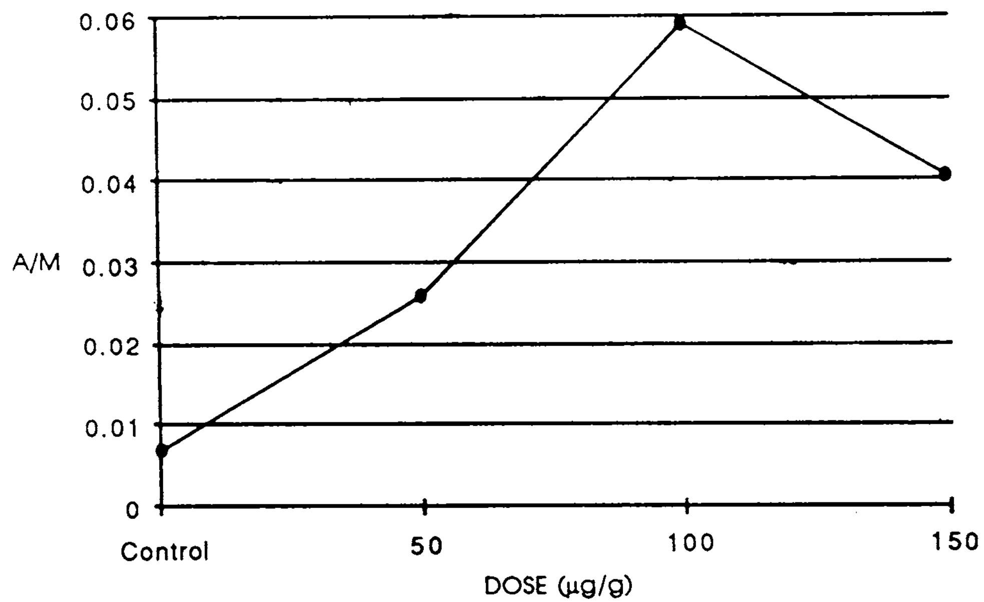
A/M = ABERRATION PER METAPHASE

FIG.4. ANALYSIS OF CHROMOSOME ABERRATIONS INDUCED BY CYCLOPHOSPHAMIDE IN *E. MACULATUS*



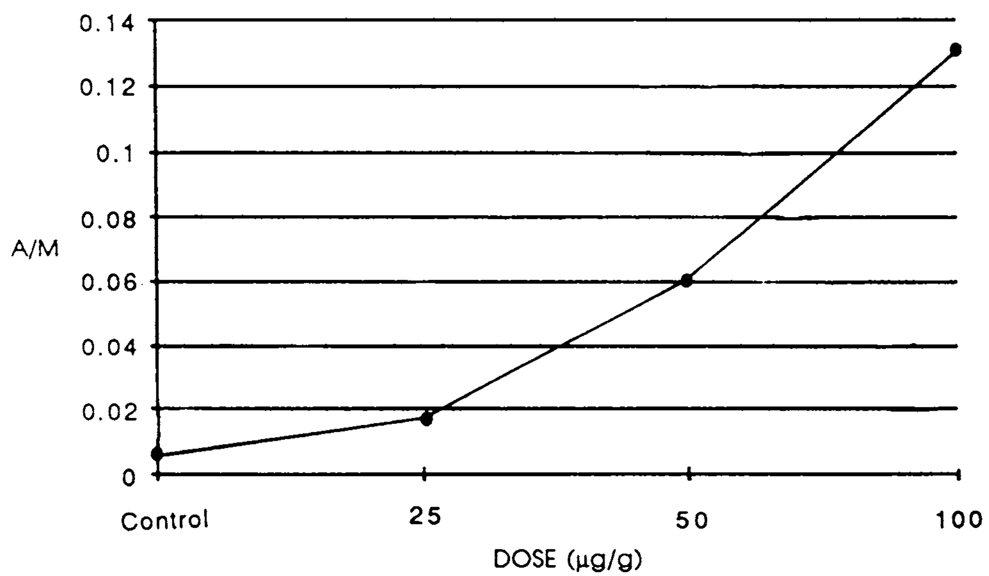
A/M = ABERRATION PER METAPHASE

FIG.5. ANALYSIS OF CHROMOSOME ABERRATIONS INDUCED BY METHYL METHANE SULPHONATE IN *E. SURATENSIS*



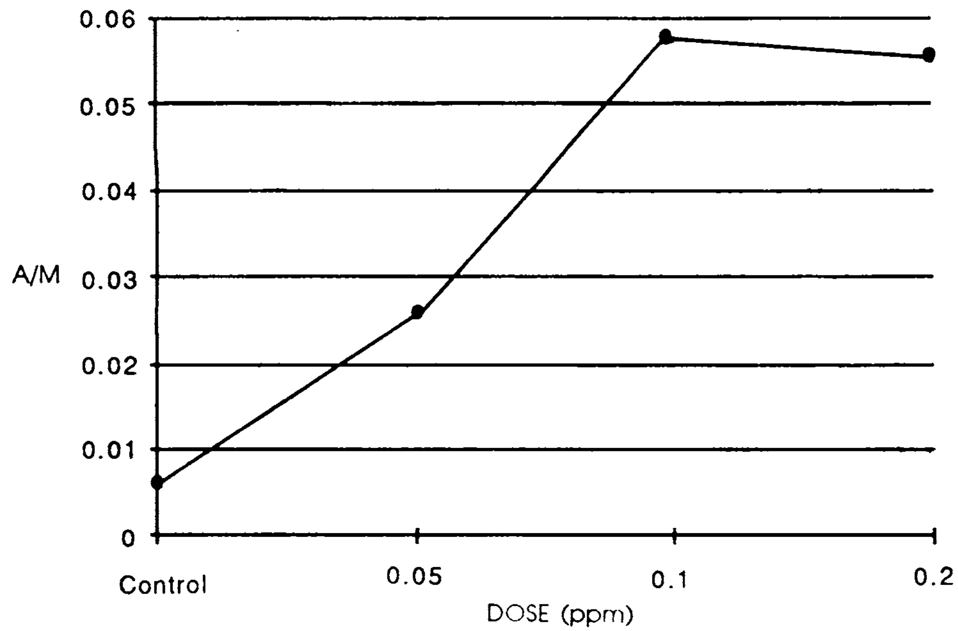
A/M = ABERRATION PER METAPHASE

FIG.6. ANALYSIS OF CHROMOSOME ABERRATIONS INDUCED BY CYCLOPHOSPHAMIDE IN *E. SURATENSIS*



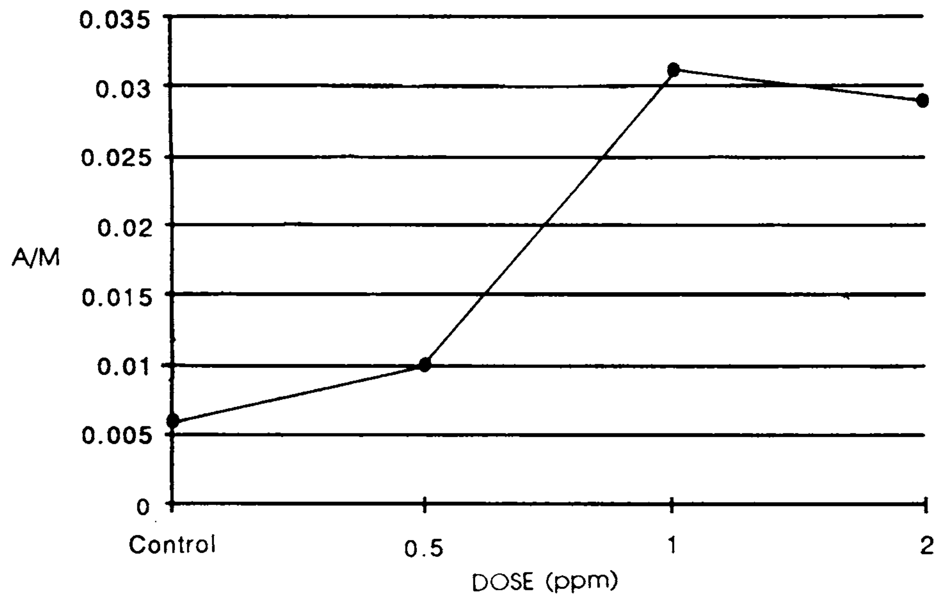
A/M = ABERRATION PER METAPHASE

FIG.7. ANALYSIS OF CHROMOSOME ABERRATIONS INDUCED BY METHYL PARATHION IN *E. SURATENSIS*



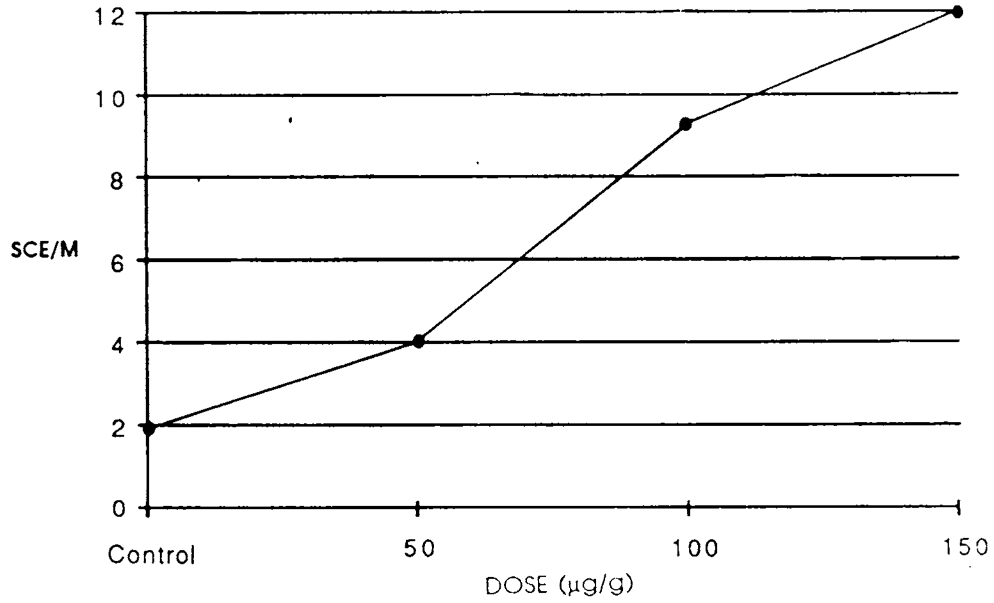
A/M = ABERRATION PER METAPHASE

FIG.8 ANALYSIS OF CHROMOSOME ABERRATIONS INDUCED BY PHOSPHAMIDON IN *E. SURATENSIS*



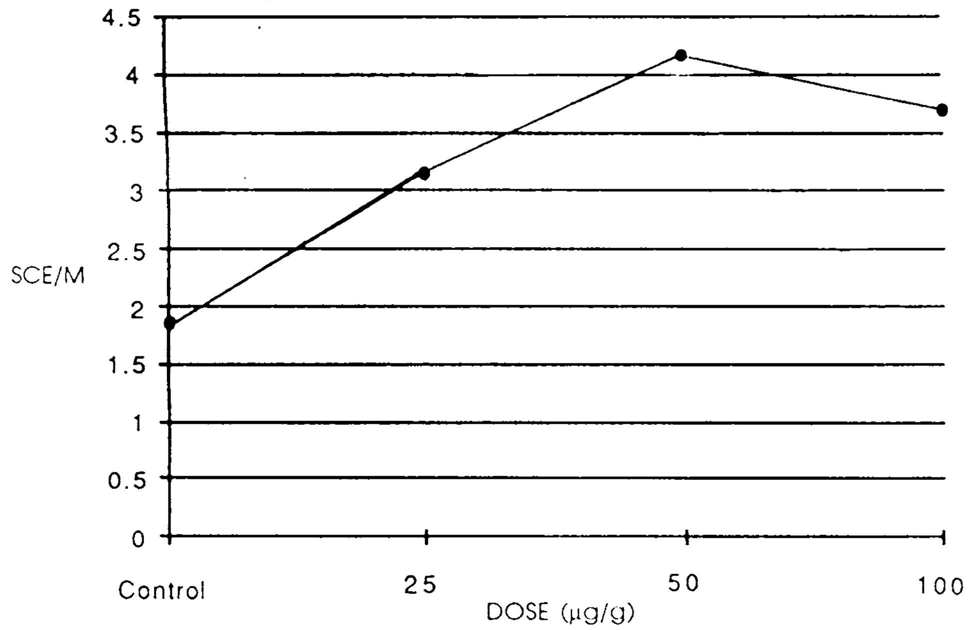
A/M = ABERRATION PER METAPHASE

FIG.9. ANALYSIS OF SCE INDUCED BY METHYL METHANE SULPHONATE IN *E. SURATENSIS*



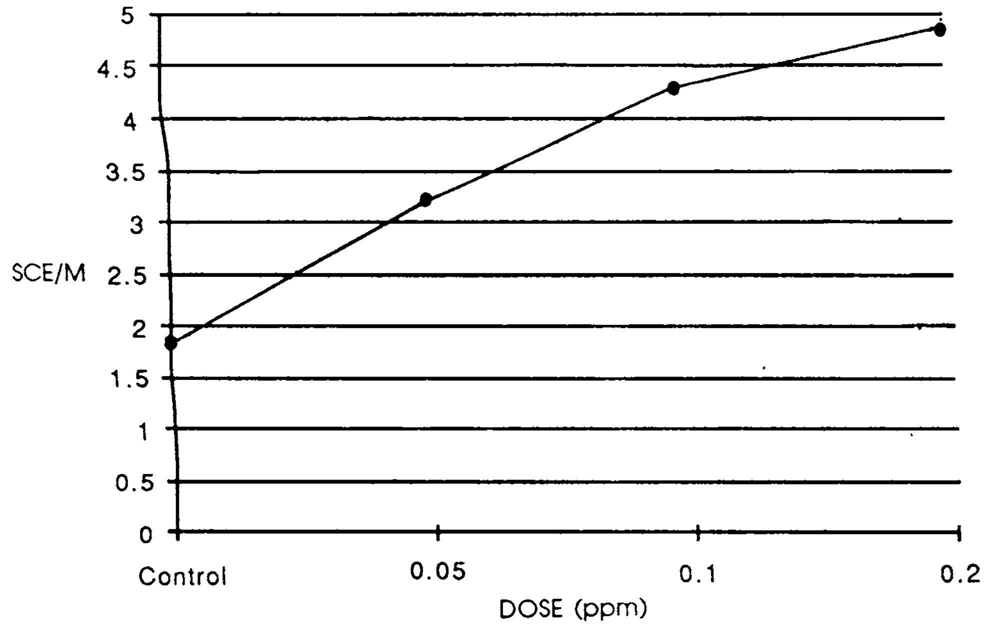
