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Genotoxic effects of endosulfan an orgnaochlorine pesticide on the silkworm *Bombyx mori* L

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Abstract
The advancement of introduction of environmental chemicals through pesticides regarded as solemn setback, present world is confronted with the indiscriminate use of the several environmental chemicals in agriculture, livestock production, conservation of plants and animals, industrialization and biodiversity. These environmental chemicals are known to affect not only the target organisms and non target organisms endangering the homeostasis mechanism but also causing severe damage to ecosystem because of long lasting residual effects. Toxicity leads to death, irritation, skin sensitization, mutagenicity, tumors etc. The primary step in assessing the toxicity of a chemical substance is to observe the physical and behavioural responses of the poisoned animal. It is in this review, the silkworm *Bombyx mori* offers as one of the best and convenient laboratory tool because of its important physiological and genetical mechanism simulates the higher eukaryotic system. The methodology developed to study the genotoxic effects of environmental chemicals/ pesticides utilizing silkworm *Bombyx mori* includes dominant lethals, specific locus and sex linked recessive test and are accepted as standard code of behaviour to evaluate the environmental chemicals/ agents. Present review focuses on genotoxic effects of end osulfan as an orgnaochlorine pesticide keeping in scrutiny the utility of the silkworm *Bombyx mori* as one of the best laboratory model organism for genotoxicity.

Keywords: Chemicals, toxicity, pesticides, genotoxic etc.

Introduction
The environmental pollution induced by the chemical substances is regarded as a serious problem. Particularly, the widespread use of pesticides is affecting the entire planet, including the human health. According to FAO (Food and Agriculture Organization of the United Nations), a pesticide is defined as any substance or mixture of substances intended for preventing, destroying or controlling the attack of various pest. This includes vectors of human or animal diseases, unwanted species of plants or animals that cause harm during the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products, or animal feedstuffs (FAO, 2005). It is recognized that World War II was responsible for the development of various pesticides that we employ at the present. In fact, some pesticides currently in use were developed for application in warfare. After World War II, these chemicals began to be used as pesticides in environmental spraying for mosquito eradication and in agriculture even when their potential hazards were unknown [163].

However, during the 1960s and 1970s, it began to emerge some evidences that these chemicals could have harmful consequences. Epidemiologists in the United States of America noted a rise in the incidence of blood cancers and when plotted on a map, these cases were clearly clustered in agricultural areas. This increase in blood cancers incidence paralleled the increase in pesticide use, has led some epidemiologists to assume that there was a causal link [163] and, in 1962, with the first publication of the Rachel Carson's revolutionary book, *Silent Spring*, it was started the slow process of raising political and public consciousness of the effects of pesticides in wildlife, humans and ecosystems. In 1972, United Nations began to recognize the risks posed to humans and to the environment by the increased usage of pesticides and decide to establish the United Nations Environment Programme (UNEP) which together with World Health Organization (WHO) and FAO promoted more sustainable agricultural practices like integrated pest management (Karabelas *et al.*, 2009) [94].

Furthermore, all over the years, there have been other efforts and initiativesto improve regulation of international pesticide trade and an example is the creation and update of an International Code of Conduct on the Distribution and Use of Pesticides (WHO, 2010).

At the present time, agriculture is an important activity and source of economic income in several countries all over the world but, at the same time, it is largely responsible for the increased consumption of pesticides. Indeed, pesticides used in agriculture are very important to reduce yield losses, maintain high product quality and sometimes improve the nutritional value of food and its safety. From this point of view, pesticides can be considered as an economic, labor-saving, and efficient tool of pest management (Cooper and Dobson, 2007) [36]. In addition a serious concerns have been raised about health risks resulting not only from occupational exposure to pesticides but also from pesticides residues on food and in drinking water for the general population (Bolognesi and Morasso, 2000) [21].

Pesticides are substances used to control pests, including insects and plant diseases. Pesticides refer to chemical substances that are biologically active and interfere with normal biological processes of living organisms deemed to be pests. They include a wide range of compounds and according to their functional class organisms designed to control, they can be classified as insecticides, fungicides, herbicides, rodenticides, molluscacides, nematicides and others that belonging to different chemical groups. They are deliberately sprayed into the environment, both in urban and rural areas, for industrial, agricultural and public health purposes and, after application, residues may persist in the environment, contaminating soils and water, remain in the crops, enter the food chain, and finally ingested by humans along with foodstuffs and water (Fascendini *et al.,* 2002, Carvalho *et al.,* 2006) [56, 31]. Naturally-occurring pesticides have been in use since centuries, but widespread production and use of modern synthetic pesticides did not begin until 1940s. On a global scale approximately over five billion pounds of conventional pesticides are used in different areas like agricultural lands, forests, rangelands management, disease control, domestic use and many more areas annually (EPA, 2001).

Use of pesticides in India began in 1948 when dichloro diphenyl trichloroethane (DDT) was imported for malaria control and benzene hexachloride (BHC) for locust control (Gupta, 2004). India started pesticide production with manufacturing plant for DDT and BHC in the year 1952. In 1958, India was producing over 5000 metric tonnes of pesticides. Currently, there are approximately 145 pesticides registered for use, and production has increased to approximately 85,000 metric tonnes (Gupta, 2004). However, it is estimated that often less than 0.1 percent of an applied pesticide reaches the target pest, leaving 99.9 percent as an unintended pollutant in the environment, including in soil, air, and water, or on nearby vegetation. The first report of poisoning due to pesticides in India came from Kerala in 1958 where, over 100 people died after consuming wheat flour contaminated with parathion. Subsequently several cases of pesticide-poisoning including the Bhopal disaster (Forget, 1991) [58] have been reported. It has been observed that their long-term, low-dose exposure are increasingly linked to human health effects such as immune-suppression, hormone disruption, diminished intelligence, reproductive abnormalities, and cancer.

Toxicology and Chemical mutagenesis

Toxicology (from the Greek words *toxicos* "poisonous" and *logos* "study") is a branch of biology, chemistry, and medicine concerned with the study of the adverse effects of chemicals on living organisms (Schrager, 2006) [166]. It is the study of symptoms, mechanisms, treatments and detection of poisoning, especially the poisoning of people.

