Discussion
Since the ultimate effect of pesticides is the elimination of pests, chemosterilants properly belong to this class of biologically active chemicals (Borkovec, 1974). Nevertheless, with respect to individual pest organisms, chemosterilants do not kill, and their effects must be restricted to damaging only one function – reproduction. The potential advantage of sterilization technique in which chemicals used to decrease the birth rate of a population over the pesticidal technique in which chemicals are used to increase the death rate of a population were recognised first by Knipling (1960) who suggested that if a portion of population of sexually reproducing organisms is killed, the survivors will continue to reproduce with unaltered vigour. However, if the same proportion is sterilised the sterile organisms will compete with fertile ones for males and the reproduction rate of the population will decrease. Thus, sexual sterilisation of insects by chemicals is a rather new and effective method of insect control. Borkovec (1966) and La Brecque and Smith (1968) demonstrated that a great variety of chemical compounds (chemosterilants) can destroy the reproducing capacity of insects without impairing their sexual competitive sex. Chemosterilants have been suggested by Klassen et al. (1968) as an alternative method of sterilisation where γ and X ray irradiation has been proved too damaging to the species.
Most of the work done so far on chemosterilants, as described earlier, has been on induction of sterility, effects on reproduction, egg viability and hatching etc. and very little has been done on induction of chromosomal aberrations. Chromosomal aberrations are frequently associated with high sterility and thus may be potentially useful as a method of genetic control. A large number of structurally abnormal chromosome in an individual may result into malformation or abortion of the resulting embryo due to chromosomal imbalance which may finally lead to sterility in the individual. Keeping the above into consideration, the present investigations were undertaken.

Numerous observations during the study for evaluating effects of alkylation and non-alkylating chemosterilants on chromosomes of Dysdercus similis, Callosobruchus chinensis, Sarris faba and Poekilocerus pictus have revealed that these chemicals have attested to their chromosome damaging properties. BHC, a chlorinated hydrocarbon, has also proved to induce chromosomal anomalies in the above mentioned insects.

The physiological effects of aziridinyl chemosterilants manifest themselves as gross effects involving all the chromosomes or the whole nucleus. This type of effect is a non specific one and has been reported as "primary effects" by Albert and Politzer (1923, 24) Marquardt (1938) and Sax
(1941). These may also be termed as nucleotoxic effects. On the other hand, sometimes chromosomes are specifically and individually affected by the chemosterilants. The effects on individual chromosomes reveal themselves after some lapse of time of administration of the chemicals. These have been termed as 'secondary effects' by the above named workers and interpreted as structural changes by Lea (1946).

The chromosomal aberrations caused by different chemosterilants and BKC in the spermatocytes of all the four insects studied have been dealt with under two categories: (a) Qualitative effects on the nucleus and chromosomes and (b) Quantitative changes involving the frequency of aberrations in different stages of the meiotic division. Qualitatively the effects have been found to be of two distinct types as mentioned above i.e. (i) gross or primary effects and (ii) effects on individual chromosomes or secondary effects. Two such distinct types of effects of chemosterilants on chromosomes of bone marrow have also been proposed by Manna and Das (1972, '73, '76, '77) in chemosterilised mice. A third type of effect of the chemosterilants has been observed in the present study in the form of spindle rearrangement which has also been described by Nambiar (1955) in urgehene treated P. pictus.

The gross or nucleotoxic effects caused by all the four chemosterilants as well as BKC in all the insects under
study are shrinkage of chromosomes, stickiness, fusion, sticky or pseudobridges, clumping, interconnected and linearly connected bivalents, pycnosis etc. In *D. similis* and *E. fabia* 'arrest of metaphase' and in the later a typical deformity of the nucleus into a serpentine form has been found. Other types of gross effects are seen in *P. pictus* in the form of tetraploidy, distorted appearance of the nuclear material, wooly appearance of the chromosomes and failure of karyolysis. The effects on individual chromosomes induced by the chemo-sterilants have generally been noticed in the form of stretching, gaps, constrictions, fragmentation and breaks, translocations and dicentric bridges. Spindle dearrangement has revealed itself in the form of unequal distribution of chromosomes at the poles and lagging chromosomes (laggards). BHC has been found to cause mostly gross effects in all the insects studied except in *P. pictus* where lower doses of BHC have induced fragmentation and also beaded appearance (constrictions) of the chromosomes. In *E. fabia* no effect on individual chromosomes by any chemical used has been observed, most probably because of their extremely small size. Only translocations have been located after 48 hours in some insects fed with .6 and .8 µl of Tepa and Henna in 10 ml water.