SCE = SISTER CHROMATID EXCHANGES
SCE/M = SCE PER METAPHASE

FIG.10. ANALYSIS OF SCE INDUCED BY CYCLOPHOSPHAMIDE IN *E. SURATENSIS*



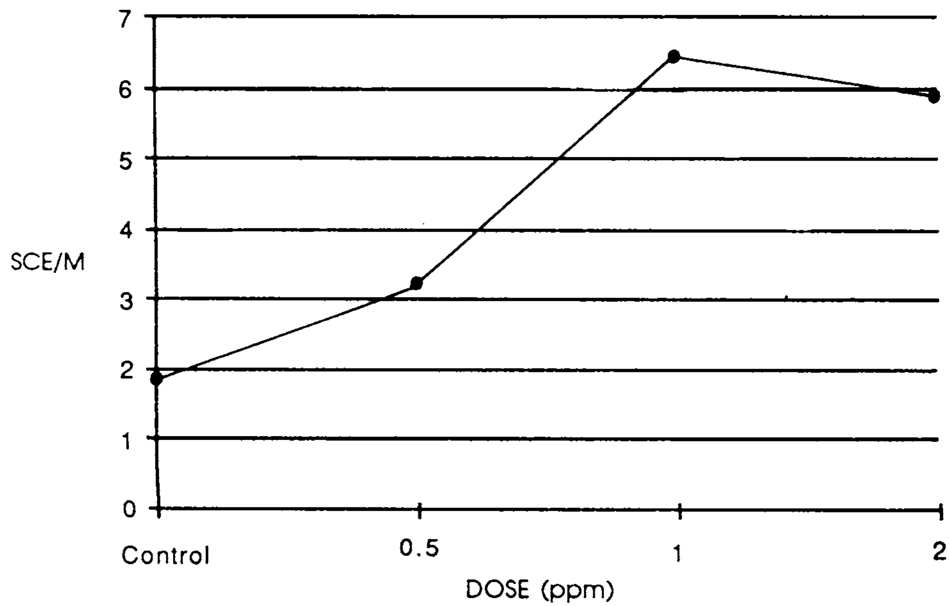
SCE = SISTER CHROMATID EXCHANGES
SCE/M = SCE PER METAPHASE

FIG.11. ANALYSIS OF SCE INDUCED BY METHYL PARATHION IN *E. SURATENSIS*



SCE = SISTER CHROMATID EXCHANGES
SCE/M = SCE PER METAPHASE

FIG.12. ANALYSIS OF SCE INDUCED BY PHOSPHAMIDON IN *E. SURATENSIS*



SCE = SISTER CHROMATID EXCHANGES
SCE/M = SCE PER METAPHASE

FIG.13. HISTOGRAM OF CHROMOSOME LENGTHS OF *E. MACULATUS*

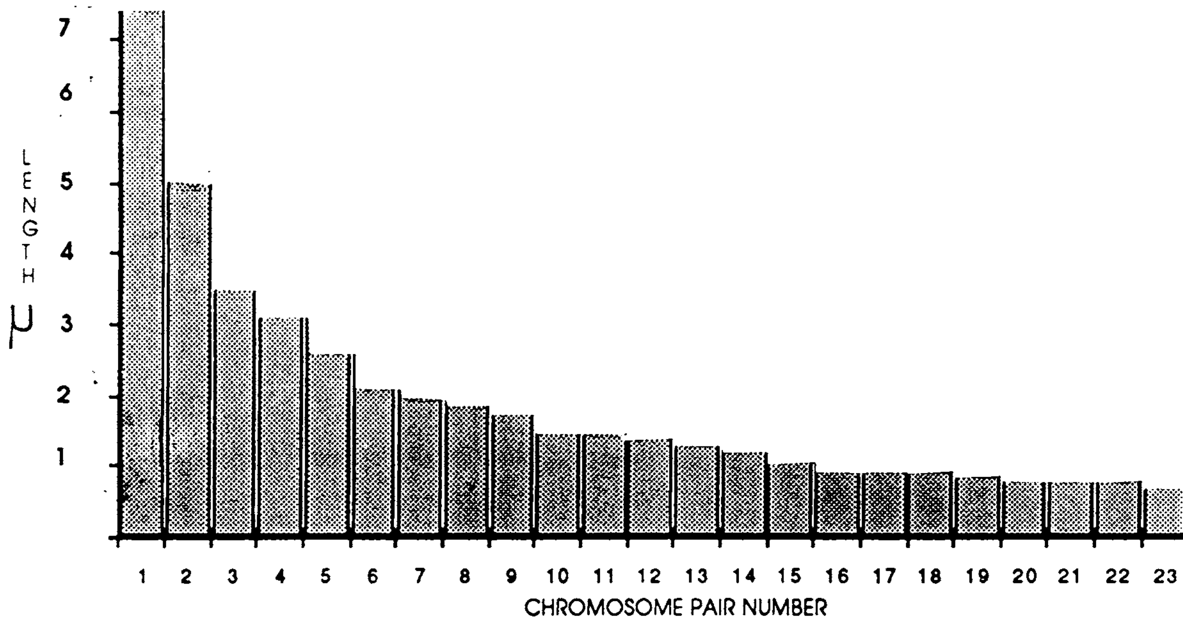


FIG.14. HISTOGRAM OF CHROMOSOME LENGTHS (μ) OF E. SURATENSIS

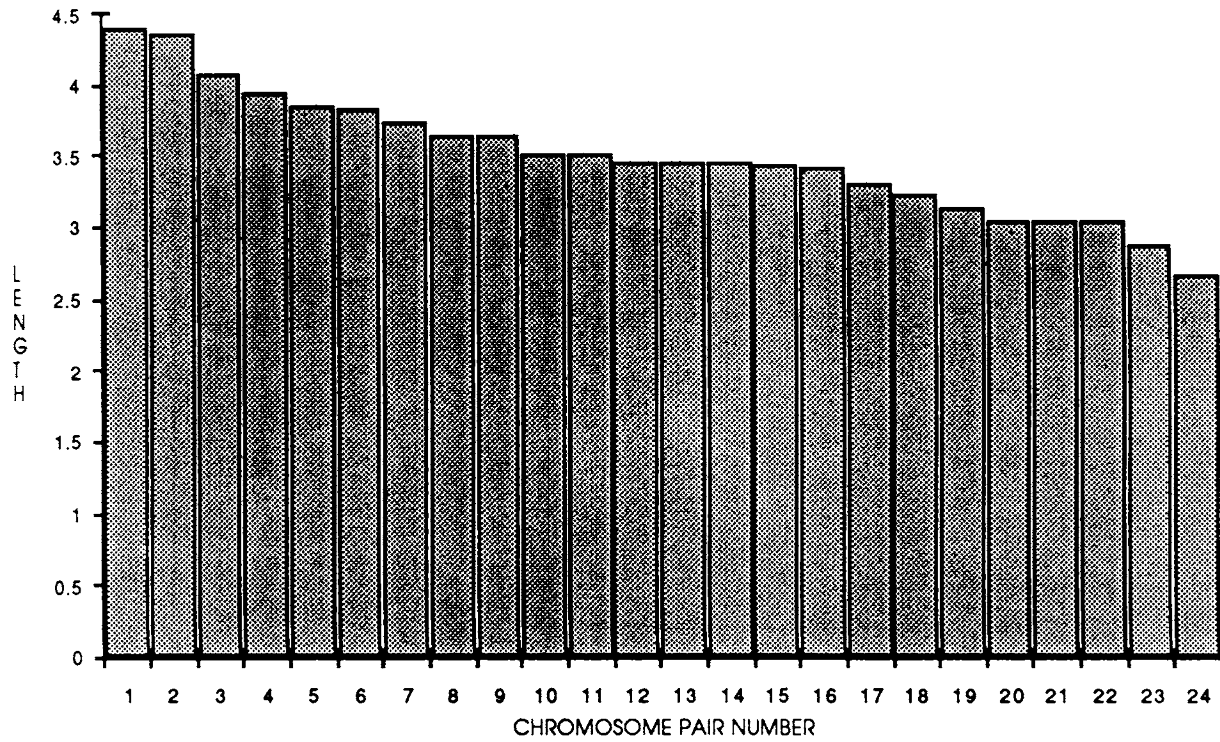
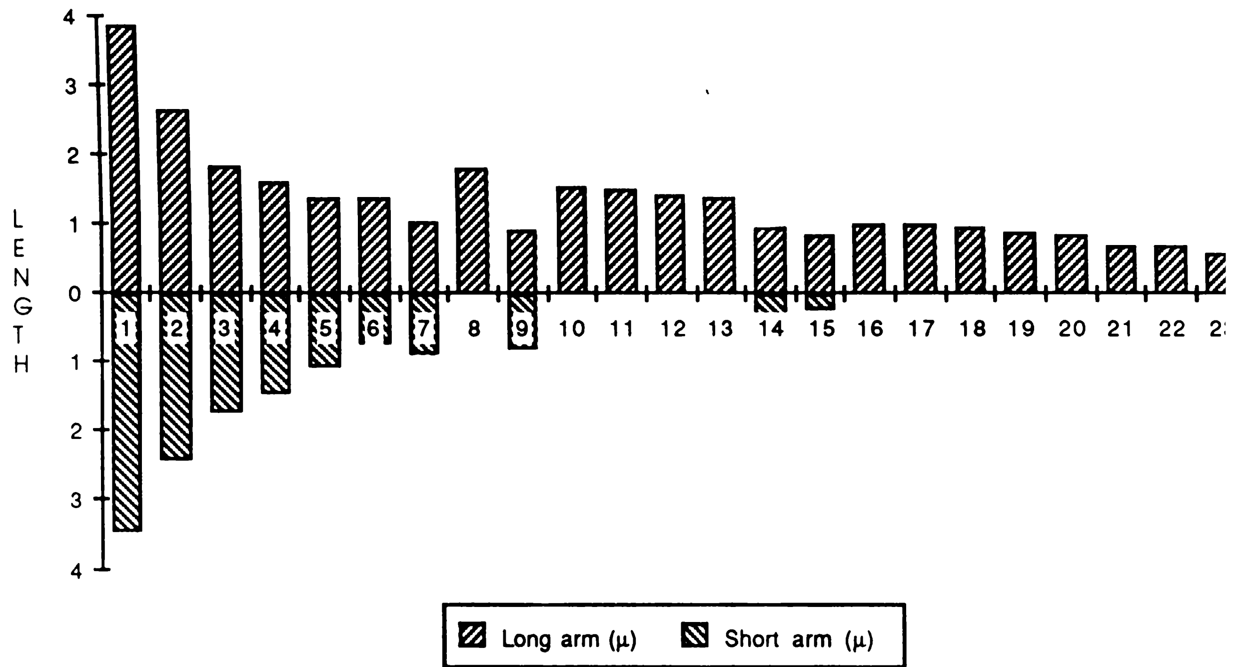


FIG.15. AN IDIOGRAM OF E. MACULATUS



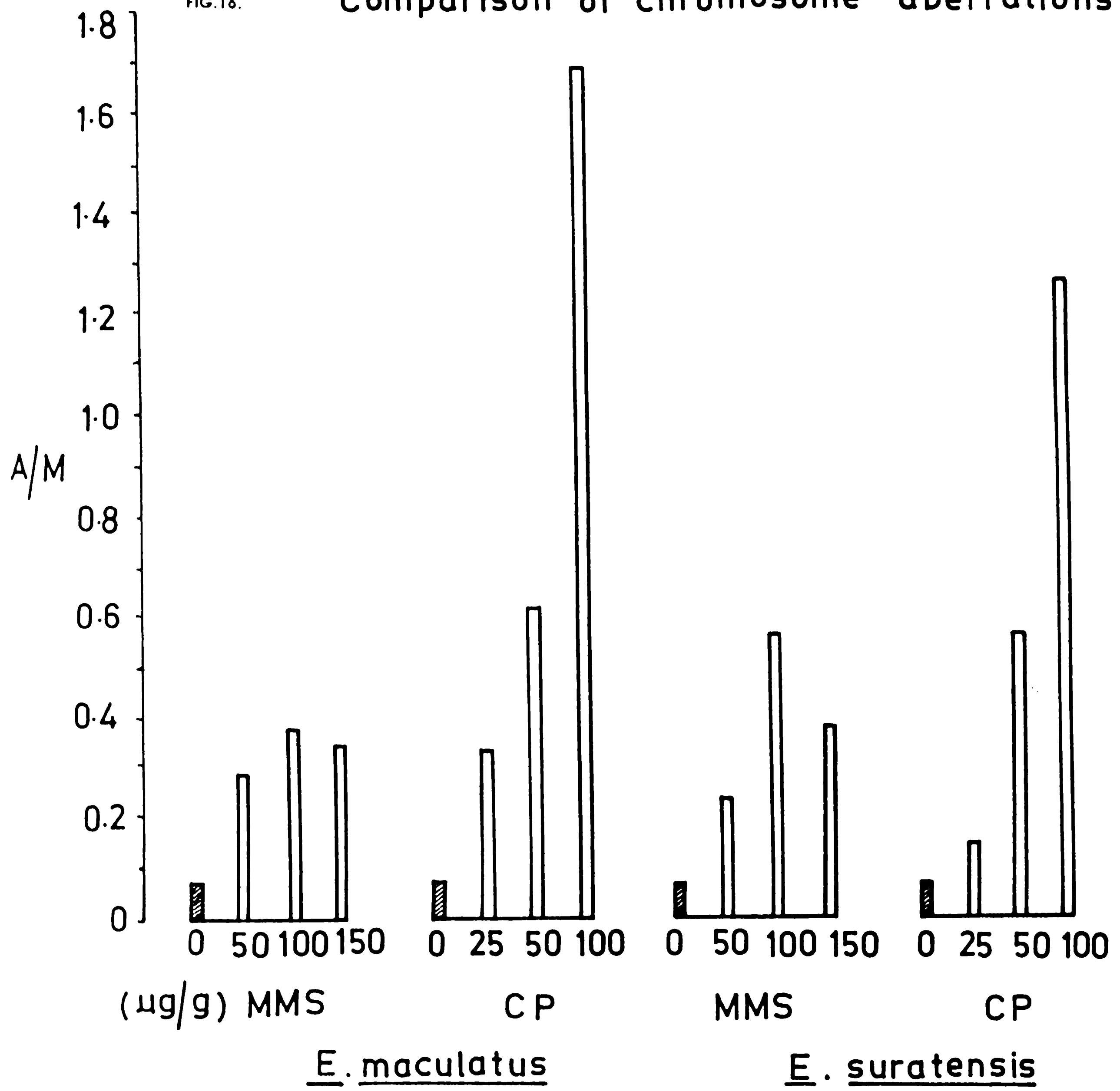


FIG.16.
Comparison of chromosome aberrations
A/M
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0.2
0
0 50 100 150
0 25 50 100
0 50 100 150
0 25 50 100
(µg/g) MMS
CP
MMS
CP
E. maculatus
E. suratensis