Mathieu Orfila is considered to be the modern father of toxicology, having given the subject its first formal treatment in 1813 in his *Traité des poisons*, also called *Toxicology general.*

Branches of Toxicology

Chemicals are used extensively in industries, homes, and crop fields to meet the growing challenges for healthy living. However, it has been reported that a vast majority of chemicals lack basic toxicity data and this is a cause for concern. Generation of quality data on the toxicity and safety of chemical substances, the proper evaluations and meaningful interpretations to human health and environmental safety demands the support of specialized branches of science. In simple terms, the chemical substance(s) under test have to pass through different branches for evaluation. These are (1) analytical toxicology, (2) aquatic toxicology, (3) biochemical toxicology, (4) clinical toxicology, (5) eco-toxicology, (6) environmental toxicology, (7) epidemiological toxicology, (8) genetic toxicology, (9) immune toxicology, (10) nutritional toxicology, (11) mammalian toxicology, (12) regulatory toxicology, and many other related branches (Fred Whit ford *et al.,* 2010).

Toxicity Characterized by Effect

Toxicity often can be described according to the observable or measurable effect it causes.

Death is the ultimate toxic effect, occurring when critical bodily functions are altered or inhibited.

Irritation is observed when a pesticide affects cells of the skin or eye; corrosion occurs when the integrity of the outer layer of cells is destroyed. The effect frequently is referred to as a "burn." Less severe irritation might appear as redness, swelling, or inflammation of the skin. Irritation/corrosion can result from a single or cumulative exposure.

Skin sensitization is an allergic reaction; sensitization requires multiple exposures over a period of time. The initial exposure "sensitizes" the person, and subsequent exposures cause the individual to react to the chemical by developing a "rash." Poison ivy is a familiar example of a skin sensitizing, natural chemical.

Mutagenicity (also called genotoxicity) results from a change in the genetic material of a cell. There are two general types: a gene mutation that changes the DNA genetic code; and a structural mutation that causes structural chromosome damage. A mutagenic compound may produce chromosomal aberrations by modifying the physical structure or number of chromosomes; the result is chromosomes that are fragmented or mismatched, or chromosomes that fail to undergo cell division. Gene mutations include the deletion, addition, or substitution of the chemical components of DNA, which contains all the coded information that allows organisms to function. Disruptions in genes or chromosomes can lead to diseases (including cancer) and birth defects. A mutagen is of

concern when it damages egg or sperm cells, enabling the defect to be passed on to successive generations.

Tumors—also called neoplasms—are abnormal growths of tissue; they can be either benign or malignant. Most benign tumors are not life-threatening because cell division usually is slow and the cells are noninvasive: They will not spread to surrounding tissue. Malignant tumors divide rapidly, in an uncontrolled fashion, and spread to other body tissues; this, coupled with their tendency to intercept nutrients needed by healthy tissue, thereby destroying it, renders them life threatening.

Genetic toxicology (Genotoxicity)

Genotoxicity is a recently developed branch of toxicology, which deals with the study of deleterious effects of toxic agents present in the environment on the structure and function of DNA. Direct damage to DNA is an increasingly more essential focus on ecotoxicology research for two reasons; firstly, because of the far reaching effects of genotoxins on the health of an organism and the possible future implications if the germ line is affected, and secondly, because extremely sensitive methods of detecting DNA damage have been developed, which allowed the improvement of early biomarkers for xenobiotic exposure. Genotoxicity can result in three types of genetic lesions. Firstly, single-gene mutations, also called point mutations, which include alterations in the nucleotide sequence of DNA, and may involve either the base substitution or frame– shift mutations. Second are the structural chromosomal mutations or genomic mutations which include changes in chromosomal structure, such as breaking of chromosome, or translocation of an arm, commonly called clastogenesis. Third are numerical changes in the genome; aneuploidy and/or polyploidy (Cajaraville *et al*., 2003) [27]. Genotoxicants are very important components to be monitored as they cause mutations that often lead to cancers. Further, understanding the changes at the DNA level of an organism exposed to pollutants is essential to demonstrate an impact at the ecological relevant population or community level (Shugart & Theodorakis, 1996). These genotoxic effects are also considered serious of the possible side effects of pesticides because they may produce DNA breakage at sites of oncogenes or tumor suppressor genes, thus playing a role in the induction of malignancies in in dividual sexposedtotheseagents(Blasiak*etal.*,1999) [20].

Mutagenesis is a process by which the genetic information of an organism is changed in a stable manner, resulting in a mutation. It may occur spontaneously in nature or as a result of exposure to mutagens. It can also be achieved experimentally using laboratory procedures. In nature mutagenesis can lead to cancer and various heritable diseases, but it is also the driving force of evolution. Mutagenesis as a science was developed based on work done by Hermann Muller, Charlotte Auerbach and J. M. Robson in the first half of the 20th century. DNA may be modified, either naturally or artificially, by a number of physical, chemical and biological agents, resulting in mutations. In 1927, Hermann Muller first demonstrated mutation with observable changes in the chromosomes can be caused by irradiating fruit flies with X-ray, and lent support to the idea of mutation as the cause of cancer. His contemporary Lewis Stadler also showed the mutational effect of X-ray on barley in 1928, and ultraviolet (UV) radiation on maize in 1936. In 1940s, Charlotte Auerbach and J. M. Robson found that mustard gas can also cause mutations in fruit flies. Later, Rapoport and others discovered mutagenic activity of formaldehyde, diethylsulfate, diazomethane and other compounds. The interaction of certain environmental chemical compounds and cell metabolism may result in genetic changes in DNA structure, affecting one or more genes. These chemical induced mutations are known as chemical mutagenesis. Many cancers and other degenerative diseases result from acquired genetic mutations due to environmental exposure, and not as an outcome of inherited traits. Chemicals capable of inducing genetic mutation (*i.e.,* chemical mutagens or genotoxic compounds) are present in both natural and man-made environments and products. For screening environmental mutagens, several convenient systems have been developed, using bacteria or cultured mammalian cells. Most of these system deal with mutagenic events that occur in somatic cells. From the genetic view point, however, we need system that can be applied for the detection of mutagenicity that affects germ cell in the gonad and produces mutation to be transmitted to the offspring.

Furthermore, various experimental data have provided evidence that pesticides are potential chemical mutagens inducing gene mutation, chromosomal alteration and DNA damage (Bolognesi and Morasso 2000) [21].