Shrinkage of chromosomes is noticed in *D. similis* and *P. pictus* quite early after treatment with apholate (4.5%).
8 ul tepa, .025% and .05% BHC and saturated apholate respectively. A number of chemicals have been shown to cause extreme contraction of chromosomes in mitosis (Nambar, 1955). It is presumed that this anomaly is due to contraction of thread-like protein molecules of the chromosomes of the particular insects under the influence of the chemicals mentioned above. The molecules thus assume a corpuscular form. This view is in conformity with that of Ostergren (1944 b) although he first (1943) believed that the contraction was due not to the action of the chemicals on the chromosomes directly but was a consequence of their action on the spindle. According to Nambar (1955) contraction occurs only in mitotic and not in meiotic chromosomes but this study has clearly revealed contraction of chromosomes in meiotic chromosomes of D. similis and P. pictus.

One of the most profound effects of almost all the chemicals used and found in the spermatocyte chromosomes of all the four insects under study is seen in the gradual manner in which the chromosomes become sticky and tend to fuse with one another. The present observations reveal that the sticky chromosomes tend to fuse with one another resulting in sticky bridges as in P. pictus, linearly connected chromosomes as in D. similis and P. pictus, interconnected bivalents as in D. similis and finally clumping chromosomes in all the four insects, because of which the individual
identity of the chromosomes is lost. Linearly connected bivalents, showing a quadrivalent chain like structure has been reported by Saha and Khudabakhsh (1974a, b) in methanol and 2', 4' dihydroxychalcone treated grasshoppers respectively. However, they explain the anomaly as being the result of exchange type aberration i.e. translocation followed by chiasma and breakage of bivalents. This anomaly involving as many as nine bivalents has been reported in barbital treated grasshopper chromosomes by Das and Mukherjee (1977). Quadrivalent structures of metaphase I have also been reported by Bhattacharya and Halder (1978). A variety of chemicals have been reported to cause stickiness in plant and animal cells. Ostergren (1944 a) obtained sticky bridges in ethylene glycol treated Allium cepa. Koller (1952) has shown chromosome stickiness in tumour cells, of nitrogen mustard fed rats whereas Revell (1953) has reported pseudo-chiasmata consequent to chromosome stickiness in Vicia faba treated with the same chemical. Stickiness has been shown by Nambiar (1955) in urethane treated P. dactylis. Sturelid (1971) in Tapa treated chinese hamster and V. faba chromosomes; Manna and Das (1972, 73, 76, 77) in bone marrow chromosomes of mice; by calcium chloride, ADHolate, Hempa, Tapa and ENT 50787 and ENT 50172 respectively; Das and Manna (1974) in Metepa treated mice; Saha and Khudabakhsh (1974) and Saha (1981) in methanol treated Oxya velox and sodium chloride treated Spathosternum respectively; Bhattacharya and Halder (1978) and Bhattacharya (1976, 81)
in proline, semicarbazide and amino acids treated Lygaeus hexas respectively and Parida, Swain and Singh (1973) in ADR treated grasshoppers.

According to Darlington and Koller (1947) chromosome stickiness is attributed to excessive nucleic acid change and failure of end gene reproduction. From the present investigations it is postulated that profound changes occur on the surface of the chromosomes most probably due to loss of viscosity in their matrix as a consequence of treatment with the chemicals. These changes on the surface result in gradual loss of individuality, the chromosomes become sticky and ultimately become massed together to form irregularly shaped clumps, from which sometimes, e.g. in P. pictus, a few strands - the only remnants of the chromosomes - project. This view is supported by the findings of Nambiar (1955). In 10 μl saturated aphpolate treated P. pictus after 24 hours, the chromosomes appear wooly and bivalents look like rings. Presumably this anomaly is also due to the changes caused by high dose of Aphpolate on the surface of the chromosomes. Clumping of chromosomes as a consequence of stickiness has been reported in various insects, plants, and mammals by all the above workers.