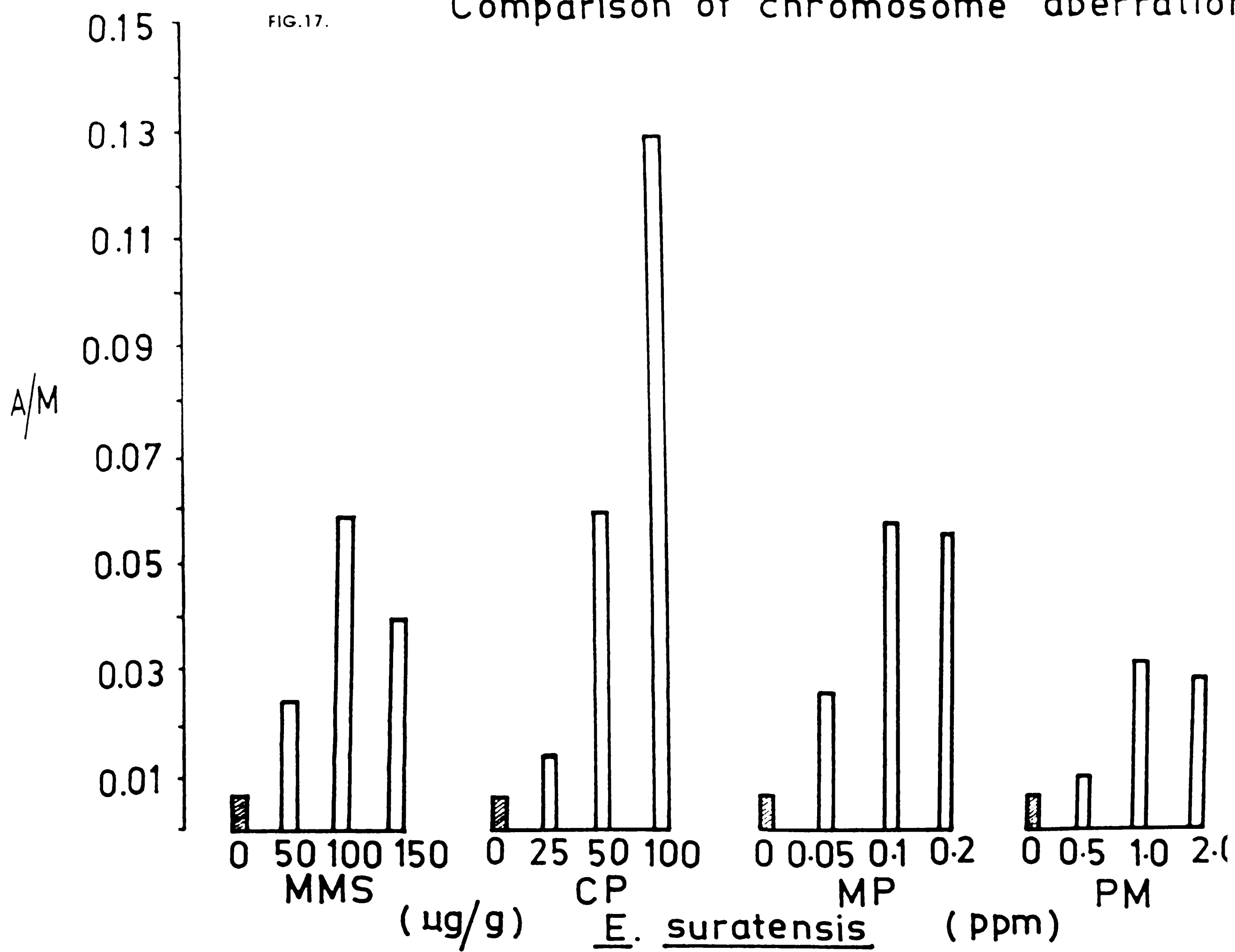
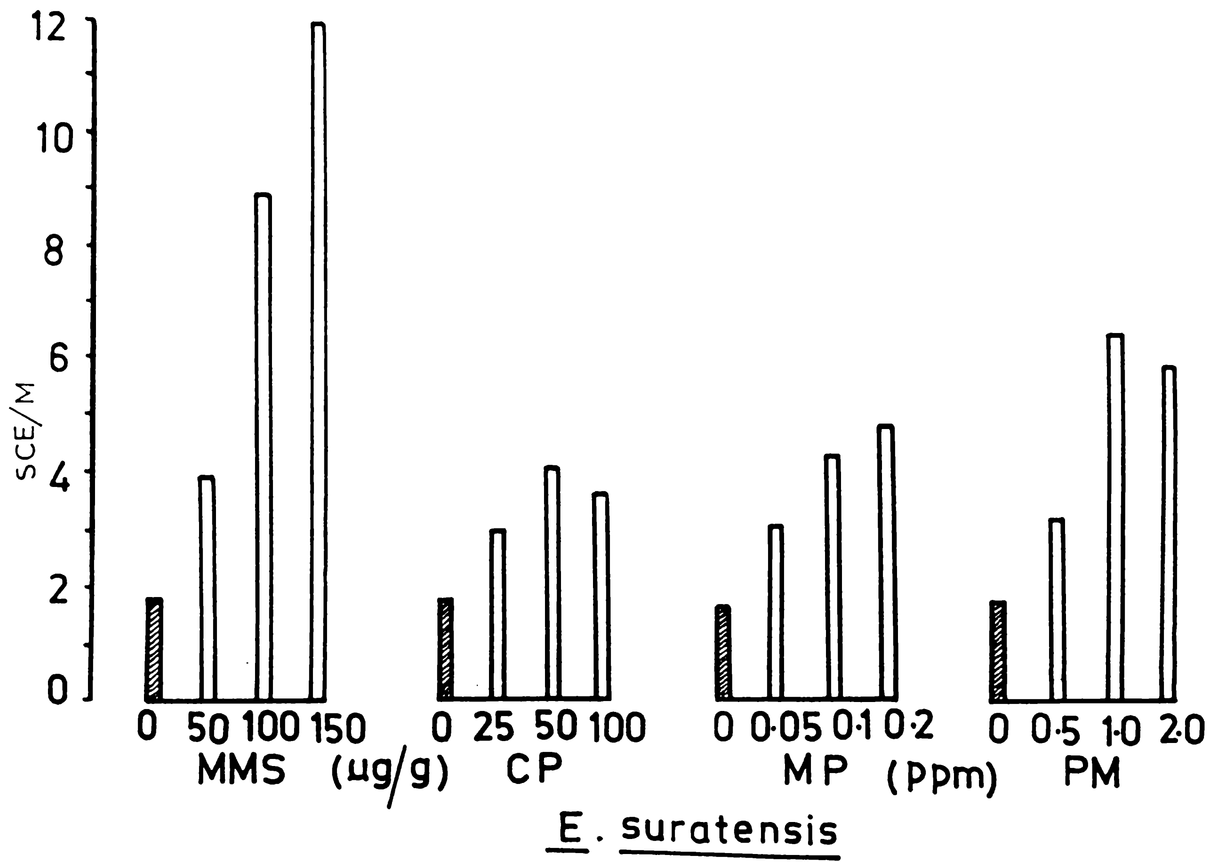


FIG.17.
Comparison of chromosome aberratio
A/M
0.15
0.13
0.11
0.09
0.07
0.05
0.03
0.01
0 50 100 150
MMS
0 25 50 100
CP
0 0·05 0·1 0·2
MP
0 0·5 1·0 2·
PM
(µg/g) E. suratensis (ppm)

FIG.18. Comparison of SCEs



DISCUSSION

1. CHROMOSOME PREPARATION METHODOLOGY

Various methods were screened for making quality preparations. Most of the methods employed deposition of cells on slides followed by air-drying or flame-drying. Squashing was also tried. The best results were obtained by modifying and incorporating the essential steps of colchicinisation, hypotonic treatment, fixation, cell suspension preparation, deposition of cells and air-drying.

Chromosomes with optimum contraction were obtained with a colchicine exposure of 60-75 minutes @ 0.005%, 1 ml/100g body wt. Shortening of chromosomes was observed with a higher dose or duration of colchicine. Optimal chromosome contraction is essential for readability of aberrations and sister-chromatid exchanges. Slide preparations were made by deposition of cells as rings. Hence, screening was less time consuming unlike other methods where entire slides have to be screened for locating plates.

Chromosome margins often displayed a fuzzy appearance. The concentration of acetic acid used for cell suspension preparations were suspected to cause this. Changes in concentration did not entirely solve the problem. Methanol storage of fixed tissues prior to preparation of cell suspensions have been advocated for improving chromosome morphology

(Bantock & Cockayne, 1975). However experience in the present study does not support their findings. Hypotonic treatment carried out in cold had definite advantages since swelling was controlled and the chances of cell bursting less when compared to hypotonic treatments at room temperature. Fixation for about 2 hours was found sufficient. Prolonged storage in fixative in a refrigerator did not confer any advantage except that slide preparation could be staggered.

Giemsa staining with a 4% staining solution yielded good results. For optimum staining, pH of the buffer was important. It was easy to get well stained slides using Giemsa working solution prepared in tap water. Consistency of results was however difficult. Increase' giemsa concentration also did not improve staining.

Slides prepared were generally observed without mounting. Gradual fading of normal chromosome preparations was seen a few days after mounting with DPX. In the case of SCD/SCE slides the problem was acute. Hence slides were screened without mounting and just after preparation.

2. METHODOLOGY FOR DIFFERENTIAL STAINING OF CHROMOSOMES

BrdU incorporation offers a simpler method for study of SCD/SCE in chromosomes as against the earlier method using tritiated thymidine. BrdU administration @ 0.5 mg/g body weight (one injection) and an exposure of 24 hours was found to give satisfactory results. This also implied that

in 24 hours atleast two replications took place. Cells with undifferentiated chromosomes also included those after only one round of replication in the presence of BrdU. Stray instances of third round cells were also seen. With the increase in duration of exposure the frequency of third replication cells were increased. Increase of BrdU dosage did not confer any advantage. On the other hand a reduction in mitotic index was indicated in such cases in the present study.

Using higher concentrations, the mitotic index and the proportion of differentially stained metaphases was greatly reduced by the toxic activity of the drugs (Vogel and Bauknecht, 1976). Kligerman and Bloom (1976) used a single injection of BrdU in U. limi. Other in-vivo studies, in mammals, showed that one injection was not enough for sufficient incorporation of BrdU into DNA due to rapid debromination and inactivation of BrdU, necessitating the use of tablet implantation method or intravenous infusion or hourly injections (Allen et al. 1977; Allen et al. 1978; Allen and Latt, 1976a; Nakanishi and Schneider, 1979). Studies by Stromberg et al. (1981), Kligerman and Bloom (1976) showed that this may not be the case with fish. Dixon and Clarke (1982) found increase of BrdU exposure time useful in enhancing the frequency of second-division cells in M. edulis.

Observations of Kerkhoff and Gaag (1985) on exposure duration of BrdU were different, ie. an exposure of 48 hours was found optimum for BrdU incorporation at a temperature of 25°C ± 1. Alink et al. (1980) found exposure to BrdU necessary for 10 days at a temperature of 12°C.

Vigfusson et al. (1983) observed that a 96 hour exposure to BrdU was necessary in U. limi at a temperature of 18°C. From these studies it is obvious that temperature plays a critical role in determining exposure duration. The present study indicated optimum exposure of 24 hours at about 28-30°C. This is in general conformity with the findings of the only other Indian study on SCE in fish (Mohanty and Prasad, 1982).

Hoechst treatment in PBS solution was useful when done for 15-20 minutes. Lesser durations did not yield proper differential staining despite appropriate U.V. treatment. Prolonged treatment reduced the quality of preparations as chromosomes became hazy. About 5-8 minutes U.V.treatment from a black bulb was good enough. Reduction in exposure resulted in poor differentiation ie., both chromatids were densely stained. Excessive exposure resulted in both chromatids getting lightly stained ie., once again reducing the differential staining. The present study has shown that the step involving U.V. treatment is critical in making good quality SCD/SCE plates. White fluorescent light or U.V. light were quite effective but prolonged exposures were necessary and chromosomes were also somehwat hazy. Haziness of chromosomes following fluorescent light treatment was corroborated by Kerkhoff and Gaag (1985).

Treatment with HCl after exposure to light has been observed to be very significant. This improved SCE quality in the present study and also as shown by Kerkhoff and Gaag (1985). According to Gonzales-Gill and Navarette (1982) who introduced HCl treatment for Allium cepa and human lymphocytes, the HCl could act by washing out most of the

nuclear and cytoplasmic RNA and some of the chromosomal proteins that interfered with giemsa staining.

The percentage of SCD/SCE metaphases among the total number of metaphases studied, ranged from 18-26% in E. suratensis and 19-28% in E. maculatus specimens examined for base-line SCE values. In animals exposed to mutagens and pesticides the percentage tended to be lower, possibly because of lesser incorporation of BrdU and subsequent failure in more metaphases to show differential staining. Kerkhoff and Gaag (1985) observed SCD in 15-50% of metaphases in N. rachowi. These findings were more or less corroborated by Hoeven et al. (1982).

3. DIPLOID NUMBERS AND KARYOTYPES

E. maculatus: The diploid number of 46 is in conformity with the earlier studies (Natarajan and Subrahmanyam, 1974). In the present study E. maculatus specimens were collected from Cochin area (west coast) while specimens from Poto Novo on the east coast were used by Natarajan and Subrahmanyam (1974). The conformity, atleast as regards the diploid number is interesting.

Detailed karyological analysis of E. maculatus showed the chromosome complement to comprise of a heterogenous group. 7 pairs were metacentrics, 2 pairs sub-metacentrics, 1 pair subtelocentric and the remaining 13 acrocentrics. (Table 1, Plate 9). This is contradictory to the findings of Natarajan and Subrahmanyam (1974) who reported pairs 8 and 16 as submedians and the remaining as medians.

Chromosome sizes ranged from 0.456 μ to 5.988 μ while a range of 0.833 μ to 5.3 μ were recorded in the earlier study. As in the earlier study the first two pairs were distinctly larger than the rest and no heteromorphic chromosomes were seen.

E. suratensis: The diploid number of 48 is in conformity with the two earlier studies of Natarajan and Subrahmanyam (1974); Rishi and Singh (1982). It is interesting to note that the specimens in the present study came from Cochin on the west coast while that for the earlier studies (Natarajan and Subrahmanyam, 1974; Rishi and Singh, 1982) from Porto Nova and Chilka respectively on the east coast.