Organisms Commonly Used in Pesticide Testing Programs

Researchers and regulators do not rely on any one animal species in conducting safety assessments. Human responses to a pesticide cannot be mimicked exactly or modeled by a single animal species; therefore, toxicologists must use multiple species—fruit fly, fish, rats, mice, rabbits, guinea pigs, dogs to predict pesticide toxicity to humans. Hamsters, monkeys, pigs, chickens, and cats are used less frequently. Toxicologists repeatedly test the same strain of animals to facilitate toxicity comparisons between new and existing pesticides. Animals are purchased from sources that document the history and purity of the genetic strain and guarantee the animals to be healthy and disease-free.

Fruit fly: (*Drosophilamelanogaster*) use of Drosophila in the modern regimen of toxicological testing, emphasizing its unique attributes for assaying neurodevelopment and behavior. Genetic manipulability and ease of detecting phenotypes made Drosophila the model of choice for mutagenesis screens of the 1980's and 90's. These same features make Drosophila ideal for toxicological screens. Indeed, flies have been, and continue to be, used routinely in mutagenicity tests. Recent investigations have propagated a number of powerful assay methods with Drosophila in developmental and behavioral toxicology.

Fish: Fishes are ideal sentinels for study of toxic chemical exposure due to their constant, direct contact with the aquatic environment where chemical exposure occurs over the entire body surface, ecological relevance in many natural systems, easy of culture. Toxicological methods, including short-term and sublethal exposure effects, mechanism of effect, interaction with environmental variables, and the potential for mortality can be studied.

Mouse: The mouse (*Mus musculus*) is commonly used in pesticide and pharmaceutical testing; in fact, current estimates indicate that 70 percent of all animals used in testing programs are mice. Mice are used for pesticide

carcinogenicity tests, predominantly, offering these advantages: They are small; they are easy to maintain; and they have relatively short life spans.

Rat: Strains derived from the Norway rat (*Rattus norvegicus*, commonly called the laboratory rat) have been used in agricultural and pharmaceutical research since the 1850s. The rat is the second most common experimental animal, comprising 20 percent of all animals used, and it offers many of the same advantages as mice.

Albino Rabbit: Albino rabbits (*Lepus cuniculus*) are used to evaluate skin and eye irritation as well as birth defects. They breed readily, produce large litters, and are easily reared in quantity. Their large bodies and eyes facilitate skin and eye exposure studies.

Guinea Pig: The guinea pig (*Cavia porcellus*), through decades of testing, has been a reliable human surrogate in identifying pesticides that induce skin sensitization— that is, allergies.

Domestic Hen: The nervous system of the domestic hen (*Gallus domesticus*) is sensitive to organophosphorus insecticides; thus, it is used to evaluate nervous system toxicity for this class of pesticides.

Dog: The beagle dog (*Canis familiaris*) is commonly used as the nonrodent species of choice. Dogs share many physiological properties with man and fully complement rodent studies. Their size facilitates difficult surgical procedures, and their ample blood supply allows larger and more frequent samples to be taken without affecting the animals' health. Dogs have longer life spans than laboratory rodents, lending them useful in pesticide toxicology studies that last a few months to a year, or longer. Some studies have lasted eight or more years.

Measuring Toxicity (LD_{50} and LC_{50} Values)

Acute toxicity of a pesticide refers to the effects from a single dose or repeated exposure over a short time (e.g. one day), such as an accident during mixing or applying pesticides. A pesticide with a high acute toxicity can be deadly even if a small amount is absorbed. Acute exposures may be referred to as acute dermal, acute oral or acute inhalation poisoning. Usually the effects of acute exposure, if any, occur within 24 hours.

LD_{50} and LC_{50} values

The test substance or preparation may be applied to the animal orally, under the skin, by inhalation, into the abdomen or into the vein. LD_{50} and LC_{50} are the parameters used to quantify the results of different tests so that they may be compared.

LD stands for "Lethal Dose". LD_{50} is the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals. The LD_{50} is one way to measure the short-term poisoning potential (acute toxicity) of a material. The test was created by J.W. Trevan in 1927, with scientist's attempt to find a way to evaluate the relative drugs and medicines poisoning potency.

LC stands for "Lethal Concentration". LC values usually refer to the concentration of a chemical in air but in environmental studies it can also mean the concentration of a chemical in water. For inhalation experiments, the concentration of the chemical in air that kills 50% of the test animals in a given time (usually four hours) is the LC_{50} value.

Toxicologists can use many kinds of animals but most often testing is done with rats and mice. In nearly all cases, both tests are performed using a pure form of chemical. Mixtures are rarely studied. It is usually expressed as the amount of chemical administered (e.g., milligrams) per 100 grams (for smaller animals) or per kilogram (for bigger test subjects) of the body weight of the test animal. The LD_{50} can be found for any route of entry or administration but dermal (applied to the skin) and oral (given by mouth) administration methods are the most common.

Procedure for Administering Various Chemicals in Toxicology

To test the toxicity of chemicals it is administered into the body of an animal. There are various methods of administering chemicals in an animal.

Oral Administration: The method chosen for administering an oral dose often depends on the chemical, the animal species, and the duration of the study. In a short-term study, the pesticide might be administered to dogs as a gelatin capsule, or to rodents through a stomach tube; these methods place the entire dose directly into the stomach. In longer-term studies, the pesticide usually is incorporated into the animals' feed or water, allowing their access to small amounts each time they eat or drink.

Topical application: It is the most common methods used for insect. In this method the chemical is dissolved in relatively non toxic and volatile solvent, such as acetone, and then placed in contact with a particular place on the body surface. A combination of varied concentrations of chemical and constant amount of solvent keeps the area of contact, as well as the solvent, constant.

Injection Method: This method is employed to know the exact amount of chemical inside the animal's body. The chemical is dissolved in a carrier material, for example, propylene glycol and then injected into the body cavity, say, within the peritoneal membrane or intraperitoneally. For insects, injections are usually made at the abdominal sterna or intersegmental membranes and not at the longitudinal centre line, in order to avoid any injury to the nerve cord. The needle is held in position for a while and then pulled away gradually so as to avoid bleeding due to internal pressure.

Dipping method: a number of test methodshave been specially designed to suit insects mode of life or its morphological arrangement. They may conflict with the standard testing methods mentioned above (Busvire, 1971).For instance, dipping method is used for diptous larvae which cannot withstand the skin injury caused by the injection, and topical application cannot deliver sufficient amount of insecticide preparation. The insecticide preparation may be either as suspension in a solvent like acetone or methyl Triton X-100. The results are expressed as LC50 rather than as LD50. Assessment of the proper range of reliability becomes important in this method, for instance, for instance, the limitation of the often does not increase beyond a certain point because of the limitation of the insecticide slow solubility or limited amount that can be suspended (Labadon 1965).