In certain instances like in 3 μl Tepa treated D. similis after 24-70 hours and saturated Aphpolate treated E. fabia after 16 hours, a typical anomaly has been met with. This has been termed by the author as 'arrested metaphase',
when all the chromosomes seem to be static on the equatorial plate. This is presumed to be due to inability of chromosome separation or due to failure of chromatid attraction to the equatorial plate. This aberration has not been reported in any insect, plant or mammal so far as the author is aware of. Another gross or nucleotoxic effect of the chemicals, specially Metopa, has been observed as deformed serpentine nucleus in *P. fabia* and distorted nuclear contents in *P. pictus*. In the later 10 µl of saturated ammoniate has also been found to distort the nuclei. Such distortion and deformity of the nucleus has not been reported, so far as the author is aware of. However, total nuclear pycnosis, as induced by almost all the chemosterilants at high doses and BHC in all the four insects investigated, at different post treatment periods, has been reported by many workers like Manna and Das (1972, '73, '76, '77); Saha and Khudabaksh (1974); Das and Manna (1974) etc. It is suggested that either continued action of lower doses of the chemicals over a period of time or non-delayed action of higher doses, or quick toxic action of a strong insecticide like BHC within ten minutes to half an hour as seen in *C. chinensis*, and *D. similis* respectively, causes very severe changes in the nucleus ending ultimately in pycnosis and cell death.

A severe cytotoxic effect of saturated solutions of ammoniate as observed in the spermatocytes of *P. pictus* 48-72 hours after treatment is failure of karyolysis i.e. the in-
ability of the cytoplasm to divide after anaphase II. Darlington and Koller (1947) have observed failure of wall formation at the end of first division in nitrogen mustard treated T. polena pollen grains leading to the formation of polyploid cells. Patterson and Thompson (1948) observed binucleate cells in urethane treated chick fibroblast tissue culture. Corman (1954) has reported the same aberration in urethane treated seaurchin eggs and has quoted Ludford's findings (in Nambar 1955) about absence of cytoplasmic cleavage leading to binucleation. This has also been reported by Nambar (1955) in P. pictus. It seems probable that Apholate has a necrotising effect also in P. pictus, preventing the final separation of the cytoplasm.

At different post-treatment periods, all the three alkylating chemosterilants have been found to induce stretching in metaphase I chromosomes in D. similis, C. chinensis and P. pictus. The anomaly has also been induced by Hempta at high doses. These stretched chromosomes show strands of faintly orcein positive material extending between chromosomes. This has also been reported as faintly feulgen positive areas by Ray Choudhuri and Manna (1950) in cold treated grasshoppers, Manna and Das (1973) in Apholate and Hempta treated and Das and Manna (1974) in Metepa treated bone marrow chromosomes of mice. Nambar (1955) has reported manifestation of this aberration in mitotic anaphase. Nambar (1955), Manna and Das (1973) and Das and Manna (1974)
have named the faintly staining areas as lesions. It is presumed that these are the parts where the chromosomes ultimately break. Metaphase stretches have also been reported by Saha and Khudabaksh (1974) and Bhattacharya (1981).

The chemosterilants, particularly the alkylating ones, cause breakage and fragmentation of spermatocyte chromosomes in all the insects studied in varying frequencies at different post-treatment periods. In *P. pictus* constrictions are also seen in the chromosomes after treatment with, Tepa, Metepa, and BHC. With the later the constrictions give a beaded appearance to the chromosomes. According to Nambiar (1955) breaks occur between these constricted portions and normal regions of the chromosomes. Usually these middle portions are faintly staining gaps in the chromosomes. These are also known as achromatic lesions as described by Sax (1938), Nambiar (1955), Das and Mukherjee (1977) or heterochromatic regions as described by Darlington and Lacour (1945). However, the author presumes that the constrictions may represent partial or potential breaks. Tepa, Metepa and BHC may be regarded to break the DNA core without in some cases completely breaking the matrix that envelopes the chromosomes. This has also been suggested in Apholate treated *Aedes aegypti* by Rai (1964) who also alternatively proposes that constrictions appear due to incomplete fusion of the broken ends of the chromosomes. In *D. simulans* and *C. chinensis* no
such constrictions or gaps have been noticed but stretched chromosomes and presence of fragments of unknown origin show that breaks do occur in the chromosomes. In _E. fabia_ achromatic lesions are impossible to observe because of the extremely small size of the chromosomes. Gaps and constrictions have also been described, particularly in grasshoppers treated with various chemicals by Saha and Khudabaksh (1974), Bhattacharya (1976), Bhattacharya and Halder (1978), Bhattacharya (1981) and Saha (1981) etc. Breaks of different kinds and fragments of unknown origin have been reported to be induced by chemosterilants and chemicals other than these by many workers like Sturelid (1971), Manna and Das (1972, '73, '76, '77); Das and Manna (1974); Saha and Khudabaksh (1974a,'74b); Bhattacharya (1976, '81); Bhattacharya and Halder (1978) and Saha (1981) in plants, insects and mammals, particularly _Vicia faba_, grasshoppers and mice respectively. Polyploidy is another change in the chemosterilised testes of _P. pictus_. Breaking up of chromosomes on a vast scale, result in the production of numerous fragments scattered over the whole plate. This anomaly has been also observed by Panjari (1955) in urathene treated _P. pictus_ and according to her it occurs through a failure of the spindle.