E. suratensis complement in the present study was found to comprise entirely of acrocentrics without much variations in size. (Plate 14) It must however be remarked that most of the chromosomes showed telomeric ends but were grouped as acrocentrics. No heteromorphic chromosomes were observed. The relative lengths varied from 3.172 to 5.23% (Table 2). Rishi and Singh (1982) also clearly showed the acrocentric nature of all chromosomes in the species with relative lengths varying from 2.3 to 8.2%. However, Natarajan and Subrahmanyam (1974) found a totally different chromosome picture. They observed median centromeres in 23 pairs and sub-terminal centromere in 1 pair. Their findings need confirmation if the possibility of intra-specific variation is ruled out.

Chromosomally both species, E. maculatus and E. suratensis show distinct karyotypes. Cytogenetically they appear to be well separated

species.

The utility of a cytogenetic model depends on the single most important factor ie. its chromosomes. A species with a small diploid number and large chromosomes would be an ideal candidate for developing cytogenetic assays. However most fish species suffer from the double disadvantage of a large diploid number and small chromosomes. This explains the relatively few studies on fish. Only in rare cases like N. rachowi, U. limi, U. pygmea have the chromosome complement found to be near ideal (Kligerman et al. 1984; Kerkhoff and Gaag, 1985).

Of the two species chosen as models in the present study, E. maculatus was less suitable because of its karyotype ie. presence of small chromosomes also. SCE can be expressed as SCE per cell or per chromosome. In cases where chromosomes are more or less uniform in size even incomplete metaphase plates can be used to compute SCE/cell. However with grossly dissimilar chromosomes and wide variations in size SCE/cell estimated on the basis of observations confined to a few larger pairs is fraught with increased likelihood of errors. This is because the number of SCE is directly related to chromosome length. If chromosomes in a cell are similar there is no problem but if they are not it might lead to a problem (Kligerman et al., 1984). This explains why in the present study E. maculatus was not subjected to the same battery of tests employing all endpoints as in E. suratensis.

4. BASE-LINE VALUES

a. Base-line CA values

The importance of base-line or reference CA values cannot be over emphasised as the picture from any treatment has to be evaluated against CA values at apparently zero levels of genotoxicity.

In the present study the base-line aberration value in *E. maculatus* was found to be 0.004 per metaphase and that in *E. suratensis* 0.006 per metaphase. From 10 animals (Table 3) studied, CA were revealed only in 2 specimens. In *E. suratensis* four animals revealed base-line aberrations (Table 4).

In-vivo systems using other species have shown a CA range from 0 - 7.3 per cell while in-vitro systems have shown upto 13% (Table 19). The findings in the present study appear to be in the lower range when compared to the other species. It is interesting to see that numerically the base-line SCE values are higher than that of CA (Tables 3-6) indicating a greater sensitivity of SCE to base-line levels of genotoxicity (present study).

b. Base-line MN values

The base-line micronuclei values in the two species in the present study were found to be zero despite a sufficient screening of a number of animals. Considering the base-line values of SCE and CA in the present

Table-18. Base-line/control Sister-chromatid exchanges (Values computed in some cases).

Species	SCE/cell	Reference
Umbra limi		
(intestine)	2.64	(Kligerman and Bloom, 1976)
(gill)	2.42	(Kligerman and Bloom, 1976)
Umbra limi		
(gill)	2.56	(Kligerman, 1979)
(kidney)	3.14	(Kligerman, 1979)
(intestine)	4.15	(Kligerman, 1979)
Umbra limi	2.90	(Bishop and Valentine, 1982)
Umbra limi	4.40	(Vigfusson et al., 1983)
Umbra pygmaea		
(gill)	1.23	(Alink et al., 1980)
(testis)	1.39	
Umbra pygmaea	0.80	(Hoeven et al., 1982)
Umbra pygmaea	2.20	(Hooftman and Vink, 1981)
Nothobranchius rachowi	1.60	(Hoeven et al., 1982)
Nothobranchius rachowi	0.88	(Gaag et al.,1983)
Nothobranchius rachowi	0.94	(Kerkhoff and Gaag, 1985)
Channa punctatus	0.17	(Mohanty and Prasad, 1982)
Parophrys vetulus	2.16	(Stromberg et al., 1981)
Etroplus suratensis	1.79	(Present study)
Etroplus maculatus	0.67	(Present study)
Opsanus tau	7.00	(Maddock and Kelly, 1980)
Leptocottus armatus (in-vitro)	1.10	(Zakour et al., 1984)
Ameca splendens (in-vitro)	4.56	(Barker and Rackham, 1979)
Mytilus edulis	4.10	(Dixon and Clarke, 1982)
Mytilus edulis	1.03	(Dixon and Prosser, 1986)
Mytilus edulis	1.20	(Jones and Harrison, 1987)
Neanthes arenaceodentata	2.52	(Pesch and Pesch, 1980)
Neanthes arenaceodentata	4.10	(Pesch et al., 1981)

Table-19. Baseline chromosome aberrations in some species.

	Species	Tissues	Base-line CA per cell or % aberration cells	References
1.	*U. limi*	Kidney Gill Gut	0.03%	Kilgerman et.al.(1975)
2.	*U. limi*	Gill Spleen	1.5%	Mong and Berra (1979)
3.	*U. limi*	Blood	0	Suyama and Etoh(1983)
4.	*N. rachowi*	Gill	0	Hooftman (1981)
5.	*B. dussumieri*	Gill	0.0017-0.0033	Krishnaja and Rege (1982)
6.	*C. carpio*	Kidney	5.8	Al-Sabti (1985a)
7.	*T. tinca*	Kidney	6.4	Al-Sabti (1985a)
8.	*C. idella*	Kidney	7.3	Al-Sabti(1985a)
9.	*M. saxitilis*	Embryo	0.00001	Daniels and Baksi(1988)
10.	*C. variegatus*	Embryo		
11.	*F. heteroclitus*	Gill Kidney Intestine		
12.	*C. variegatus*	Embryo	1%	Daniels and Means(1989)
13.	*S. gairdneri*	Gill Kidney	0 7.3%	Al-Sabti (1985b) Al-Sabti (1985b)
14.	*S. gairdneri* (in-vitro)	Gonad cell-line	8-13%	Kocan et.al. (1985)
15.	Bluegill fish (in-vitro)	Tissue cell line	8-13%	Kocan et.al.(1985)

study, the absence of MN is not necessarily indicative of the absence of genotoxicity. It is rather a reflection on the sensitivity of the different tests to detect genetically active compounds. There were rare instances in the present study when fish sampled from apparently unpolluted sites revealed micronuclei. These were considered as an outcome of possible chronic exposure to unknown mutagens rather than a reflection of base-line values.

Studies by Manna and Sadhukhan (1986) on kidney cells of O. mossambicus and peripheral blood cells of O. mossambicus (Manna et. al., 1985) showed no base-line values. MN frequencies of reference fishes averaging from 0.6-0.8‰ are lower than spontaneous MN frequencies of 1-3‰ reported in mice (Heddle et.al., 1983). Hose et.al. (1987) reported an MN frequency of 0.8‰ in fish studied from reference (control) site.

Since, in general the base-line MN values are seen to be nil or very low in fish species, it is not surprising that base-line values of the two species in the present study have been zero.

c. Base-line SCE values

Spontaneous SCE measurements serve as a reference for evaluating SCE caused by genotoxic conditions. Even an animal apparently unexposed to any form of toxicity does reveal a low incidence of base-line SCE. This is indicative of DNA repair mechanisms operating at base-line levels. These values may vary from species to species (Table 18). Some workers

have opined that spontaneous exchanges observed are caused by BrdU incorporation or other DNA labelling techniques. Perhaps under ideal conditions no exchanges take place. Occurrence of spontaneous exchanges has remained a controversial issue especially with techniques involving DNA labelling using tritiated thymidine and some workers like Gibson and Prescott (1972) felt that a majority if not all such SCE were radiation induced. Studies by Kato (1974), Latt (1974), Wolff and Perry (1974) showed that SCE increased with increasing BrdU concentration. SCE frequencies have also been found to be approximately proportional to chromosome lengths (Kihlman and Kronberg, 1975).

In the present study the base-line SCE values were found to be 1.79 per metaphase in E. suratensis and 0.67 SCE per metaphase in E. maculatus (Table 5,6). From Table 18 it is obvious that base-line values vary from species to species. Some variations are apparent with tissues also. Probably this may be explained by the physiological functions resulting in a differential effect of BrdU or perhaps due to differential distribution of BrdU. Metabolic activation and de-activation processes or differences in sensitivities can be alternative explanations.

With in-vivo systems there are undoubtedly differences in DNA content, exposure distribution and BrdU availability with different tissues. Consequently it is not surprising that different organisms have different base-line frequencies (Pesch and Pesch, 1980).

Some dependence of base-line SCE values on DNA content have been indicated (Barker and Rackham, 1979); (Schvartzman et. al., 1979).

This provides a reasonable explanation for species variation. Individual variations also do exist in base-line values as shown in the present study (Table 5 & 6). Possibly this is linked to the physiological status of animals as opined by Stromberg et. al. (1981).

For explaining differences in base-line SCE values of the two species in the present study, supportive evidence can be drawn from the fact that the total chromosome length in E. maculatus is 43.903 μ and the base-line SCE value is 0.67 per cell, while the total chromosome length in E. suratensis is 84.149 μ with a base-line exchange rate of 1.79 per cell (Tables 1, 2, 5, 6). However, SCE dependence on DNA values in the two species can be established only after quantifying the cellular DNA contents.

5. CA INDUCTION

The response of E. maculatus to MMS and CP shows clearly the greater genotoxicity of CP. At identical doses (100 μg/g) MMS induced 0.038 CA per metaphase while CP induced 0.17 CA per metaphase. thus CP was approximately 4 times more genotoxic than MMS while at 50 μg/g, genotoxicity of CP was about twice that of MMS (Figs. 3,16 and Table 7).

In E. suratensis also, as in the case of E. maculatus, CA was induced to a greater extent by CP. The toxicity of CP was more than twice that of MMS at the 50 and 100 μg/g levels. When considering

the relative toxicity of MMS and CP separately in both species, cP was more toxic in E. maculatus while MMS was generally more toxic in E. suratensis (Fig. 17).

Between the two pesticides tested in E. suratensis, M.P.appeared more toxic than P.M. At a dose of 1 ppm PM induced 0.031 C.A per metaphase as against the 0.058 per metaphase induced by a much lower dose of 0.1 ppm of MP. A similar trend was seen at the second dose levels of MP and PM (Figs. 7, 8, 16, 17 and Tables 11, 12). The overall responses to MP and PM were similar.

The effect of MP and PM on the two endpoints CA and SCE seem to be different. MP appeared more effective in inducing numerically more CA while more SCE were induced by PM (Figs. 17, 18). This apparent shifting of trends as regards the number of inductions of the two endpoints can be attributed to be mechanisms involved in SCE and CA induction. The increase of aberration tends to increase with dose of chemical pollutants and at the same time the frequency of a particular kind of aberration is pollution specific (Al-Sabti, 1985).

The general dose dependent trends observed in the present studies have been reported elsewhere also. The effect of EMS on N. rachowi was found to be dose dependent and caused significant increase in aberration at all concentrations (Hooftman, 1981). Elevation of CA in response to pollutants was demonstrated also in O. mossambicus by Mukherjee (1984).

The greater efficacy of CP in inducing CA in E. suratensis (present study) could possibly be linked to the promutagen activation processes as in the case of M. saxitilis larvae exposed to CP (Daniels and Baksi, 1988). A dose related trend in CA observed by them suggests that the developmental stage of fish may be susceptible to the action of promutagens.

When considering the effect of MMS and CP on CA and SCE in E. suratensis, the picture is interesting. CP was found to be more genotoxic as regards CA induction while MMS was more effective as a genotoxicant in inducing SCE. In the case of MP and PM also the shifting of trends is somewhat similar. SCE is undoubtedly more sensitive than CA as obvious from the number of inductions. At the same time this reversing of trends with the two cytogenetic endpoints CA and SCE despite uniform treatments is interesting. It could be that the phenomenon is related to the mechanisms of SCE and CA inductions.

6. MICRONUCLEUS TEST

All treatments in the present study drew a blank as regards MN induction. It appears that the chemicals tested do not reveal genotoxicity atleast as far as MN is involved. To a good extent this can be attributed to the lesser sensitivity of the MN test in relation to SCE and CA as evidenced from the present study. However the possibility exists that the duration of exposure (96 hours) may not nave been adequate to reveal micronucleated erythrocytes in the blood if one were to give credence

to the assumption that MN test is sensitive to the doses tested in the present study. It is also likely that the damaged erythroblasts may not have reached the peripheral blood.

U. pygmaea exposed for 48 hours to mutagens revealed MN (Hooftman and Vink, 1981). After a 3-week exposure of *U. limi* to chemicals Hooftman and Raat (1982) did not observe MN in any of the treatments except one. In *O. mossambicus* MN formations were seen 30 hours after exposure (Manna et. al. 1985). Since *O. mossambicus* and the two species in the present study are tropical species and quite related, the argument that the duration of exposure in the present study was too short stands invalidated. On the other hand the lesser sensitivity of MN to genotoxicants appears more plausible since with the same duration CA and SCE elevations were observed.

7. SCE INDUCTION

a. Mechanism of SCE induction

Numerous theories have been put forward to explain the mechanism of SCE. Though none of them really explain all aspects of the phenomenon it is generally recognised by various workers that SCE incidence is in response to mutagenicity ie., presence of genetically active compounds.

Kligerman (1979) while examining the various hypothesis opined that SCE appears to be a way in which cells can proceed through the S-phase when DNA is replicated from a damaged template. It is also clear

that SCE formation is critically associated with DNA replication since passing through the S-phase is a pre-requisite for its expression (Wolff et.al., 1974) and Figs. 1, 2.

Theoretically SCE represents a reciprocal exchange of identical DNA material between two sister-chromatids. SCE responses have been correlated with induced point mutations and may be useful as a quantitative indicator of mutagenesis (Carrano et. al., 1978). A similar view point was reflected by Latt et. al. (1981) who suggested that SCE represent the interchange of DNA replication products at apparently homologous loci and presumably involve DNA breakage and reunion, although little is known about its molecular basis.