Dermal Administration: The animals' fur is clipped prior to placing a pesticide dose directly on the skin. Solid materials are crushed and mixed with a liquid to form a paste, slurry, or solution, then applied to the skin. The site of application is bandaged to keep the animals from licking the treated area and ingesting the chemical. Another method employed to deter licking is the placement of a large "collar" around the neck to restrict the animals' access to the application site.

Inhalation Method: Animals are confined in air-tight chambers into which pesticide vapor, aerosol mists, or dusts are introduced. It is critical that the test substance be uniformly distributed throughout the chamber for the time period during which animals are obliged to breathe the treated air. The pesticide concentration and particle size in the air is monitored regularly. If the particles are too large, they are ground to assure accessibility to the lungs. Placing animals in chambers exposes not only the respiratory system, but all external body surfaces, as well. Alternative testing systems are available for limiting exposure to the animal's nose or face.

General Toxicity Symptoms

The primary step in assessing the toxicity of an insecticide is to observe the physical and behavioral responses of the poisoned animal. The general toxicity symptoms are

Paralysis, vomiting, fatigue, weakness, restlessness, nausea, diarrhea, loss of appetite, loss of weight, excessive saliva, stomach cramps, excessive perspiration, trembling, increased rate of breathing, uncontrollable muscle twitches, inability to breathe *etc*.

Different Types of Pesticides and Their Genotoxic Effects

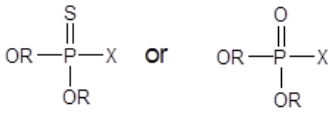
Pesticides form an important group of environmental pollutants and the genotoxic effects of several chemical groups of pesticides have been shown by *in vivo* and *in vitro* experiments (Bolognesi, 2003; Abdollahi *et al.,* 2004; Kaushik & Kaushik 2007) [22, 124, 167]. However, genotoxicity data for a great majority of pesticides are scanty (Gandhi *et al*., 1995), and where ever exist; the findings from different laboratories are contradictory for many formulations. Among pesticides, organophosphates and organochlorines are constantly a matter of worry because of their wide use. Both groups of chemicals bear the potentiality to cause genotoxicity and carcinogenicity (Kaushik & Kaushik, 2007) [97]. In a survey including halogenated hydrocarbons, organophosphates, carbamates and other classes of pesticides, Borzsonyi *et al*., (1984) found 29 pesticides to be definite or suspected genotoxic agents.

Induction of DNA damage is one of the primary events in the initiation of carcinogenesis by chemicals. Several chemical pollutants can produce carcinogenic effects through the induction of genetic lesions. Compounds that react directly or indirectly with DNA are, in most cases genotoxic carcinogens for example alkylating agents, food additives and contaminants, drugs and antibiotics, Pesticides etc. are called as genotoxic compounds. Among the most commonly used pesticides and their persistence in the environment, pesticides can be classified as organochlorine, organophosphates, carbamates *etc.* Which are also of economic importance, are the organophosphorus, organochlorine and carbamate insecticides.

Organophosphates

The word "organophosphates" refers to a group of insecticides or nerve agents acting on the enzyme acetylcholinesterase (the pesticide group carbamates also act on this enzyme, but through a different mechanism). The term is used often to describe virtually any organic phosphorus (V)-containing compound, especially when dealing with neurotoxic compounds. Many of the so-called organophosphates contain C-P bonds. For instance, sarin is *O*-isopropyl methylphosphonofluoridate, which is formally derived from phosphorous acid ($HP(O)(OH)_2$), not phosphoric acid ($P(O)(OH)_3$). Also, many compounds which are derivatives of phosphoric acid are used as neurotoxic organophosphates.

Organophosphates General Structure



X: SR' or OR' group

Organophosphate pesticides (as well as sarin and VX nerve agent) irreversibly inactivate acetylcholinesterase, which is essential to nerve function in insects, humans, and many other animals. Organophosphate pesticides affect this enzyme in varied ways, and thus in their potential for poisoning. For instance, parathion, one of the first organophosphate commercialized, is many times more potent than malathion, an insecticide used in combatting the Mediterranean fruit fly (Med-fly) and West Nile Virus-transmitting mosquitoes.

Organophosphate pesticides degrade rapidly by hydrolysis on exposure to sunlight, air, and soil, although small amounts can be detected in food and drinking water. Commonly used organophosphates have included parathion, malathion, methyl parathion, chlorpyrifos, diazinon, dichlorvos, phosmet, fenitrothion, tetrachlorvinphos, and azinphos methyl. Two of the most & widely used organophosphorus insecticides are Malathion and Methyl Parathion. Both have been applied to bean, corn, sorghum, and tobacco crops to exterminate, green flies, harvest bugs, and other insects. These insecticides inhibit the enzymatic activity of cholinesterase, which is responsible in hydrolyzing the acetylcholine generated in axon terminals to choline.

Malathion is one of the earliest developed organophosphate insecticides, introduced in 1950. It is a nonsystematic, broad-spectrum; general-use pesticide that disrupts the nervous system function by inhibiting cholinesterase, an enzyme that normally terminates nerve transmissions by cleaving the neurotransmitter acetylcholine and resultant acetylcholine accumulation. Malathion is widely used in agriculture, residential landscaping, public recreation areas, and in public health pest control programs such as mosquito eradication. In the US, it is the most commonly used organophosphate insecticide (Bonner *et al.*, 2007) [119]. Forty organophosphate pesticides are registered in the U.S., with at least 73 million pounds used in agricultural and residential settings (Maugh and Thomas, 2010)