In all the insects studied except _E. fabia_, bridges have been observed, mostly in anaphase I and few in anaphase II. These are found to be of two distinct types. When they are seen as interconnected irregular DNA positive strands
between the poles indicating fusion of sticky chromosomes they are called as pseudobridges or sticky bridges and are included as gross effects. But when they are found as specifically straight DNA positive bands extending from one pole to the other they are termed as dicentric bridges and are included under effects on individual chromosomes.

Dicentric chromosome bridges can arise in two ways: a chromosome can be broken and when the end of the centromere piece heals during replication it is continuous with the newly formed chromatid (McClintock, 1937) or when two chromosomes are broken, the subsequent translocation and re healing leaves one chromosome with two centromeres and one fragment with none (Curtis, 1971). In each case, centromeres during separation produce dicentric chromosomal bridges extending from one anaphase pole to the other. In D. pictus translocations are also observed but in D. similis and C. chinensis only stretching, gaps, breaks and fragments and finally bridges after reunion have been located. Earias fabia being a Lepidopteran has holocentric chromosomes having diffuse instead of localised centromeres. According to Hughes-Schrader and his (1941) and Bauer (1967) this type of chromosomes of Lepidoptera are notoriously resistant to dominant lethal mutations by radiation and as acentric or dicentric chromosomes are not possible in them, chromosome bridges do not occur. However, during meiosis in their progeny the chromosome pairing and segregation of holo-
centric chromosomes follow the rules for chromosomes with only one centromere (North and Holt, 1968). In *L. fabia*, in certain instances translocations have been located in spermatocyte chromosomes like after hours of treatment with Tepa and Hempa. McClintock's experiments (1938) on maize had led to the concept that bridges can cause loss of chromosomes during division and this can cause death because of genetic imbalance. However, according to Müller (1954) most dominant lethals result from the bridge formation itself and not through loss of chromosome or chromosomes involved. A concurrent experiment on *Drosophila* (Smith and Borstel (1972) suggests that early deaths from radiation induced dominant lethals are caused by chromosome bridges and these consequently slow down the process of mitosis sufficiently for concurrent cytoplasmic differentiation and nuclear production to get out of phase. Although the present investigations have been limited to the studies on chromosomal aberrations only and not extended to studies on reproduction, the author agrees with the views of Smith and Borstel (1972) on the consequences of bridge formation, whether induced by radiation or chemicals, on the development of the embryo.

Apparently all the chemosterilants used have a profound effect on the centromere also and its relation with the centrosomes and the spindle. One of the most characteristic expressions of this effect is lack of synchronisation of centromere movement during anaphase. Consequently
the bivalents appear un-coordinated on the spindle and while some have reached the poles, the others are left behind at the equator or near the poles. The author has termed the aberration as 'unequal distribution' (segregation) of chromosomes and 'laggards'. Laggards and unequal segregation of chromosomes have been reported by Darlington and Koller (1947) in nitrogen mustard treated Tradescantia; Nambiar (1955) in urethane treated P. pictus; Parida et al. (1973) in ADR treated grasshoppers; Bhunya and Jash (1973) in dimethoate treated P. pictus and Nath and Mittal (1975) in Tepa treated Locusta migratoria.