Cleaver (1981) and Painter (1980) have proposed models which explain SCE on the basis of the functional organisation of DNA replication units. Painter (1980) also suggested that junctions between DNA replication clusters are unstable sites prone to SCE formations and DNA double strand breakage at these junctions and subsequent joining of the daughter strands of the replicated replicon clusters to the strands of the unreplicated clusters may be responsible for SCE formation. Therefore it follows that the unit of SCE formation may be related to a visible counterpart of DNA replication ie., DNA replicon cluster. It is also generally accepted that DNA replication is controlled at the level of replication clusters i.e, tandem sets of 10 - 100 DNA replication units, each with an average length of 100 Kb and replicates as a group (Hand, 1978). These clusters contain enough DNA

(1-10 megabases) to be resolved at the light microscopic level and this corresponds to the DNA present in a high resolution chromosome band (Holmquist, 1978). Recent studies by Lugo et al. (1989) have suggested chromosomal replicons to be units of sister-chromatid exchanges.

Numerous workers have indicated the greater sensitivity of SCE to mutagens than the traditional chromosome breakages. It has been estimated that in the case of a number of mutagens like MMS and CP, the in-vivo SCE analysis is 10-600 times more sensitive than most commonly used in-vivo tests like dominant lethal, micronucleus and chromosome aberrations (Renner, 1979). A dose of mutagen that gives a highly significant doubling of SCE produces oniy a minimal and barely noticeable effect on CA incidences. Doses giving a 10 - fold increase in SCE only slightly increased the low incidence of CA (Perry and Evans, 1975). The findings of the present study also provide some support to these views.

b. SCE induction by MMS and CP

MMS is known as a direct acting mutagen, but CP is not active unless metabolized into mutagenic compounds and therefore is an indirect mutagen (Perry and Evans, 1975). CP is a watersoluble promutagen that is dependent for its mutagenic activity in mammals on transformations accomplished via. the cytochrome P-450 pathway (Dixon, 1985). Both components were administered by injections in the present study.

The response of SCE in *E. suratensis* to MMS has been a typical dose response. Against the control value of 1.82 per metaphase the response

showed a direct correlation to dosage. The highest value of SCE, 12 per cell was observed with a dose of 150 µg/g (Tables 13, 14; Fig.9, 10, 18). The response of E. suratensis to CP was not in a typical dose related manner (Table 14; figs. 10, 18). The highest dose of (100 µg/g) yielded 3.69 SCE per metaphase against a value of 4.14 per metaphase at the lower dose of 50 µg/g.

The response of the species to MMS and CP appears somewhat different. From the 50 to 100 µg/g dosage, the values increased with MMS while in the case of CP a slump was noticed. Beyond the 50 µg/g (CP) dosage the SCE values did not go more than 4.14 per metaphase, but dropped, while in the case of MMS despite a high SCE rate of 9 per metaphase at the 100 µg/g dose level, the increasing trend continued (Fig. 18, Tables 13, 14). It is seen that the SCE rate for MMS was slightly lower than that induced by CP at the comparative dose level of 50 µg/g. It can be inferred that MMS is more or less similar in genotoxicity to CP as indicated by SCE inductions in the comparative dose of 50 µg/g.

The above findings pertaining to relative genotoxicity of MMS and CP are interesting against the backdrop of reports by Kligerman (1979) that CP is about 2-4 times, more potent inducer of SCE than MMS. At doses as low as 7 µg/g (MMS) gill, kidney and intestine tissues showed statistically significant increases in SCE. The effect was more pronounced with CP, where even at doses as low as 2.1 µg/g significant increases in SCE were seen. The greater potency of CP than MMS has been reported by Stetka and Wolff (1976), Bloom (1978) and Bishop and Valentine (1982).

The apparent departure in the present study may be indicative of the absence of microsomal activation systems in E. maculatus as inferred from the observation of Kligerman (1979). The fact that CP causes highly significant increases in SCE in U. limi indicates that the fish (mud-minnow) possesses microsomal activation system to convert promutagens into active moieties (Kligerman, 1979).

Studies by Bishop and Valentine (1982) on U. limi once again indicated the greater potency of CP than MMS in SCE induction. They observed the highest SCE value of 11.3 per metaphase at a dose of 250 μg/g MMS while it was 19.9 per metaphase at a dose of only 200 μg/g CP. Since these studies also happen to be in a mud-minnow (U. limi), the probability of existence of microsomal activation systems is strengthened and also corroborates the earlier findings of Kligerman (1979).

Studies by Hoeven et. al. (1982) on N. rachowi on the comparative potency of CP and EMS showed that on a molar basis CP was almost three times more effective an inducer of SCE than EMS.

When comparing the results of U. limi obtained by Bishop and Valentine (1982), with the present study it is seen that MMS (50 μg/g) induced 4.05 exchanges per cell in E. suratensis and 8.3 per cell in U. limi. At the 100 μg/g dosage E. suratensis responded with 9 exchanges per metaphase. While U. limi showed only 8.1 exchanges per metaphase. Probably this can be attributed to the differential responses in a tropical

species (*E. suratensis*) and a temperate species (*U. limi*). CP at a dosage of 30 µg/g in *U. limi* showed an average response of 7.1 SCE per metaphase as against the comparatively low rate of 4.14 SCE per metaphase in *E. suratensis* with a dose of 50 µg/g (present study). The findings once again point to the probable absence of enzyme activation systems in *E. suratensis* to convert promutagens like CP into genetically active compounds.

A direct correlation between SCE induction and doses is not indefinite but is limited by cytotoxicity responses. Consequently the highest doses always do not show the highest frequencies.

A decline observed in SCE incidence despite increase in CP dosage from 50 µg/g to 100 µg/g in *E. suratensis* is likely due to selective killing of sensitive cells (Fig. 18). A decline in SCE response with increasing concentrations is likely due to selective killing (Pesch et al., 1981). In a heterogenous population of cells some are more sensitive to both killing and mutation. Thus at higher doses the sensitive cells are destroyed while the remaining cells exhibit fewer SCE. Hence a reduction in SCE rate at the highest dose when compared to the previous lower dose does not mean a reduction in genotoxicity.

c. SCE induction by MP and PM

Methyl parathion is a widely used organophosphorus insecticide in agriculture. It is a contact poison. Phosphamidon is also an organo-

phosphorus compound acting as a systemic insecticide. These two pesticides are extensively used in agriculture. The implications of this for aquatic ecosystems lie in the fact that most terrestrially used pesticides reach the aquatic system through surface run offs. Hence the need for testing these compounds for genotoxicity is obvious,

The three doses tested with both pesticides on E. suratensis did not yield responses quite identical. With MP the dose response was typical while with PM it was not entirely so, especially with the highest dose (2 ppm). With MP at a dose of 0.05 ppm the SCE rate was 3.17 while it was 3.22 with a PM dosage of 0.5 ppm ie., a dosage 10 times higher. It may hence be inferred that MP is 10 times more potent than PM as far as SCE induction is concerned (Figs. 11, 12, 18 and Tables 15, 16).

The higher doses of MP (0.1 and 0.2 ppm) induced 4.23 and 4.88 exchanges per metaphase. The values with PM were 6.46 SCE per metaphase and 5.89 SCE per metaphase at doses of 1 ppm and 2 ppm which happen to be 10 times higher than that of MP. This again clearly shows that MP is more genotoxic, atleast as regards SCE induction (Fig. 18).

With the highest dose (2 ppm) of PM only 5.89 SCE per metaphase were observed as against the 6.46 in the lower dose (1 ppm). The decrease in SCE response with higher dose may be attributed to selective killing of sensitive cells, with the remaining cells exhibiting fewer SCE (Pesch et. al., 1981).

The percentage of cells exhibiting high SCE frequencies ie., the outlier cells or ,HFC cells increase with higher genotoxic concentrations and this may prove to be a more sensitive measure of SCE induction and thus genotoxic exposure than mean SCE frequencies (Harrison and Jones, 1982). Despite the lowering of mean SCE in the highest PM dose (2 ppm) to 5.89 per metaphase in the present study the possibility of increase in outlier cells ie., higher genotoxicity cannot be excluded unless this is specifically looked into.

8. FIELD STUDIES

E. suratensis were studied from a site suspected to be polluted. The SCE values in the individuals ranged from 2.27 - 3.16. The mean value was 2.69 SCE per metaphase (Table 17). When compared to the base-line value of 0.179 per metaphase (Table 6) the elevation is pronounced. While it is difficult to quantitatively or qualitatively categorise the pollutants it is obvious that there is some mutagenic load. While screening animals from natural environments it is difficult to point out the actual cause of genotoxicity. At best the studies can point out the presence or absence of an elevation in values with reference to mutagensis.

Although there is no way to relate a specific level of environmental contaminant to a corresponding SCE rate, a linear relation is evident. It can then be assumed that a high rate of SCE indicates a significant level of mutagenic contaminant or contaminants (Stromberg et.al., 1981). While a variety of polluting agents are known to be genetically active either directly through mutation (Parry et.al.,1976) or by exerting selection

pressures (Beardmore et al.,1980), information comes largely from lab studies (Dixon and clarke, 1982). However, there have been some field studies on SCE values in relation to environmental mutagenesis.

Stromberg et al. (1981) observed an SCE value of 3.25 per cell in fish (P. vetulus) sampled from contaminated sites. The base-line value in the species was 2.16 per cell. Exposure of fish to polluted river water has been found to significantly increase SCE values as shown by some studies. In a study on the effect of Rhine water on N. rachowi, Gaag et. al., (1983) found SCE values to double from the base-line value of 0.055 per chromosome to 0.104 SCE per chromosome. Another study on the effect of Rhine water on U. pygmaea showed SCE elevation from 0.063 per chromosome to 0.161 per chromosome.

The present attempt to study SCE in fish, in field conditions has been the first in Indian species. This approach can be adapted suitably for other species and should form a vital compone of all environmental monitoring programmes.

S U M M A R Y

1. An introduction to the topic of research, scope of the work and its relevance have been presented.

2. A historical resume on the landmark developments in fish cytogenetics and genotoxicity testing has been provided.

3. Detailed description of methods used and material employed for the study are given.

4. The methods developed for routine chromosome analysis, karyotyping and chromosome aberration analysis have been detailed.

5. The various steps in development of the protocol for inducing differential staining in the two test species for SCE analysis have been documented in detail.

6. Two known mutagens MMS and CP were tested on the two species chosen for the study. The relative genotoxicities of these compounds are examined using the different cytogenetic endpoints.

7. Two pesticides methyl parathion (MP) and phosphamidon (PM) were evaluated for their genotoxicity based on the various

cytogenetic end points. The SCE assays developed for the first time in Indian species can be adapted for others in laboratory or field conditions.

8. The findings based on field studies indicate the possibility of genotoxic contaminants at the site selected. The usefulness of this approach in monitoring environmental mutagenesis is elaborated.

CONCLUSION

The present study has shown that cytogenetic assays can be developed using sensitive endpoints like chromosome aberrations and sister chromatid exchanges. The development of an SCE-based assay for genotoxicity testing in fish is the first work in the country. The method developed can be extended to other fish cytogenetic models. It is suggested that large scale genotoxicity testing of chemicals may be undertaken based on SCE assays in fish.

The field studies in the present work and the demonstration of SCE assays for genotoxicity testing in natural environments is significant. It offers a method for assessing the synergistic genotoxic effect of a complex mixture and large number of pollutants found in most of the polluted sites.

Aqua-pollution studies in the country aimed at understanding the link between pollutants and genotoxicity have largely been confined to laboratory studies and have relied only on conventional cytogenetic endpoints like chromosome aberrations. While it would be imprudent to convey the impression that understanding genotoxicity based on routine chromosome aberrations have limited value, it is stressed that a more precise picture of genetically active compounds in natural environments can be obtained by SCE analysis.

The pollution scene is rather disquieting since many compounds even at sub-lethal levels are genetically active and can cause DNA damage, the consequences of which get manifested in the long run. It would not be out of place to surmise that decline or drastic fluctuations in fisheries are linked to this phenomenon.

As has been opined by Alink et. al. (1980), mutagenic compounds may lead to changes in the gene pool with unpredictable population genetic consequences. A variety of polluting agents are known to be genetically active, either directly through mutations (Parry et. al., 1976) or by exerting selection pressures (Beardmore et. al., 1980). Because of the universality of the DNA molecule, an agent which is genotoxic for one group of living organisms is typically genotoxic for other groups (Landolt and Kocan, 1983). More potent would be the picture if studies corroborate the hypothesized transgenerational transfer of genotoxic agents (Harrison and Jones, 1987) via. the females in natural populations found in polluted environments. Cytogenetic damage in fish may lead to changes in the gene component of the fish including the risk of their accumulation in the fish organs which then becomes a serious danger for human health (Al-Sabti, 1985).

REFERENCES

AHOKAS,J.T. 1979. Cytochrome P450 in fish liver microsomes and carcinogen activation. In: M.A.Q. Khan. J.J.Lech and J.J.Menn (Eds), Pesticide and xenobiotic metabolism in aquatic organism. American Chemical Society, Washington.

ALINK,G.M., FERDERIC-WALTERS and E.M.H.VAND, 1980. Induction of Sister Chromatid Exchanges in fish exposed to Rhine Europe water. Mutation Research, 78: 369.

ALLEN,J.W., C.F.SHULER, R.W.MENDES and S.A.LATT, 1977. A simplified technique for in vivo analysis of Sister Chromatid Exchanges using 5 - Bromodeoxyuridine tablets. Cytogenetics and cell Genetics, 18: 231-237.

ALLEN,J.W., C.F.SCHULER and S.A.LATT, 1978. Bromodeoxyuridine tablet methodology of in vivo studies of DNA synthesis. Somatic Cell Genetics, 4: 393-405.

ALLEN,J.W. and S.A.LATT, 1976a. In vivo BrdUrd-33258 Hoechst analysis of DNA replication kinetics and Sister Chromatid Exchange formation in mouse somatic and meiotic cells. Chromosoma, 58: 325-340.

ALLEN,J.W. and S.A.LATT, 1976b. Analysis of Sister Chromatid Exchange formation in vivo in mouse - spermatogonia as a new test system for environmental mutagens. Nature, 260: 449-451.

AL-SABTI,K. 1984. Frequency of chromosome aberrations in the rainbow trout (Salmo gairdneri, Richardson) exposed to detergent and benzene. Veterinary Archives, 54: 83-89.

AL-SABTI,K. 1985a. Carcinogenic-mutagenic chemicals induced chromosome aberration in the kidney cells of three cyprinids. Comparative Biochemistry and Physiology, 82C(2): 484-493.

AL-SABTI,K. 1985b. Frequency of chromosomal aberration in the rainbow trout (S. gairdneri Richardson) exposed to 5 pollutants. Journal of Fish Biology, 26(1): 13-19.

AL-SABTI, K. 1985c. Chromosomal studies by blood leucocyte culture technique on three salmonids from Yugoslavian waters J. Fish. Biol. 1985, 26: 5-12.