Richardson and Imamura (1999) demonstrated malathion-induced breakage of plasmid or bacteriophage DNA in vitro. Malathion has the potential to produce chromosome

aberration and sister-chromatid exchange in Chinese hamster ovary cells (Galloway *et al*., 1987).Malathion induced DNA damage in cerebral tissue and peripheral blood in the gill, kidney, and lymphocytes of teleost fish *Channa punctatus* (Kumar *et al.,* 2010) [135]. Malathion can cause chromosomal damage in rat bone marrow cells, spermatogonia, and spermatocytes (Degraeve *et al.,* 1979) [45]. When Malathion is administered to rats for two generations, there is a decrease in progeny survival and body weight (Kalow and Marton 1961) [91]. It also inhibits cell growth in primary cultures of chicken embryo fibroblasts (Wilson and Walker 1966) [200]. Mice skin exposed to Malathion induces a micronuclei formation in bone marrow cells (Dulout *et al.,* 1982) [48].Malathion interferes with mouse testicular function, being toxic both to the somatic (Leydig and Sertoli) and spermatogenic cells (mainly spermatogonia and maturing spermatids). The damage may result from a variety of mechanisms, mainly affecting the DNA structure and function (Bustos *et al*., 2003) [51]. Studies on Micronucleus induction in humans and animals indicated malathion to be genotoxic on somatic and germ cells in bone marrow cells of mice (Salvadori *et al*., 1988) [162]. Moreover, malathion induced DNA damage in cerebral tissue and peripheral blood in rats (Reus *et al.,* 2008). (Hoda and Sinha 1991) in buffalo blood cultures(Gupta *et al*., 1988) [81] in human peripheral blood lymphocytes (De Ferrari *et al.,* 1991) [43] as in cultured human lymphocytes (Nicholas *et al*., 1979; Herath *et al*., 1989; Garry *et al.,* 1990;Rupa,1991 and Balaji and Sasikala 1993) [134, 85, 66, 157, 10] and caused specific mutations (deletions) anddecreased the mitotic index in human T-lymphocytes (Pluth *et al*., 1996) [147]. Malathion poisoned individual yielded a larger number of chromosomal aberrations (Yoder *et al.,* 1973, Van Bao *et al.,* 1974.) [206, 192]. Sister chromatid exchanges, and the delay of the cell cycle were induced in human cell cultures (Nicholas *et al.,* 1979; Chen *et al*., 1981) [134, 35]. A significant difference in frequency and distribution of MN was observed between the malathion exposed workers and control workers (Garaj-Vrhovac and Zeljezic, 2002) [165]. The commercially available malathion has been shown to produce DNA lesions *in vivo*, potentially comprising DNA breakage at sites of oncogenes or tumor suppressor genes, and might play a role in the induction of malignancies in exposed individuals (Błasiak and Trzeciak, 1998). Malathion has therefore been shown to interact with DNA but the major malathion is a potent genotoxic agent and may be regarded as a potential germ cell mutagen (Giri *et al.,* 2002) [74]. Additionally, several studies indicated the genotoxic effect of malathion in both human and animals, such as the induction of DNA in the form of chromosomal aberrations (Reus *et al.,* 2008) and micronuclei formation (Kumar *et al.,* 2010; Giri *et al.,* 2011) [135].; in human liver carcinoma cells (Moore *et al.,* 2010) [137].

Methyl parathion an effective organic phosphate was reported to induce chromosomal breaks in root tips of *Allium cepa* (Epstein and legato, 1971) [54], In some mutagenic tests with microorganisms, methyl parathion was positive (Fahrig, 1974) [55] and also produce DNA breaks in *Escherichia coli* plasmid (Griffin and Hill 1978) [78], it is also mutagenic in *Salmonella Typhimurium* and *Saccharomyces cerevisiae* (Waters *et al.,* 1980) [198]. Chen *et al.,* (1981) [35] found significant increase sister chromatid exchange in a Burkitt lymphoma in V79 chinese hamster cell line and also a strong cell delay but it was unable to chromosome mutations in *Drosophila melanogaster* (Velazquez *et al*., 1990) [195]. Methyl parathion in polychromatic erythrocytes of mice bone marrow induced micronuclei (Grover and Malhi, 1985) [79], and also resulted in an increase the number of sister chromatid exchange (SCE) and chromosomal aberration (CA) in fish and rats, induced micronuclei in mice (Das and John, 1999; Undeger *et al.,* 2000) [40, 190]. Genotoxic and cytotoxic effects of methyl parathion on male reproduction cells in mice Increased abnormal sperm number (Narayana *et al.,* 2005). Chen *et al.,*(1981) [35] found significant increase sister chromatid exchange in a Burkitt lymphoma B35M human cell line,but on the other hand, no clastogenic effects were observed in peripheral blood cultures from men occupationally exposed to Methyl Parathion (de Cassia Stocco *et al*., 1982) [42]. Sobti *et al.,*(1982) [173] obtained an increase in sister chromatid exchange of human lymphoid cells in culture. Although methyl parathion was reported to increase the number of SCEs and CA in fish and to induce micronuclei in rats and human Lymphocytes (Geetanjali *et al.,* 1993; Dolora *et al.,* 1992; O Mohamed *et al.,* 1995). Undeger, U; Basaran, N (2004) [68] induction of DNA damage by methyl parathion in human peripheral lymphocytes *in vitro*: Edwards *et al*., (2011) found that methyl-parathion is induced DNA damage in human $HepG_2$ cells.

One of the widely used organophosphate insecticides is dimethoate (O, O-dimethyl-S-N-methylcarbamoylmethyl)-phosphorodithioate, dimethoate) which has a contact and systemic action. It is frequently used against a broad range of insects and mites and is also used for indoor control of houseflies. The dimethoate or rogor, in barley it induced chromosomal aberrations in root tip meristems and in gametic cells (Grover 1985) [79]. In *Vicia faba*, it produced a decrement of the mitotic index and also chromosomal alterations (Amer and Farah 1974) [3] as well as SCE (Gomez-Arroyo *et al.,* 2004) [204]. In E. *coli*, it caused resistance to 5-methyl tryptophan (Mohn 1973) [70]. It induced mitotic gene conversion in S. *cerevisiae* (Fahrig 1973, 1974) [55]. It is positive in the bacteria reversion test (Moriya *et al.,* 1983; Ladhar *et al.,* 1990; Bianchi *et al*., 1994). Chen *et al.,* (1981) [35] showed an increment in SCE frequency of V79 cell line of Chinese hamster. Dimethoate induced a concentration dependent increase in sister chromatid exchange frequency in toadfish lymphocytes (Ellingham *et al*., 1986) [52], and it was also found to increase the incidence of numerical but not structural chromosomal aberration in male Wistar rats (Undeger *et al*., 2000; Nehéz and Dési 1996) [190]. It was also reported that dimethoate was non-mutagenic in some other genotoxicity tests in mice (Gillot-Delhalle *et al*., 1983) [73]. It also induces chromosomal damage in bone marrow cells and spermatogonial cells of mice (Degraeve and Moutschen, 1983) [73]. Oxidative stress can induce many kinds of negative effects including membrane peroxidation, protein cleavage, and DNA strand breakages, which could lead to cancer (Mittler 2002; Collins and Harrington, 2002). Oxidative DNA damage is the most frequently occurring damage and includes oxidized bases, DNA single- and double-strand breaks, abasic sites, and DNA–protein cross-links (Cadet *et al*., 2003a, b; Marnett 2000; Bjelland and Seeberg, 2003). Recent investigations have pointed out that oxidative stress and DNA damage are possibly linked to

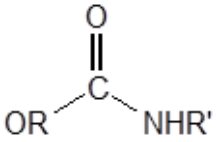
pesticides-induced adverse health effects in agricultural workers (Muniz *et al.*, 2008).

Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate) is a common organophosphate insecticide with anti-cholinesterase activity. It is applied on crops (mainly tobacco), to control parasites in livestock, and against flies and mosquitoes inside the house. DDVP decreased MI in *Allium cepa* roots (Sarı, 2007). It is found to be mutagenic in *Escherichia coli* and *Salmonella typhimurium* (Ashwood *et al.,* 1972; Voogd *et al.,* 1972).Dichlorvos was shown to induce chromosomal aberrations in *Alleum cepa* and *Vicia faba* root tip cells (Sax and Sax, 1968). Amer and Ali (1986) [52] showed that DDVP decreased the MI and increased chromosomal aberrations in *Vicia faba* meristem cells. Kontek *et al*. (2007) reported that after the treatment of *Vicia faba* meristem cells with DDVP the mitotic activity in all series significantly decreased as compared to control, which indicated a mitodepressive effect. Induction of chromosomal aberrations and micronuclei *in vivo* has been reported in Syrian hamster and rat (Mennear, J. H, 1998). Dean and Blair (1976) reported dichlorvos to induce dominant lethal mutations in mice but its *in vivo* mutagenic activity has been confirmed only in the liver of lLacZ transgenic mice (Goldsmith, D. F, 2000). Induction of chromosomal aberrations and micronuclei *in vivo* has been reported in Syrian hamster and rat (Mennear, J. H, 1998).

Carbamates

Carbamate insecticides feature the carbamate ester functional group. Included in this group are aldicarb, carbofuran (Furadan), carbaryl (Sevin), ethienocarb, and fenobucarb. These insecticides kill insects by reversibly inactivating the enzyme acetyl cholinesterase. The organophosphate pesticides also inhibit this enzyme, although irreversibly, and cause a more severe form of cholinergic poisoning (Robert L. Metcalf, 2002).

Carbamates General Structure



Mad from carbamic acid. Control pests by acting on the nervous system (interfere with nerve-impulse transmission by disrupting the enzyme (cholinesterase) that regulates acetylcholine (a neurotransmitter). In general, are less persistent in the environment than the organochlorine family. Carbaryl, Propoxur, Methomyl, Carbofuran, thiodicarb, barban, EPTC, propham, triallate, maneb, nabam etc

Carbaryl a carbamate insecticide was found to induce breaks in plant root tips (Epstein and Legator 1971) [54], other carbamate pesticide like isopropyl phenyl carbamate (IPS) and dimethyl-N-methyl carbamodithioic phosphate (Rogar) were shown to induce chromosomal abnormalities in *Vicia faba* (Amer and Farah 1976). Franekic *et al*., (1994) [59] reported that ziram, zineb and thiram are mutagenic in a battery of bacterial test systems. Zineb, a carbamate fungicide, has been reported to be mutagenic in both somatic and germ-line cells in Drosophila (Tripathy *et al.,* 1988) [187]. In another report, the same research group has reported that the fungicide ziram is mutagenic in the wing, eye and female germ-line mosaic assays, and in sex linked recessive lethal test in *Drosophila melanogaster* (Tripathy *et al.,* 1989) [188]. Carbaryl caused the disruption in female reproductive system in fish, Channa striatus. It induced the reduction and deformity of oocytes and follicular atresia (Kulreshtha and Arora 1984) [101]. It also caused the homeostatic unbalance of the reproductive regulatory system in Channa punctatus (Ghosh *et al.,* 1990) [72]. The thiocarbamate pesticide malinate and vernolate have been reported to cause chromosomal changes like SCE and chromosomal aberrations *in vitro* and increased frequency of polychromatic erythrocytes in mouse bone marrow cells (Pinter *et al.,* 1989) [145]. Studying on the genotoxicity of aldicarb, aldicarb sulfone, aldicarb oxide, carbofuran and propoxur, Canna-Michaelidou & Nicolaou (1996) [28] reported that all the pesticides were 'suspect genotoxic' directly and after S9-actvation in mutation test. Pant *et al*. (1996) [139] observed a decrease in spermatozoa number and sperm motility when rats were exposed to carbaryl. Wyrobek *et al*., (1981) [203] determined that exposure to carbaryl increased abnormal sperm morphology in Humans.

Carbosulfan belongs to the benzofuranyl methyl carbamate group of pesticide and has been widely used in agriculture for broad spectrum control of insect pests of crops such as caterpillars, green leafhoppers, white-backed plant hoppers, brown plant hoppers, gall midges, stem borers, leaf folder of paddy, white aphids of chilies (Giri *et al.,* 2002) [74]. It has been reported to be effective against certain insect pests not controlled by organo-chlorine or organo-phosphorus insecticides (Sahoo *et al.,* 1990) [161] and has also been proposed for the control of pyrethroidresistant mosquitoes (Guillet *et al.,* 2001) [80]. Very few studies have been carried out on the potential cytogenetic effect of carbosulfan. It also induced cell necrosis, degeneration and oedemas in liver, kidney and spleen of rainbow trout (Capkin *et al.,* 2010) [29]. Carbosulfan is reported to induce genotoxicity in freshwater fish *Channa punctatus* (Nwani *et al.,* 2010) [135]. Stehrer-Schmid and Wolf (1995) [177] reported that carbosulfan induced a positive micronucleus response in polychromatic erythrocytes (PCEs) in the bone marrow cells of mice at different expression times. It has been reported to induce chromosome aberrations in rat (Topaktas *et al*., 1996) and in mouse (Stehrer-Schmid and Wolf, 1995; Geri *et al.,* 2002) [177] bone marrow cells. Increase in the frequency of sister chromatid exchanges following carbosulfan treatment has also been reported (Giri *et al.,* 2003) [76]. Carbosulfan induced a positive micronucleus response in polychromatic erythrocytes in the bone marrow cells of mice at different exposure times (Giri *et al.,* 2002) [74]. Studying on the genotoxicity of Marshal and its effective ingredient carbosulfan, Topakatas and Rencüzogullari (1993) reported that both the agents significantly induced chromosome aberration (CA) in human peripheral lymphocytes *in vitro*. In another report, Rencuzooullari and Topakatap (2000) reported that mixture of carbosulfan with that of ethyl methosulfate or ethyl carbamate show synergistic effect in inducing chromosome aberrations in human peripheral Lymphocytes. Karami-Mohajeri and Abdollahi (2010) [95] have reviewed other toxic effects of carbamate pesticides on cellular metabolism of lipids, proteins, and carbohydrates.