Spindle dearrangement or disturbances are mostly observed in first meiotic anaphases and are frequently caused by Apholate and Tepa and also sometimes by Metepa and Hempe. The second division figures are mostly without this anomaly. The evidences of spindle disturbances in anaphase I are scattered or multipolar distribution and unequal segregation of chromosomes with resulting laggards as already discussed. Scattered or multipolar distribution of chromosomes has been observed in C. chinensis after 48 hours of treatment with Tepa and P. pictus after 72-96 hours of treatment with Metepa, where bivalents grouped in three or four groups (tripolar or tetrapolr mass) have been seen. Although this may reveal the clumping tendency of the chromosomes but the possibility can not be ruled out that Tepa and Metepa in the insects mentioned above may form irregular
spindles on which the chromosomes clump to form irregular masses. It is possible that Apholate, Tenpa and sometimes Metopa and Hempa delay the formation of spindle in some particular insects and hence the chromosomes which are organised well in time are unable to assume a co-ordinated disposition on it. Corman's studies (1954) reveal the same and according to him it is usual for the delayed figures to be tri-or multipolar. Other chemicals are also known to produce multipolar spindles (Levan and Ostergren, 1943; Koller, 1952; Nambiar, 1955). In E. fabia the chromosomes do not have localised centromeres (Smith and Borstel, 1972). Most probably this is the reason that spindle dearrangement and consequent laggards are not seen in this insect. However, arrested metaphase which is presumed due to unability of chromosome separation or failure of chromatid attraction to the equatorial plate, as stated earlier, may be a result of spindle dearrangement in this insect as well as in D. similis. Failure of chromatid attraction of metaphases in urethane treated P. pictus has been reported by Nambiar (1955) but this has not been found in chemosterilised or B:K treated P. pictus and C. chinensis in the present study.

It has been generally found that there is a direct correlation between the amount of any chemosterilant or B:K given and the frequency of aberration when the post-treatment is kept constant. Similarly for a particular dose, the aberrations increase with prolonged post-treatment period.
In some cases the aberrations increase up to the period the insects survive but in others they increase up to a particular period as shown in the tables. This period has been termed by the author as the peak period of activity — after which the aberrations decrease in most of the cases or in certain other cases they remain more or less the same. With very high doses or with a strong insecticide like 3:4C the aberrations only increase up to the period the insect survives. However, in P. pictus which has a longer life span than the other insects studied, the aberrations are clearly traceable up to 144 hours but almost negligible after this period.

Most of the insects studied show the highest frequency of aberrations by almost all the alkylating agents at 48 hours with higher doses and at 72 hours, if the insects survive, with lower doses, although aberrations may appear as early as 1-6 hours as has been described. Thus the effects seem to be non-delayed if not immediate ones. Hempa induces aberrations by using comparatively high doses as compared to the alkylating chemosterilants and the peak period for this non-alkylating agent is generally 72 hours except in C. chinensis for which Hempa has proved to be very toxic and the aberrations, although not of a considerable frequency, have been observed up to 36 hours only. In the other three insects the aberrations are gradually lost after 72 hours. It seems probable that the frequency of chromosomal aberrations depends upon the number of cells available for division after the necrotic effect on the gonad and also on the
amount/concentration of the chemical to act upon them. Most probably recovery from the effects is not possible in the gonads treated with alkylating agents and B-E. Hempe treated gonads may recover, although to a certain extent only, from the damaging effects because its degradation in the body tissues is faster than the chemicals mentioned above (Borkovec 1974).

According to Davidson (1974), spermatogenesis being a continuous process, recovery of fertility in sterilised males is expected, but this does not occur in chemosterilised males until well after the mating efficiency peak is passed. The present study has also revealed that the chromosomal aberrations which cause genetic imbalance are considerably frequent till the mating peak of the insects is passed i.e. 48-72 hours in C. chinensis, D. similis and E. fabia which mate within 24 hours of emergence and 144 hours in P. nictus which mates 120 hours after emergence. It can be certainly stated from the present observations that only a minute amount, particularly of alkylating chemosterilants and B-E, and short exposure is necessary to initiate genetic damage in the gonads.

The present investigations have revealed that Tepe is the most effective chemosterilant for the insects studied. Very minute doses have caused severe damage to the gonad and induced maximum chromosomal aberrations - both qualitatively
and quantitatively. Metepa has been found to be almost of
the same effectiveness although saturated solution of
Apholate has some times proved to be more effective than it.

Although interestingly, Hempa is the mildest type of chemosterilants, enough, its
fumes have proved to be quite toxic to C. chinensis. Hafez
et al. (1970) regard Hempa to be more effective than Apholate
or Tepa in houseflies. If a comparison is made between all
the chemosterilants and B.C, the later is found to be most
effective in hampering reproduction, not solely because of
its sterilising effect by causing chromosomal anomalies but
obviously because of its highly toxic effect by increasing
acetyl choline in the body. This comparison also shows that
aziridinyl group is not solely responsible for hampering
reproduction by inducing chromosomal breaks. If it would
have been so then apholate having the maximum (six) aziri-
dinyl groups would have been the most effective in causing
chromosomal aberrations. This view has also been supported
by Manna and Das (1972, '76, '77) as discussed later.