AL-SABTI,K. 1986a. Comparative micronucleated erythrocyte cell induction in 3 cyprinids by 5 carcinogenic-mutagenic chemicals. Cytobios, 47(190/191): 147-154.

AL-SABTI,K. 1986b. Clastogenic effect of 5 carcinogenic mutagenic chemicals on the cells of the common carp, Cyprinus carpio. L. Comparative Biochemistry and Physiology, 85C(1): 5-9.

AMES, B.N. 1971. The detection of chemical mutagens with enteric bacteria. In: Chemical mutagens, Principles and methods for their detection. Hollaender (Ed.) 1: 267-287. New York.

AMES,B.N. and H.J.WHITFIELD, 1966. Frameshift mutagenesis in Salmonella. Cold Spring Harbor Symp. Quant. Biol., 31: 189-201.

AUERBACH,C.H. & J.M. ROBSON, 1946. Nature, (London), 157: 302.

BANTOCK, C.R. and W.C.COCKAYNE, 1975. Chromosome polymorphism in Nucella lapillus. Heredity, 34: 231-245.

BARAT, A. and A.R.KHUDA-BUKSH, 1986. Karyomorphometrical studies in two species of fishes, Lepidocephalichthys guntea (Fam:cobitidae) and Mystus corsula (Fam : Bagridae). In Perspectives in Cytology and Genetics G.K.Manna and U. Sinha (Eds) Hindasia Publishers New Delhi 5: 407-412.

BARKER, C.J. 1972. A method for the display of chromosomes of plaice, *Pleuronectes platessa* and other marine fishes. *Copeia*, 2: 365-368

BARKER, C.J. and B.D.RACKHAM, 1979. The induction of Sister Chromatid Exchanges in cultured fish cells (*Ameca splendens*) by carcinogenic mutagens. *Mutation Research*, 68: 381.

BEARDMORE, J.A., C.J.BARKER, B.BATTAGLIA, A.C.LONGWELL, J.F.PAYNE and A.ROSENFIELD, 1980. The use of genetical approaches to monitoring biological effects of pollution. *Rapp., P.V. Reun. Cons. int. Explor. Mer*; 179: 289-305.

BERRY, R.J. 1980. Genes, Pollution and monitoring. *Rapp., P.V. Reun. Cons. int. Explor. Mer.*, 179: 253.

BISHOP, W.E. and L.C.VALENTINE, 1982. Use of central mudminnow (*Umbra limi*) in the development and evaluation of a Sister chromatid test for detecting mutagens *in vivo*. Fifth Conference, on Aquatic Toxicology and Hazard Assessment, (J.G.Pearson *et. al.* Editors), American Society for Testing and Materials. 99-108.

BLOOM, S.E. 1978. Chick embryos for detecting environmental mutagens. In: Chemical Mutagens : Principles and methods for their detection Vol. 5. (Eds) Hollaender and de Senes. Plenum Press, N.Y.

BLOOM, S.E. 1982. Avian and Aquatic Systems for *In vivo* detection of sister chromatid exchange. Allan R. Liss Inc. N.Y. 249-277.

BLOOM, S.E. and T.C.HSU, 1975. Differential flourescence of Sister chromatids in chicken embryos exposed to 5-Bromodeoxyuridine. *Chromosoma* (Berlin), 51: 261-267.

BOHM, 1891. Sitz Ges Morph Phy Minchen. Quoted from Makino (1951).

BROOKES, P. and P.D.LAWLEY, 1971. In: Chemical Mutagens-Principles and methods for their detection : 1: 121 Ed. A. Hollaender plenum press, New York.

BRUSICK,D.J. 1978. The role of short-term testing in carcinogen detection. Chemosphere ,No.5. pp. 403-417.

BRUSICK,D.J. 1980. Principles of genetic toxicology (First edition) Plenum Press. New York.

BRUSICK,D.J. 1987a. Principles of genetic toxicology (Second Edition). Plenum Press. New York.

BRUSICK,D.J. 1987b. Implications of treatment condition induced genotoxicity for chemical screening and data interpretation. Mutation Research, 189: 1-7.

BURKE,P.M. 1982. Some effects of crude and No.2 fuel oil on the chromosomes of Smooth flounder, Liopsetta putnami (Gill). Dissertation Abstracts International. P.T.B-Science and Engineering, 44(5): 1375B.

CARRANO,A.V., L.H.THOMPSON, P.A.LINDL and J.L.MINKLER, 1978. Sister chromatid Exchange as an indicator of mutagenesis, 271: 551-555.

CHAGANTI,R.S.K., S.SCONBERG and G.GERMAN, 1974. A manifold increase in Sister Chromatid Exchange in Bloom's syndrome lymphocytes. Proceedings of National Academy of Science (Washington), 71: 4508-4512.

CHAKRABORTY, S.K. and P.V.KAGWADE, 1989. Somatic chromosomes of two marine teleosts. In Fish Genetics in India., 63-68. Das and Jhingran (Eds). Today and Tommorrow's Printers and Publishers, New Delhi.

CHEN, T.R. and A.W.EBELING, 1968. Karyological evidence of female heterogamety in the mosquito-fish, Gambusia affinis. Copeia, 1:70.

CHIARELLI,A.B. and E.CAPANNA, 1973. Check list of fish chromosomes. In: Cytotaxonomy and vertebrate evolution, 205. Academic Press, N.Y.

CUCCHI,C. and A.BARUFFALDI, 1989. A simple in vivo method for increasing mitoses in teleost fishes. Cytobios, 60: 165-169.

CUCCHI, C. and A. BARUFFALDI, 1990. A new method for karyological studies in teleost fishes. J. Fish Biol., 37: 71-75.

DAS, B. and M.D.L.SRIVASTAVA, 1973. The meiotic chromosomes of certain Indian teleosts. Proc. Natl. Acad Sci. India, 43B: 17, 29.

DAS,C.C., 1983. chromosomes in marine fishes. Proc. 70th Ind. Sci. Cong 2:1.

DAS,R.K. and A.K.NANDA, 1986. Induction of micronuclei in peripheral erythrocytes of fish H. fossilis. Mutation Research, 175(2): 67-71.

DENTON,T.E. 1973. Fish chromosome methodology. Charles C. Thomas (Publ.) Springer Field, III: PP.166.

DENTON,T.E. and W.M.HOWELL, 1969. A technique for obtaining chromosomes from the scale epithelium of teleost fish. Copeia, 392-393.

DIJCK,R.V. and H.V. VOORDE, 1976. Mutagenicity versus carcinogenicity of organochlorine insecticides. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent. 41: 1491-1492.

DIXON, D.R. 1982. Aneuploidy in mussel embryos (Mytilus edulis) originating from a polluted dock. Mar. Biol. Lett., 3: 155-161.

DIXON,D.R. 1985. Cytogenetic evidence of inducible processes linked with metabolism of a xenobiotic chemical in adult and larval Mytilus edulis. The Science of Total Environment 46: 1-8.

DIXON,D.R. and H.PROSSER, 1986. An investigation of the genotoxic effects of an organotin antifouling compound Bis Tributylin oxide on the chromosomes of the edible mussel Mytilus edulis. Aquatic toxicol.,8: 185-195.

DIXON,D.R. and K.R.CLARKE, 1982. Sister chromatid Exchange : A sensitive method for detecting damage 'caused by exposure to environmental mutagens in the chromosome of adult M. edulis Marine Biological Letter, 3(3): 163-172.

DREWRY,T.E. & W.M.HOWELL, 1964. Appendix I, Chromosome number. Tex. mel. Mus. Bull., 8, 5-72.

DUTKA,B.J. and K.H. SWITZER, 1978. Distribution of mutagens and toxicants in Lake Ontario waters as assayed by microbiological procedures. J. Great Lake Res., 4(2): 237-241.

ETLINGER,H.M., H.O.HODGINS & J.M. CHILLER, 1976. Rainbow trout leucocyte culture: a simplified method in vitro, 12, 599-601.

EVAN, E.P., M.D.BURTENSHAW and C.E.FORD, 1972. Chromosomes of mouse embryos and new-born young: preparations from membranes and tail tips. Stain Technol., 47: 229-234.

EVANS,H.J. 1976. Cytological methods for detecting chemical mutagens. In Hollaender (Ed) Chemical Mutagens: Principles and methods for their detection. Vol. 4: 1-29. Plenum Press, N.Y.

FOX,M. and D.SCOLT, 1980. The Genetic toxicology of Nitrogen and Sulphur mustard. Mutation Research, 75: 131-168.

GAAG VAN DER, M.A., G.M.ALINK, P.HACK, J.C.M.HOEVEN VAN DER and J.F.J.KERKHOFF VAN DE, 1983. Genotoxicological study of Rhine water with the Killifish Nothobranchius rachowi. Mutat. Res,. 113: 311.

GIBSON,D.A. and D.M.PRESCOTT, 1972. Induction of Sister chromatid Exchanges in Chromosomes of rat kangaroo cells by Tritium incorporated into DNA. Experimental Cell Research, 43: 255-267.

GONZALES-GILL,G. and M.H.NAVARRETE, 1982. On the mechanism of differential Giesma staining of BrdU substituted chromatids. Chromosoma, 86: 375-382.

GOODIER,J., HAI-FEIMA & FUMIO YAMAZAKI, 1987. Chromosome fragmentation and loss in two salmonoid Hybrids. Bulletin Fac. Fish, Hokkairo Univ. 38(3) 181-184.

GRINFIELD,S., A.JAYLET, R.SIBOULET, P.DEPARIS, and I.CHOUROULINKOV, 1986. Micronuclei and red blood cells of the newt Pleurodeles walt after treatment with benzo(a) pyrine. Environmental Mutagens. 8(1): 41-51.

HAND,R. 1978. Eukaryotic DNA. Organisation of the genome for replication. Cell, 15: 317-325.

HARRISON,F.L. 1987. Effects of radiation on frequency of chromosomal aberrations and SCE in Neanthes arenaceodenta. In: Oceani Process in Marine Pollution-1. Biological processes and wastes in the ocean. Capuzz, J.M. et al. FL (USA): 145-156.

HARRISON,F.L. and J.M.JONES, 1982. An in vivo Sister Chromatid Exchange assay in the larvae of the mussel Mytilus edulis: Response to 3 mutagens. Mutation Research, 105: 235-242.

HECKMAN, J.R., F.W.ALLENDORF and J.E.WRIGHT. 1971. Trout leucocytes: Growth in oxygenated cultures. Science, 173: 246-247.

HECKMAN, J.R. and P.E.BRUBAKER, 1970. Chromosome preparation from fish blood leucocytes. Progressive Fish Culturist, 32: 206-208.

HEDDLE, J.A., M.HILE, B.KIRKHART, K.MAVOURIN, J.T.MACGREGOR, G.W.NEWELL and M.F.SALAMONE, 1983. The induction of micronuclei as a measure of genotoxicity. Mutation Research, 123: 61-118.

HOEVEN VAN DER JCM., I.M.BRUGGEMAN, G.M.ALINK, J.H. KOEMAN. 1982. The Killifish Nothobranchius rachowi, a new animal in genetic toxicology. Mutat Res., 97: 35-42.

HOLMQUIST, J. 1978. Role of replication time in the control of tissue specific gene for expression. Am. J. Hum. Genet., 40: 151-173.

HOOFTMAN, R.N. 1981. The induction of chromosomal aberrations in Notobranchius rochowii after treatment with methanesulphonate or Benzo (a) pyrene. Mutation Research, 91: 347-352.

HOOFTMAN, R.N. and G.VINK, 1981. Cytogenetic effects on the eastern mud minnow, Umbra pygmea exposed to Ethylmethanesulphonate (EMS), Benzo (a) Pyrene and river water. Ecotoxicology and Environmental Safety, 5: 261-269.

HOOFTMAN, R.N. and W.K.ROAT, 1982. Induction of nuclear anamalies (micronuclei in the peripheral blood erythrocyte of the eastern mud minnow, Umbra pygmea by Ethylmethanosulphonate (EMS). Mutation Research, 104(1/3): 147-152.

HOSE, J.E., J.N.CROSS, S.G.SMITH and D.DIEHL, 1987. Elevated circulating erythrocyte micronuclei in fishes from contaminated sites off Southern California. Marine Environ. Res., 167-176.

HOSE,J.E., V.M.LIGOURI and H.W.PUFFER., 1984. A method for evaluating pollutant teratogenecity and genotoxicity using freshwater or marine embryos. Presented at the Seventh Symposium (Aquatic toxicology) of the American Society for Testing and Materials, Milwaukee, USA.

HYNES,H.B.N. 1960. The Biology of Polluted waters. Liverpool Univ. Press.

ISHI,Y. and M.BENDER, 1978. Caffeine inhibition of pre-replication repair of mitomycin C induced DNA damage in human peripheral lymphocytes. Mutat Res.,51: 419-425.

JAYLET,A., P.DEPARIS and D.GASCHIGNARD, 1986. Induction of micronuclei in peripheral erythrocyte of axolotl larvae following in vivo exposure to mutagenic agents. Mutagenesis, 1(3).

JONES,I.M. and F.L.HARRISON, 1987. Variability in the frequency of Sister Chromatid Exchange in larvae of Mytilus edulis. Implication for field monitoring. Journal of Experimental marine Biology and Ecology, 113(3): 283.

KASTCHENTO, 1890. Zeit. f Mikros Anatomische Forschung, 50. Quoted from Makino (1951).

KATO,H. 1974. Spontaneous Sister Chromatid Exchange detected by a Budr-labelling method. Nature, 251: 70-72.

KATO,H. and H.SHIMADA, 1975. Sister Chromatid Exchange induced by mitomycin : A new method of detecting DNA damage at chromosomal level. Mutation Research, 28: 459-464.

KAUR,D. and M.D.L. SRIVASTAVA, 1965. Structure and behaviour of chromosomes in five freshwater teleosts. Caryologia, 18: 181-191.

KERKHOFF,J.F.J. and M.A.GAAG, 1985. Some factors affecting optimal differential staining of sister-chromatids in vivo in the fish Nothobranchius rachowi. Mutat. Res., 143: 39-43.

KHUDA-BUKSH,A.R. 1979a. Karyology of two hill stream fishes, Barilius bendelisis and Rasbora daniconius Cyprinidae. Curr. Sci., 48: 793.

KHUDA - BUKSH, A.R. 1979b. Chromosomes in three species of fishes, Aplochelius panchax (Cyprinodontidae), Lates calcarifer (Percidae) and Gadusia chapra (Clupeidae). Caryologia , 32: 161.

KHUDA-BUKSH, A.R. 1980. A high number of chromosomes in the hill stream cyprinid, Tor putitora (Pisces) Experientia, 36: 173.