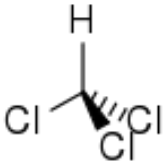
Carbofuran has been shown to be a potent genotoxic agent in several studies (Kar and sing, 1986; Ahmed *et al.,* 1999). The mutagenic potential of carbofuran was found to be positive in Salmonella *typhimurium* JK 947 strain by lactam

assay (Hour *et al.*, 1998) [87]. The quantitative assessment of cells containing micronucleus serves as a good indicator for the induction of structural and numerical chromosomal aberrations. Single cell gel electrophoresis, more commonly known as comet assay, is a simple, sensitive and rapid method for the detection and quantization of DNA damage by strand breaks, open repair sites, cross links and labile site at individual cell level (Kumari *et al.*, 2002) [102]. It has also been shown to be mutagenic in *Saccharomycestyphimurium* following metabolic activation with S9 (Moria *et al.*, 1983) and a weak mutagenic response in Chinese hamster cells have also been reported (Gridelet *et al.*, 1982; Chauhan *et al*., 2000) [71, 34]. Increased frequency of Chromosomal Aberration, Micronucleus, sperm abnormalities and decreased mitotic index in mice. Since, the digestive tract comprises the primary target tissue interacting with pesticides entering the body through an oral route (Chapalmadgu and Chaudhry, 1992; Heaton *et al.*, 2002) [33]. Carbofuran, a structural analogue of carbosulfan has been reported to be embryo toxic and causes alterations in spermatozoa of rat (Barr *et al*., 2010; Gallegos-Avila *et al*., 2010) [13, 63]. Gentile *et al.*, (1982) [69] described that carbofuran is responsible for unscheduled DNA synthesis in human lung fibroblasts. Carbofuran, a structural analogue of carbosulfan has been reported to be teratogenic and embryo toxic (Gupta, 1994) [82]. Genotoxic potential, including chromatin instability, of carbofuran has also been reported in other studies (Zeljezic *et al.*, 2009; Mladinic *et al.*, 2009; Chauhan *et al*., 2000) [207, 34]. Carbamate pesticides have been implicated as causative agents for certain types of cancer (Andreotti *et al.*, 2010) [4].

Organochlorines (Chlorinated Hydrocarbons)

Organochlorine insecticides, solvents, and fumigants are widely used around the world. This class comprises a variety of compounds containing carbon, hydrogen, and chlorine.

Organochlorine General Structure



These compounds can be highly toxic, and the over whelming majority have been universally banned because of their unacceptably slow degradation and subsequent bioaccumulation and toxicity. The toxicity of these agents varies according to their molecular size, volatility, and effects on the CNS. In general, they cause either CNS depression or stimulation, depending upon the agent and dose (Reigart JR, and Roberts JR, 1999) [154].Generally organochlorine persistent in soil, food, and in human and animal body's. They can accumulate in fatty tissues. Traditionally used for insect and mite control, but many are no longer used due to their ability to remain in the environment for a long time. Organochlorine pesticides includes DDT, Aldrin, chlordane, Chlordecone, dieldrin, endosulfan, endrin, lindane, Heptachlor, Hexachlorobenzene, methoxychlor, Mirex, Pentachlorophenol *etc*.

A number of organochlorine insecticides have had a great impact on ecology, not only because of their persistent in the environment for extended periods of time, but also for their high accumulation potential in living organisms (Maslanskyand Williams 1981, Siddiqui *et al.*, 1981) [117, 170]. An example of this group of compounds is Heptachlor, which has been used for the extermination of grasshoppers, locusts, soil mosquitoes and still other insects (Negherbon 1959, Barbera, 1976) [133, 12]. Most organochlorine compounds cause serious harmful effects to certain tissues of animals (*i.e*. rodents) fed with crops contaminated with these agents. In mammals, and so in humans, these compounds accumulate in several tissues and are detectable in milk and urine (Randaleff 1970, De la Jara and De la Parra, 1977, Siddiqui *et al.*, 1981) [44, 170]. The evaluation of the possible mutagenic effects of Heptachlor has been based on the results of dominant lethal assays and on the records of cytogenetic alterations in rat bone marrow cells (Cerey *et al.*, 1973) [32]. Data from these studies showed that there were an increased number of resorted fetuses and chromosomal abnormities. *i.e* .translocations and DNAaberrations.