The physiological effects of aziridinyl chemosterilants
are undoubtedly complex but they are more or less easily
observable under a microscope whereas the biochemical effects
are extremely difficult to detect. It is known that when the
aziridinyl chemosterilant is an alkylating agent, a true
chemical reaction occurs. A covalent bond is formed between
the electrophilic center of the aziridine and the nucleo-
philic centre of the acceptor which may be a constituent of
a chromosome protein or an enzyme complex (Borkovec, 1974). The target nucleophilic centre may be nitrogen in nucleotides, nucleic acids or proteins; sulphur in proteins and oxygen in nucleotides and nucleic acids. However, the nucleic acids, particularly DNA, are the main centre of attraction because of the fact that they replicate on the basis Watson-Crick theory. Presumably a single hit on the DNA chain of a single chromosome could bring about a mutation that could change or even destroy the reproductive potential of the cell. In nucleic acids, nitrogen in the seventh position in guanine is the most possible site for electrophilic attack (Brookes and Lawley, 1963; Köhn and Spears, 1967; Borkovec, 1974). Fahmy and Fahmy (1964) has elaborately given the pathways of alkylation by the aziridine groups of alkylating agents. However, direct alkylation of DNA by alkylating agents has been postulated by Wheeler (1962).

Various alkylating agents are known to induce chromosomal aberrations (Kihlman, 1966) and Apholate, Tepa, and Metepa being alkylating chemosterilants are obviously expected to induce damaging effects on the chromosomes of insects. Sturelid (1971) has argued that after alkylation, a considerable portion of the alkylated DNA is cross linked through guanine of opposite strands and aberrations result from replication of such damaged DNA. According to Sturelid (1971) the delayed aberrations caused by Tepa in Vicia faba and Chinese hamster cells is explained on the assumption
that a period of DNA synthesis is required for alkylation and cross linking and after that the aberrations reveal themselves in the following mitosis. The cross linking mechanism was also given by Fahmy and Fahmy (1964). From the present observations the author is unable to support this view. In this study it has been found that the effects of any chemosterilant on the chromosomes of almost all the insects taken are non-delayed if not immediate. Secondly, if chemosterilants with alkylating groups, cause aberrations then Hempa—a non alkylating agent can induce no aberration and in fact Sturelid (1971) has found no chromosomal anomaly induced by this chemosterilant in V. faba and Chinese hamster cells. However, aberrations caused by Hempa have invariably been observed in the present study which are qualitatively almost similar to those caused by alkylating agents. Lastly, if cross linking through guanine moieties after alkylation of DNA was the factor for the induction of aberrations in chromosomes it was not expected to see so much difference in the frequency of aberrations when same doses of two tri-functional aziridine compounds Tepa and Metepa were used because Sturelid’s (1971) hypothesis suggests that the damage depends upon the amount of DNA alkylated and cross linked by the different number of constituent aziridine (alkylating) groups. Further, the aziridine groups are not solely responsible is proved, as already stated, by the frequency of aberrations caused by
hexa-functional, Apholate as has also been observed by Manna and Das (1973, '76, '77). The effects of all the chemosterilants have continued, mostly up to the period the insects have survived except a few cases, like in P. pictus, although quantitatively different peak periods have been noticed as already stated. This shows that the affected cells can not undergo a normal division otherwise the aberration types could not have been there many hours after the treatment. These views have received a strong support from Manna and Das (1973, '76, '77) who postulate that the effect of the chemosterilants on chromosomes is a direct one and as fresh batch of cells - those surviving the necrotic effect on the gonad - come in contact with the chemosterilants during division, the aberrations are inflicted because of the fact that the chromosomes during division do not have a direct covering of the nuclear membranes over them. This basis of action i.e. a general cytotoxic one, has been given by Manna and Das (1976) for both gross effects and effects on individual chromosomes. The author, however, differs on this point. It is suggested by the present investigations that the effects on chromosomes can not be induced by the chemosterilants because of any one but more than one reactions going on simultaneously. The gross effects are supposed to be non-specific biochemical ones and are suggested to be induced because of the alkylating action of the chemosterilants disturbing the synthetic machinery of
the cell causing excessive nucleic acid change and changes in protein constituents of the chromosomes resulting in an inactivation of essential enzymes. Because of these changes the surfaces of the chromosomes are also affected. The synthetic imbalance leads to physiological effects like, different types of fusions, clumping and ultimately pycnosis. Thus the gross effects seem to be inflicted through an indirect pathway. As far as the effects on individual chromosomes is concerned, the author is of the view that a direct action of physico-chemical stress on the naked chromosomes during division causes stretching and breaks are inflicted on some inherently weaker sections of chromosomes. This has been supported by Manna and Das (1973, '76, '77) and Das and Manna (1974) who have found a non-random distribution of chromatid breaks in bone marrow chromosomes of mice treated with chemosterilants and various other mutagens (Manna, 1971, '75; Manna and Bardin, 1973 b). It is presumed that breakage may result from disruption of bands important for integrity. Once breaks are inflicted, other aberrations like translocations and bridges follow in an attempt of healing up of the broken ends. Disruption in the integrity of bands may also result in the failure of some coding mechanism when spindles are not properly formed resulting into multipolar masses and inability of the chromosomes to move in unison and forming laggards. Disturbed coding may also result in failure of karyolysis.
Although Hempa is chemically entirely different from Tepa, its physiological effect in some insects are qualitatively indistinguishable from those of Tepa (Borkovec 1974). Thus it appears that the physiological mode of action of these two compounds is identical. Borkovec (1974) has given two explanations for this similarity. One is that the response of a reproducing cell to any attack is limited and the physiological and microscopically or genetically observable effects are identical even if the biochemical pathways involved are different. The second explanation (Chang et al., 1967; Chang and Borkovec, 1969) is that Hempa, although chemically and thermally stable, is rapidly demethylated in cells via a highly reactive and unstable hydroxy-methyl derivatives. This intermediate could function as an alkylating agent and perform a role similar to that of the aziridinium ion resulting from protonation of Tepa. A possible alternative according to Borkovec (1974) is the decomposition of the hydroxymethyl intermediate to formaldehyde which is a mild alkylating agent. However, formaldehyde had no sterilising effect when injected into house-flies (Borkovec, 1974). Chang et al. (1964) and Chang and Borkovec (1966) have shown Hempa to be an active chemosterilant though less effective than Tepa and also has low toxicity to mammals. Chang and Klassen (1968) have shown chromosome breaking activity of Hempa in human leukocyte cultures only when applied in a very high concentration
Thus it is a very weak mutagen as compared to the alkylating agents.