KHUDA-BUKSH, A.R., 1982. Karyomorphology of two species of Tor (Pisces: Cyprinidae) with a high number of chromosomes. Experientia, 38:82.

KHUDA-BUKSH, A.R. and A. BARAT., 1987. Chromosomes in 15 species of Indian teleosts (Pisces). Caryologia, 40: 131-144.

KHUDA-BUKSH,A.R. and G.K.MANNA, 1976a. The chromosome analysis in two species of Indian mullets. Indian Biol., 8: 23.

KHUDA-BUKSH,A.R. and G.K.MANNA, 1976b. Chromosome analysis of two Indian hybrid carps. Proc. 63rd Ind. Sci. Congr. 3: 151.

KHUDA - BUKSH, A. R. and K.NAYAK, 1982. Karyomorphological studies in two species of hill streams from Kashmir, India. Occurrence of a high number of chromosomes. Chromosome Inf. Ser., 33: 12.

KIHLMAN,B.A. and D.KRONBERG,1975. Sister chromatid Exchanges in Vicia faba I. Demonstration by a modified fluorescent plus giemsa (FPG) technique. Chromosoma, 51: 1-10.

KLIGERMAN,A.D. 1977. Umbra limi: An aquatic model for the study of Sister Chromatid Exchange and exchanges in vivo. Ph.D. Thesis., Cornell University, Ithaca New York.

KLIGERMAN,A.D. 1979a. Cytogenetic methods for the detection of radiation induced chromosome damages in aquatic organisms.

KLIGERMAN,A.D. 1979b. The use of aquatic organisms to detect mutagens that cause cytogenetic damages. Symposium on radiation effect on aquatic organisms, 11th May 1979, Japan.

KLIGERMAN,A.D. 1979c. Induction of sister chromatid exchanges in the central mudminnow following in vivo exposure to mutagenic agents. Mutation Research, 64: 205-217.

KLIGERMAN,A.D., J.WILMER and G.EREXSON, 1981. Characterisation of a rat lymphocyte culture system for assessing Sister Chromatid Exchange after in vivo exposure to Xenotoxic agents. Environmental Mutagensis, 3: 531-543.

KLIGERMAN,A.D. and S.E.BLOOM, 1976. Sister Chromatid differentiation and exchanges in adult mudminnows (Umbra limi) after in vivo exposure to Bromodeoxyuridine. Chromosoma (Berl), 56: 101-109.

KLIGERMAN,A.D. and S.E.BLOOM, 1977. Rapid chromosome preparations from solid tissues of fishes J. Fish Res. Bd. Canada, 34: 266-269.

KLIGERMAN, A.D., S.E.BLOOM and W.M.HOWELL, 1975. Umbra limi: A model for the study of chromosome aberration in fishes. Mutat Res., 31: 225-233.

KLIGERMAN, A.D., W.E.BISHOP and L.C.VALENTINE, 1984. Use of the mudminnow, Umbra sp., in an in vivo sister-chromatid exchange test. Proc: Symposium on the Use of Small Fish Species in Carcinogenecity Testing p. 111-118. Maryland, USA.

KOCAN,R.M. and D.B.POWELL, 1985. Anaphase aberrations: An in vitro test for assessing genotoxicity of individual compounds and complex mixtures. In: Short term genetic bioassays in the analysis of complex environmental mixtures. Waters M.D. et al. (Eds.). Vol. IV. Plenum Press. N.Y.

KOCAN,R.M., K.M.SABO and M.L.LANDOLT, 1985. Cytotoxicity/Genotoxicity: The application of cell culture techniques to the measurements of marine sediment pollution. Aquatic Toxicology, 6(3): 165.

KOCAN,R.M., M.L.LANDOLT, J.A.BOND and E.P.BENDITT, 1981. In vitro effect of mutagens/carcinogens on cultured fish cells. Archieves of Environmental Contaminant and Toxicology, 10: 663-671.

KOCAN,R.M., M.L.LANDOLT and K.M.SABO, 1982. Anaphase aberration: A measure of genotoxicity in mutagen treated fish cells. Environmental Mutagen, 4: 181-189.

KRISHNAJA,A.P. and M.S.REGE, 1980. some observations of certain teleosts using a simple method. Indian Journal of Experimental Biology, 18(3): 268-270.

KRISHNAJA,A.P. and M.S.REGE, 1982. Induction of chromosomal aberration in fish Boleopthalmus dussumeri, after exposure in vitro to mitomycin C and heavy metals Mercury, Selenium and Chromium. Mutation Research, 102: 71-82.

LABAT,R., G.LARROUY and L.MALASPINA, 1967. Technique de culture des leucocytes de Cyprinus carpio L. C.R.Acad Sci. Paris Ser. D, 264: 2473-2475.

LANDOLT,M.L. and R.M.KOCAN. 1983. Fish cell cytogenetics: a measure of the genotoxic effects of environmental pollutants. In: Advances in Environmental Science and Technology, Vol. 13, Aquatic Toxicology edited by J.O. Nriagu, John Wiley and Sons, New York, PP. 336-353.

LANDOLT,M.L. and R.M.KOCAN, 1984. Anaphase aberration in cultured fish cells as a bioassay of marine sediments. Marine Environmental Research, 14(1/4): ISSN 0141-1136.

LATT,S.A. 1974a. Sister Chromatid Exchanges indices of human chromosome damage and repair : Detection by fluorescence and induction by mitomycin-C. Proceedings of National Academy of Science (USA), 71: 3162-3166.

LATT,S.A. 1974b. Localisation of Sister Chromatid Exchanges in human chromosomes. Science, 185: 74-76.

LATT,S.A., G.STETTEN, L.A.JUERGENS, H.F.WILLARD and C.D.SCHER, 1975. Induction by alkylating agents of Sister Chromatid Exchanges and chromatid breaks in Falconi's anaemia. Proceedings of National Academy of Science (USA), 72: 4066-4077.

LATT, S.A., J.ALLEN, S.BLOOM, A.CARRANO, E.FALKE, D.KRAM, E. SCHNEIDER, R.SCHRECH, R.TICE, B.WHITFIELD & S.WOLFF 1981. Sister chromatid exchange - a report of the GENE-TOX workshop. Mutat. Res, 87, 17-62.

LEVAN, A, K. FREDGA and A.A.SANDBERG, 1964. Nomenclature for centromeric position on chromosomes. Hereditas, 52: 201-220.

LONGWELL,A.C. 1976. Chromosome mutagenesis in developing eggs sampled from the New York Bight. NOAA Tech. Memorandum 7: 64.

LONGWELL,A.C., D.PERRY, J.B.HUGHS and A.HEBERT, 1983. Frequencies of micronuclei in mature and immature lymphocytes of fish as an estimate of chromosomes mutation rate results of field surveys on Window pane flounder, Winter flounder and Atlantic mackerel. Proceedings of council Meeting of the International Council for the Exploration of sea (Sweden), 14(7):

LUCHNIK,N.V., N.A.PORJADKOVA, T.V.KONDRASH. VA and A.S.SEILANOV, 1987. Production of sister-chromatid exchange by irradiation during the GI Stage: The probable role of 5 - bromodeoxy-uridine. Mutat. Res, 190: 149-152.

LUGO,M.H., H.S.RAUCHFUSS, H.R.ZAKOUR, J.W.ALLEN and J.C.HOZIEF 1989. Evidence for chromosomal replicons as units of sister-chromatid exchanges. Chromosoma, (Berl), 98: 69-76.

Mac GREGOR,J.F., C.M.WEHR and D.H.GOULD, 1980. Clastogen induced micronuclei in peripheral blood erythrocytes: Of the basis of an improved micronuclei test. Environmental Mutagen, 2: 509.

Mac PHAIL,J.D. and R.L.JONES, 1966. A simple technique for obtaining chromosomes from teleost fishes. Journal of Fisheries Research Board Canada, 23(5): 767-768.

MADDOCK,M.B. 1986. Induction of sister-chromatid exchanges and chromosomal aberrations in haematopoetic tissue of a marine fish following in vivo exposure to genotoxic carcinogens. Mutation Research, 172(2): 165-175.

MADDOCK,M.B. and J.J.KELLY, 1980. A sister chromatid exchanges: Assay for detecting genetic damage to marine fish exposed to mutagens and carcinogenes: "Water chlorination, Environ-mental Impact Health effects". Jolly Ral, et al. (Eds). Annual Arbor Science (Publ.) 3: 835-837.

MAJONE,F., R.BRUNETTI, I. GOLE and A.G.LEVIS, 1987. Persistence of micronuclei in the marine mussel, M. galloprovincialis after treatment with MMC. Mutation Research, 191 (314): 157.

MAKINO,S. 1951. An atlas of the chromosome numbers in animals. IOWA State College Press.

MANNA,G.K. 1982. The new horizon of mutagenesis. Current Science, 51: 1082.

MANNA G.K. 1984. Progress in fish cytogenetics Nucleus, 27(3): 203-231.

MANNA,G.K. 1986. Tilapia as a model for testing genotoxic agents. In: Perspectives in Cytology and Genetics, 395-406.

MANNA,G.K. 1989. Fish cytogenetics related to taxonomy, evolution and monitoring aquatic genotoxic agents. In: Fish Genetics in India Das and Jhingran (Eds): 21-46. Today and Tommorrow's Printers and Publishers, New Delhi.

MANNA,G.K. and A.SADHUKHAN, 1986. Use of cells of gill and kidney of tilapia fish in micronucleus test. Curr. Sci, 55: 498-501.

MANNA G.K. and A.R.KHUDA-BUKSH, 1974. Somatic chromosomes of two carp hybrids. Chrom. Inf. Ser, 16: 26.

MANNA,G.K. and A.R.KHUDA-BUKSH, 1977a. Karyomorphology in eleven species of cyprinid fishes and cytological evaluation of the family. Nucleus, 20: 119.

MANNA,G.K. and A.R.KHUDA-BUKSH, 1977b. A check-list of chromosomes in cyprinid fishes. J. Zool. Res, 1: 34-43.

MANNA,G.K., G.BANERJEE and S.GUPTA, 1985. Micronuclei test of different genotoxic agents in the peripheral erythrocytes of the exotic fish Sarotherodon mossambica. Nucleus, 28: 176-179.

MANNA, G.K. and P.K.MUKHERJEE, 1984. Effect of organophosphate malathion on Tilapia Proc. Fifth All India Cong. Cytol. Genet. 225-235.

MANNA,G.K. and R.PRASAD, 1968. A study of somatic chromosomes of a common Indian live-fish Channa punctatus by a kidney technique Proc. 55th Ind. Sci. Congr. 3: 468.

MANNA,G.K. and R.PRASAD, 1971. A new perspective in the mechanism of evolution of chromosomes in fishes. Proc. first All India cong. Cytol. Genet.

MANNA,G.K. and R.PRASAD, 1973a. Chromosomes in three species of fish (Channa) Nucleus, 16: 150.

MANNA, G.K. and R.PRASAD, 1973b. Somatic and germinal chromosomes of the tropical perch, Anabas testudineus (Bloch) Indian J. Zool., 2:1.

MANNA,G.K. and R. PRASAD, 1973c. Somatic and germinal chromosomes of two species of fishes belonging to the genus Puntius. J. Cytol. Genet, 8: 145.

MANNA,G.K. and R.PRASAD, 1974a. Chromosome analysis in three species of fishes belonging to the family Gobi[illegible]. Cytologia, 39:609.

MANNA,G.K. and R.PRASAD, 1974b. Cytological evidence of two forms of Mystus vittatus (Bloch) as two species. Nucleus, 17:4.

MANNA,G.K. and R.PRASAD, 1976. Chromosome analysis in five species of freshwater fishes. Nucleus, 20: 274.

MEREDITH, R. 1969. A simple method for preparing meiotic chromosomes from mammalian testis. Chromosoma, (Berl), 26: 254-258.

MINGDE,T., Y.YIZHEN and Z. ZHIYOUNG, 1987. Chlorothalonile induced micronucleus rate of eel erythrocytes. Bulletin of Human medical college, Vol. 12, No.2, 154.

MITAMI, H. 1983. Lethal and mutagenic effect of radiation and chemicals on cultured fish cells from the erythrophoroma of gold fish, C. auratus. Mutat. Research, 107(2): 279-288.

MOHANTY, L. and R.PRASAD, 1982. Sister chromatid exchanges in a live fish Channa punctatus. Nucleus, 25(3): 161-164.

MONG,S.J. and T.M.BERRA, 1976. The effect of increasing dosages of X-irradiation on the chromosomes of the central mud minnow, Umbra limi. J. Fish. Biol, 14(6): 523-527.

MORTIMER,R.K. and T.R.MANNEY, 1971. Mutation induction in yeast. In Chemical mutagens: Principles and methods for their detection, Vol.1, pp. 289-310. Ed. by A. Hollaender, Plenum Press.

MUKHERJEE,P.K. 1984. A study on the cytogenetic effects of a few environmental chemical pollutants in the exotic fish Sarotherodon mossambica. Ph.D. Thesis, Kalyani University.

MULLER,H.J. 1927. Artificial transmutation of the gene. Science, 66: 84-87.

NAKANISHI,Y. and E.L.SCHHEIDER, 1979. In vivo SCE: A sensitive measure of DNA damage. Mutation Research, 60: 329-337.

NATARAJAN,R. and K.SUBRAHMANYAM, 1968. A preliminary study on the Chromosomes of Tilapia mossambica (Peters). Curr. Sci, 37(9) 262-263.

NATARAJAN, R. and K.SUBRAHMANYAM, 1974. A karyotype study of some teleosts from Porto Novo Waters. Proc. Ind. Acad. Sci, (B) 5: 173-196.

NAYAK,K. and A.R. KHUDA-BUKSH, 1988. Karyomorphology of a crocodile fish Platycephalus tuberculatus (Platycephalidae, Pisces). J. Inland fish Soc. India, 20(2) 35-37.

NAYYAR,R.P. 1962. Karyotype studies in two cyprinids. Cytologia, 27(2): 229-231.

NAYYAR, R.P. 1964. Karyotype studies in seven species of Cyprinidae. Genetica, 35: 95-104.

NAYYAR,R.P. 1965. Karyotype studies in the genus Notopterus (Lacepede). The occurrence and fate of univalent chromosomes in spermatocytes of N. chitala. Genetica, 36: 398-405.

NAYYAR,R.P. 1966. Karyotype in thirteen species of fishes. Genetica, 37: 78-92.

NEWSOME,C.S. 1980. A multigeneration fish toxicity test as an aid in the hazard evaluation of aquatic pollutants. Ecotoxicology and environmental safety. 4, 362-369.

NOGUSA,S. 1960. A comparative study on the chromosomes in fishes with particular consideration on taxonomy and evolution. Mem. Hyogo. Univ. Agric., 3:1.