The literature on insecticide induced chromosomal aberrations in insects is very limited. Effects of DDT and dimethoate on spermatocyte chromosomes have been reported by Sharma and Sharma (1968) and Bhunya and Dash (1976). These workers also suggest a disturbance in the synthetic machinery indirectly leading to chromosomal aberrations. However, a direct action of the chemical on DNA, RNA, or proteins can not be ruled out.

The insecticide BHC has been found to produce mostly gross effects and also fragmentation in some cases as described. The infliction of gross effects implies that the chemical has a non-delayed effect on the synthetic machinery of the nuclei. Chemically BHC is a chlorinated hydrocarbon and like DDT it also inhibits physiologically the synthesis of the enzyme cholinesterase and thus produces toxic effects within the body. The chemical may thus hamper reproduction more due to its acute toxicity and its stable nature than its breaking properties.

Another possibility of induction of chromosomal aberrations by BHC may be that it affects directly the oxidation-reduction system of cells or indirectly its oxidation products might be responsible for the phenomenon.

Since the investigations on chromosomal aberrations suggested a strong sterilant action of Apololate, Tema, Metene
and also by Hempa and B.C., it was decided to study the pathology of the testes at corresponding stages. The visible and measurable damage to the male gonads due to the four chemosterilants appears to be the general necrosis of the germinal epithelium, loss of sperm motility (hyper- trophyed sperms) and degeneration and resorption leading to azoospermia. There has been some controversy about when the development and maturation of sperms are most severely affected. Some researchers opine (La Brecque and Fye, 1978) that sperms already formed at the time of treatment have the highest of incidence of dominant lethal mutations. The gonial cells usually appear less susceptible and there is eventual recovery of fertility. Others claim (La Brecque and Fye, 1978) that the spermatogonial zone is the most receptive and the damage is irreversible. The author accepts this view, and suggests that there is a progressive testicular degradation and resorption. B.C causes severe necrosis of the male gonad most probably due to its toxic effect via inhibition of cholinesterase synthesis.