OHNO,S. 1970. Evolution by gene duplication Berlin. Speringer-Verlag.

OJIMA,Y. and S.HITOTSUMACHI, 1967. Cytogenetic studies in lower vertebrates IV. Japan J. Genet, 42: 163.

OJIMA,Y., S.HITOSUMACHI and M.HAYASHI, 1970. A blood culture method for fish chromosomes. Jpn. J. Genet, 45, 161-162.

PAINTER, B. 1980. A replication model for SCE. Mutat Res, 70: 337-341.

PARK,E.H. and H.GRIMM, 1982. Elevated SCE rate in lymphocytes of the European eel (Anguilla anguilla) with cauliflower tumour. Cancer Genetics & Cytogenetics, 5: 137.

PARRY,J.M., D.J. TWEATS and A.J. Al-Mossawi, 1976. Monitoring the marine environment for mutagens. Nature, 264: 538-540.

PECHKURENKOV,V.L. 1976. Chromosome aberrations in fish embryos induced by continuous irradiation. Radiobiologia, 16(6): 842-846.

PERA,F. and P.MATTIAS, 1976. Labelling of DNA and differential sister chromatid staining after BrdU treatment in vivo. Chromosoma, 57:13.

PERRY,P. and H.J. EVANS, 1975. Cytological detection of mutagen carcinogen exposure by sister chromatid exchange. Nature, 258: 121-125.

PERRY, P. and S. WOLFF, 1974. New giemsa method for differential staining of sister chromatids. Nature, 251: 156-158.

PESCH,G.G. and C.E.PESCH, 1980. Neanthes arenaceodentata (Polychaeta: Annelida) a proposed cytogenetic model for marine genetic toxicology. Can. J. Fish Aquatic Sci, 37: 1225-1228.

PESCH,G.G., C.E.PESCH and A.R.MALCOLM, 1981. Neanthes arenaceodentata. A cytogenetic model for marine genetic toxicology. Aquatic Toxicol, 1: 000-000.

PREIN, A.E., G.M.THIE, G.M.ALINK, J.H.KOEMAN and C.L.M.POELS, 1978. Cytogenetic changes in fish exposed to water of river Rhine. Science of total Environment, 9: 287-291.

PROTIC,S.M. and B.KURELEC, 1983. High mutagenic potency of several polycyclic aromatic hydrocarbons induced by liver post mitochondrial fractions from control and xenobiotic treated immature carp. Mutat. Res., 118: 3.

REDDY,P.V.G.K. and G. JOHN, 1986. A method to increase mitotic metaphase spreads and permanent chromosome preparations for karyotype studies in fishes. Aquacultura Hungarica (Szarvas), 5: 31-36.

REGAN,J.D., M.M.SIGEL, W.H.LEA, K.A.LEMES and A.R.BEASKY, 1968. Chromosomal alterations in marine fish cells in vitro. Canadian Journal of Genetics and cytology, 10: 448.

RENNER,H.W. 1979. Arzneimittel - Forsch, 29, 1971.

RETZIAT, 1890. Biol. Foren Stockholm. (Quoted from Makino (1951).

RISHI,K.K. 1973. A preliminary report on the karyotypes of 18 marine fishes. Res. Bull. Panjab Univ, 24: 161.

RISHI,K.K. 1975. Somatic and meiotic chromosomes in Trichogaster fasciatus (Bl & sch). Genen Phaenen, 18: 49.

RISHI, K.K. 1976a. Mitotic and meiotic chromosomes of a teleost, Callichrous bimaculatus (Bloch) with indications of male heterogamety. Cienciae cultura, 28(10): 1171-1173.

RISHI,K.K. 1976b. Karyotypic studies on four species of fishes. The Nucleus, 19: 95-98.

RISHI,K.K. 1979. Somatic g-banded chromosomes of Colisa fasciatus (Perciformes, Belontidae) and confirmation of female heterogamety. Copeia, 1:146.

RISHI,K.K. and J.SINGH, 1982. Karyological studies on five estuarine fishes. Nucleus, 25(3): 178-180.

RISHI,K.K. and P.GAUR, 1976. Cytological female heterogamety in jet-black molly, Molliensia sphenops. Curr. Sci, 45(18) 669-670.

RISHI,K.K. and S.RISHI, 1981. Giemsa banding in fish chromosomes. In: Perspectives in Cytology and Genetics. G.K.Manna and U. Sinha (Eds). Hindasia, Delhi.

ROBERTS,F.L. 1964. A chromosome study of twenty species of centrarchidae. J. Morphol, 115: 401.

ROBERTS,F.L. 1966. "Cell culture of fibroblasts from clupea harengus Gonads". Nature, 1966, 212 (5070), 1592-93.

ROBERTS,F.L. 1967. Chromosome cytology of Osteichthys. Prog. fish. cult, 29: 75.

ROHRBORN,G. 1970. In: Chemical Mutagenesis in Mammals and Man, (1970) 148. Eds. f. Vogel & G.Rohrborn, Springer-Verlag, Berlin, New York.

SAVAGE, J.R.K. 1975. Chromosomal aberrations as tests for mutagenicity. Nature, Vol. 258: 103-104.

SCHMID,W. 1975. The micronucleus test. Mutat Res, 31: 9.

SCHMID,W. 1982. The micronucleus test: An in vivo bone marrow method. In: Cytogenetic Assays of Environmental mutagens. T.C.Hsu, ed. Allnheld, Osmun and Co. New Jersey. pp.221-229.

SCHRODER,J.H. 1969. X-ray induced mutations in the poecillid fish, Lebister reticulatus. Peters. Mutat Res ,7: 75.

SCHRODER,J.H. 1979. "Radiation induced mutation in fish. International Symposium on Radiation" : Effects on aquatic organism. Japan In: Radiation effects in aquatic organism.

SCHROEDER,T.M. 1966. Cytogenetische and cytologische Befunde bei enzymopenischen Penmyelo Pathien and Pancytopenien (amiliare Panmyetopathien Fanconi Glutathion reduktasemangel Anamie, Megaloblastare vitamin B folsauremangel Anamie) Humangenetik, 2: 287.

SCHROEDER,T.M. 1970. Investigations of bone marrow smears In: chemical Mutagenesis (ed G. Vogel and G. Rohrborn) Berlin Springer-verlag.

SERRES,F.J.D. 1976. Prospects for a revolution in the methods of toxicological evaluation. Mutat. Res, 38: 165-176.

SHAH,M.S. and J.A.BEARDMORE, 1987. Induction of dominant lethal mutation by MMS in the fish tilapia Oreochromis niloticus (Linneas). Indian Journal of Experimental Biology, 25(6): 375.

SHIRASU,Y., M.MORIYA, K.KATO, F.LIENARD, H. TEZUKA & S. TERAMOTO, 1977. Mutagenicity Screening on pesticides and modification products: a basis of carcinogenicity evaluation. In Origins of human Cancer, Book A. pp. 267-285. Ed. by H.H. Hiatt. J.D. Watson, and J.A. winsten. Cold Spring Harbor Laboratory.

..ICK,K., M.WESTERGAARD and O.FRYDENBERG, 1962. Haemoglobin pattern and chromosome number of american, European and Japanese eels (Anguilla) . Nature, 193 (4819), 1001-02.

SIMON,R.C. 1963. Chromosome morphology and species evolution in the five North American species of Pacific salmon (Oncorhynchus). J. Morphol, 112, 77-97.

SIMON,R.C. and A.M.DOLLAR, 1963. Cytological aspects of speciation in two North american teleosts, Salmo gairdneri and Salmo clarki lewisi. Can. J. Genet. Cytol ,5, 43-49.

SOBELS,F.H. 1976. Some thoughts on the evaluation of environmental mutagens. Mutat. Res, 38: 361-366.

SOFRADZIJA,A., T.VUKOVIC and R.HADZISE, LIMOVIC, 1980. Effects of some pesticides and detergents on the fish chromosomes sets. Results of the preliminary investigations. Ichthyologia 12: 71-76.

SOM,R.C. and G.K.MANNA, 1984. Preliminary study of somatic chromosome aberrations in F1 offspring and their E. irradiation parents of Tilapia. In: Perspectives in cytology and Genetics. Manna, G.K. and U. Sinha (Eds.) 4:

SRIVASTAVA,M.D.L. & B. DAS, 1969. Somatic chromosomes of teleostean fish J. Heredity, 60(2): 57-58.

STETKA,D.G. and S.WOLFF, 1976. Sister chromatid exchange as an assay for genetic damage induced by mutagen - carcinogens. II. In-vitro tests for compounds requiring metabolic activation. Mut. Res, 41: 343-350.

STICH,H.F. and A.B.ACTON, 1976. The possible use of fish tumours in monitoring for carcinogens in the marine environment. Prog. Exp. Tumour. Res, 20: 44-54.

STOCK,A.D., D.B. BURNHAM and T.C. HSU, 1972. Giemsa banding of meiotic chromosomes with description of a procedure for cytological preparations from solid tissues. Cytogenetics, 11: 534-539.

STROMBERG,P.T., M.L.LANDOLT and R.M.KOCAN, 1981. Alterations in the frequency of sister chromatid exchanges in flatfish from Puget Sound. NOAA Technical Memorandum OMPA-10. Washington, USA.

SUBRAHMANYAM, K. 1969. A karyotypic study of the estuarine fish Boleopthalmus boddaerti (Pallas) with calcium treatment. Curr. Sci, 38(18): 437-39.

SUBRAHMANYAM,K. 1970. Effect of rotenone on fish chromosomes. J. Annamalai Univ.,28: 243-245.

SUBRAHMANYAM and NATARAJAN, 1970. A study on the somatic chromosomes of Therapon Cuvier (Teleostei : Perciformes). Proc. Ind. Acad. Sci, 1970. 72(B) 6: 288-294.

SUGATT,R.H. 1978. Chromosome aberrations in eastern mudminnow U. pygmaea exposed in vivo to Trenimon or river water. Rep. M.D. and E. 78/3 of Central Lab. Netherlands.

SUTOU,S.1981. Spontaneous sister chromatid exchanges in chinese hamster cells in vivo and in vitro. Mutat Res. 82: 331-341.

SUTOU,S. 1986. Sex difference in micronuclei test: The collaborative study group for micronucleus test. Mutation Research, 172(2): 151-164.

SUYAMA,I. and H.ETOH, 1983. X-Ray induced dicentric yields in lymphocytes of the teleost, Umbra limi. Mutation Research, 107, 111-118.

SVARDSON,G. 1945. Chromosome studies in Salmonidae. Rep. Inst. Freshwater Res. Drottningholm 23:1.

SWARUP, H. 1959. Production of triploidy in Gastrosteus aculeatus J. Genet, 56: 143.

TAYLOR,J.H., P.S.WOODS and W.L.HUGHES, 1957. The organisation and duplication of chromosomes as revealed by autoradiographic studies using Tritium-labelled thymidine. Proceedings of National Academy of Science (USA), 43: 122-128.

TICE,R., J.CHAILLET and E.L.SCHNEIDER, 1976. Demonstration of spontaneous SCE in vivo. Experimental Cell Research, 102: 426-429.

TJIO,J.H. and A. LEVAN, 1956. The chromosomes of man. Hereditas, 42:1

TYSTSUGIMA,V.G. 1972. In: Marine Ecology 157 (Ed.) G.G.Polikarpov. AECtr 7299 Washington D.C., USAEC.

VIGFUSSON,N.V., E.R.VYSE, C.A.PERNSTEINER and R.J.DAWSON, 1983. In vivo induction of SCE in Umbra limi by the insecticide endrin chlordane diazinon and guthion. Mutation Research, 118: 61-68.

VOGEL,W. and T.BAUKNECHT, 1976. Differential chromatid staining by in vivo treatment as mutageneticity test system. Nature, 260: 448-449.

WALTON,D. 1984. Fish cell DNA breakage and repair assays for detecting genotoxic activity. Aquatic Toxicol-workshop

WALTON,D.G., A.B.ACTON and H.F.STICH, 1983. DNA repair synthesis in cultured mammalian and fish cells following exposure to chemical mutagens. Mutation Research, 124 (2): 153-161.

WARDHAUGH,A.A. 1981. Dominant Lethal Mutations in Tilapia mossambica elicited by myleran. Mutation Research, 88 191-196.

WATERS, M.D., V.F.SIMMON, A.D.MITCHELL, T.A.JORGENSON and R.VALENCIA, 1980. An overview of short term test for mutagenic and carcinogenic potential of pesticides. J. Environ. Sci. Health, (B) 15: 867-906.

WITSCHI,E. and R.LAGUENS, 1963. Chromosomal aberrations in embryos from overripe eggs. Developmental Biology,7: 605-616.

WOLFF,S. 1977. Sister Chromatid Exchanges. Annual Review of Genetics, 11: 183-201.

WOLFF,S. and J.BODYCOTE, 1977. In: Human Molecular Cytogenetics Sparkes, D, E. Comings and C.F.Fox (Eds), Academic Press, N.Y. 1977, p. 335.

WOLFF,S. and P.PERRY, 1974. Differential Giemsa staining of sister - chromatids and the study of SCEs without autoradiography. Chromosoma (Berl.), 48: 341-353.

WOLTERS,W.R., C.L.CHRISMAN and G.S.LIBEY, 1982. Erythrocyte nuclear measurements of diploid and triploid channel catfish, Ictalurus punctatus Journal of Fish Biology, 20: 253-258.

WOODHEAD,D.S. 1976. Influence of acute irradiation on induction of chromosome aberrations in cultured cells of the fish Ameca splendens In: Biological and environmenal effects of low level radiation. Vienna. IAEA. 1:67.

WEI,Y.L. 1987. Preliminary studies on frequencies of Sister Chromatid Differentiation (SCD) and exchanges (SCE) in renal cells of grass carp (Ctenopharyngodon idellus) in vivo. Acta Hydrobiologica, 11(1): 29-33.

YAMAZAKI,F. 1983. Chromosomal changes in salmonids. Changes in chromosome number and morphology due to over ripening of eggs and irradiation. Can. Trans. Fish. Aquatic Sci., 4962: 1-22.

ZAJICK,K.D. and R.B.PHILLIPS, 1984. Mitotic inhibition and anaphase aberrations in rainbow trout embryos treated with MNNG and gamma radiation. Genetics, 107(3):´2.

ZAKOUR,H.R., M.L.LANDOLT and R.M.KOCAN, 1984. Sister Chromatid Exchange analysis in cultured peripheral blood leucocytes of the coldwater marine fish, Pacific staghorn sculpin (Leptocottus armatus) A feasible system for assessing genotoxic marine pollutants. Basic Life Sci., 29(B): 493-508.

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