The present investigation clearly indicates that the chemosterilants inhibit DNA synthesis in the testes. This causes fewer gonial cells to divide in the testis and thus the number of spermatocytes produced are minimal. The inhibition of DNA synthesis also causes chromosomal aberrations in the spermatocytes that remain. These aberrations lead to break down of meiosis and death of spermatocytes as
suggested by La Chance et al. (1969) on their abnormal growth. Kihlman (1966) has suggested that Amololate may inhibit DNA synthesis by cross linking directly or by alkylation of nucleic acid precursors and this has been already discussed by the author.

Necrosis caused by Amololate and Teena as described in the present study has been reported by Tai (1964) in Aedes aegypti, Sugai and Hirano (1965), Sugai (1967); Sugai and Moritoishi (1974) in Bombyx mori; Saxena and Aditya (1971) in P. pictus; Schwartz (1966) in Hippolates musilo; Fedin et al. (1967); von (1976) Teena in Anthomonas grandis and Henarey et al. (1972), who has also studied the effect of Teena on testes of cabbage looper moth. All the above workers agree that earlier stages of development are more susceptible to the effects of the chemosterilants.

It is interesting to note that the non-alkylating chemosterilant Hemna also inhibits DNA synthesis in the insects studied though less effectively. This has also been shown in A. aegypti by Madhukar et al. (1974) and Grover and Pillai (1975). This identical mode of action of Hemna supports the suggestion of Burckovec (1968, '74) that Hemna may be probably converted into an alkylation metabolite within the insect which has been mentioned earlier. The greater effectiveness of the alkylating agents in inhibition of DNA synthesis and induction of necrosis of testes may be correlated with their higher biological activity and
sterilising potency. Campion and Lewis (1971) has shown a high sterility index of Annolate and Tena in *Cirropus castanea* and an extremely low sterility index of Hempa in the same insect. Also, the sterility caused by Tena is permanent. Hafez *et al.* (1970) regards Hempa to be more effective than Annolate or Tena in houseflies. It is a weak sterilant for *Dacus cucurbitae* (Hooper, 1969) and is completely ineffective in *Anthonomus grandis* (Klassen *et al.*, 1968) and *Tetranychus urticae* (Redfern, 1970). Chang *et al.* (1967) attributes the low sterilizing activities of Hempa to its slow action as compared to Tena and rapid metabolism within the organism.

The metabolism may be even faster (Nath and Mittal, 1973) in cases where no sterility is caused by Hempa.

Crovet and Pillai (1973) have shown that action of Hemna is less drastic than Annolate and Metena in somatic cells of *Culex pipiens fassigna*; Palmquist and La Chance (1965) have reported that both Tena and Hemna induce recessive mutations but Tena is a more efficient mutagen. Chang and Borkovec (1964) suggests that the high efficiency of Tena as a sterilant is not weighed by low toxicity of Hemna.

This has been confirmed in the present study except in the case of *C. chlorocephala* where Hemna has proved to be considerably toxic.

Most of the work done so far on chemosterilants, as described earlier has been on induction of sterility, effects
on reproduction, egg viability, hatching etc. and very little has been done on induction of chromosomal aberrations as already stated. In the light of the present findings it may be suggested that chemosterilants act both directly and indirectly by way of disturbance in the DNA synthesis in spermatocytes. This results in the general necrosis of the testis and non completion of meiotic division due to chromosomal aberrations which cause dominant lethal mutations and sperm inactivation as also suggested by La Chance (1969). Structurally abnormal chromosomes in an individual may result into malformation or abortion of the resulting embryo due to chromosomal imbalance which finally leads to sterility in it. Also hypertrophied sperm or non-motile abnormal sperm may not be able to fertilize the ova and thus reproduction is hampered. To hamper the process of reproduction is the main principle behind the sterilisation technique the advantage of which was first recognised by Kinling (1960) over the pesticidal technique of killing the organisms.

Although the chemosterilants used and JC varied considerably in effectiveness as sterilisers, all of them produced lethal mutations through abnormal chromosomes and also induced significant sperm inactivation. All the chemicals used have attested to their mutagenic properties (La Brecque and Fye, 1978). Therefore these may not be
acceptable for field use. However, the laboratory experiments can add to the knowledge of the physiological and cytogenetic information about the effects of such compounds on reproductive and somatic tissues of various species.