DDT (1, 1, 1-trichloro-2, 2-di (4-chlorophenyl ethane) was one of the most used pesticides in the mid 20th century. In the period of almost 35 years, 2 million tons of DDT was used to control malaria and typhus, thus contaminating water, soil and air. Genotoxicity of DDT was evaluated in a variety of test systems. Results obtained by studying cytogenetic effects of DDT on DNA of shrimp larvae (*Litopenaeus stylirostris*) indicated that DDT causes DNA adducts and/or breaks (Galindo Reyes *et al.*, 2002). DDT and its Insecticides – Pest Engineering 136 metabolites DDE and DDD showed a clear genotoxic effect on haemocytes of zebra mussel (*Dreissena polymorpha*) specimens in different concentrations that have been found in several aquatic ecosystems worldwide, with a greater genotoxic potential of the DDE in respect to the other two chemicals (Binneli *et al.*, 2008a, 2008b). DDT has also the ability to induce chromosomal aberrations in mouse spleen indicating its genotoxicity (Amer *et al.*, 1996) [174]. DDT also induces cellular and chromosomal alterations in the rat mammary gland, which is consistent with the hypothesis that it can induce early events in mammary carcinogenesis (Uppala *et al.*, 2005) [191]. In addition, DDT was genotoxic towards lymphocytes and mammary epithelial cells of female rats showing an increase in lipid peroxidation, the outcome of the growth level of free oxygen radicals, which lead to an oxidative stress (Canales-Aquirre *et al.*, 2011) [1]. Additionally, beluga whales (*Delphinapterus leucas*) inhabiting the St. Lawrence estuary are highly contaminated with environmental pollutants including DDT which can induced significant increases of micronucleated cells in skin fibroblasts of an Arctic beluga whale (Gauthier *et al.*, 1999) [89]. Regarding human test system, the cytogenetic effect of DDT was investigated both *in vitro* and *in vivo*. *In vitro*, certain DDT concentrations have the effects on human leukocyte functions (Lee *et al.*, 1979) [107], are causing chromosomal aberrations (Lessa *et al.*, 1976) [108], DNA strand breaks (Yáñez *et al.*, 2004), and apoptosis induction which is preceded by an increase in the levels of reactive oxygen species (Pérez-Maldonado *et al.*, 2004, 2005). *In vivo*, DDT is able to induce chromatid lesions (Rabello *et al.*, 1975) [150], increase in chromosomal aberrations and sister chromatid exchanges (Rupa *et al.*, 1989, 1991) [38, 158],

DNA strand breaks (Yáñez *et al.*, 2004; Pérez-Maldonado *et al.*, 2006), apoptosis (Pérez-Maldonado *et al.*, 2004) as well as cell cycle delay and decrease in mitotic index (Rupa *et al.*, 1991) [157]. DDT induced unrepaired lesions will interfere with DNA replication process forming DNA strand breaks thus, chromatid breaks, but also chromosome breaks which may result from DNA breaks due to additional topoisomerase II impairment (Maynard *et al.*, 2009). DDT induces cytogenetic damage to human peripheral blood lymphocytes (Gerić *et al*., 2012) [115].

Endosulfan is one of such pesticide that belongs to organochlorine group of pesticides. It exhibits in two isomeric forms, Alpha – endosulfan and Beta-endosulfan. It is widely used as an insecticide with Trade name, Afidan, Beosit, Cyclodan, Devisulfan, Endocel, Endocide, Endosol, FMC 5462, Hexasulfan, Hildan, Hoe 2671, Insectophene, Malix, Phaser, Thiodan, Thimul, Thifor, and Thionex in both developing and developed countries due to its several advantages of being a broad spectrum, fast-acting and cost-effective pesticide. Endosulfan is used as pesticide mainly in agricultural crop plant. Being residual in nature, their circulation in the ecosystem is a great concern and is a challenge to environmental toxicologists. In recent years, the use of this pesticide has invoked huge concerns from all over the world due to its highly toxic nature and also its dangerous effects at molecular level. It has affected living organisms of lower and higher groups including human beings. Many death cases have been reported in humans due to their direct or indirect exposure to the pesticide. Also it has affected the internal organ systems like central nervous system (Karats *et al.*, 2006), hepatic system, lymphatic systems and induced immunosuppressant, neurological disorders, congenital birth defects, chromosomal abnormalities, mental retardation, impaired learning and memory loss, *etc*.

It has also been reported that endosulfan causes DNA damage in bacterial systems like *Salmonella, E. coli* and *Saccharomyces* (Martins *et al*., 2003) [116] and dose-related decrease in bacterial polar lipid dispersion in *Bacillus stearothermophilus* (Martins *et al.*, 2003) [116]. Reports obtained from endosulfan effects in plant systems clearly highlight DNA damage (in white clover plants, Liu *et al*., 2009). Chromosome aberrations in anaphase–telophase (CAAT) were determined in root tips of the wetland macrophyte, *Bidens laevis* exposed to environmentally relevant concentrations of endosulfan (Perez *et al.*, 2008) [144]. Endosulfan has inhibitory effect on the nitrogenase activity in *Arachis hypogea* (Darure, 2012) [39]. In earthworms, it decreased the success of the immune reaction (Kelvie *et al.*, 2009) [120]. Endosulfan was considered to be an efficient mutagen in *Drosophila*, since it exhibited pronounced clastogenic effect in sperm (Velazquez *et al.*, 1984). Some physiological effects were observed in terms of reproduction (partial spawning) and histopathology (atrophy of the digestive tubule epithelium) in oysters (Buisson *et al.*, 2008) [26].

In fishes, histopathological lesions were observed in gills, liver, spleen and trunk kidney of rainbow trout (Altinok *et al.*, 2007) [2]. Endosulfan damaged sertoli cells of testes and possibly has a negative impact on spermatogenesis and male fertility and also resulted in very high DNA damage in all the tissues of fresh water fish, *Mystus vittatus* (Sharma *et al.*, 2007, Dutta *et al.*, 2006) [167]. Endosulfan significantly decreased feeding, growth and predator avoidance in tadpoles (Broomhall, 2004) [25]. In chick embryos, endosulfan exhibits embryotoxic and teratogenic effects (Mobarak *et al.*, 2011) [123]. Endosulfan and its metabolites caused DNA damage in Chinese hamster ovarian cells (Bajpayee *et al.*, 2006) [9]. In rats, endosulfan induced behavioral aberrations and changes in neurotransmitter activities (Paul *et al.*, 1997) [141], genotoxic effects on liver hepatoblastoma cells, f*etal* liver cells and spermatogonial cells (Karatas *et al.*, 2006& Pandey *et al.*, 1990) [138]. In sheep, inhibitory effects of endosulfan were seen on the metabolic activity of peripheral blood neutrophils, monocytes and phagocytic cells (Pistl *et al.*, 2002). Endosulfan has caused mutagenic and genotoxic effects in human lymphocytes, estrogenic effects on human estrogen-sensitive cells and HepG2 cells (Bajpayee *et al*., 2006, Soto *et al.*, 1994 & Lu *et al.*, 2000) [9, 175, 110] and also causes mitochondrial dysfunction and oxidative stress (Kannan *et al.*, 2000) [93].

Silkworm as a Model Organism for Toxicological and Mutagenic Studies

Pesticide toxicology is concomitant with the needs and demands in agriculture and related fields such as sericulture, aquaculture, etc. Several pesticides are being synthesized, more are more and introduced into the market for use and it becomes impracticable to ban and eliminate each one of them. Hence there is a need for environmental monitoring as well as evaluating their toxicity, mutagenicity and the changes they induce at molecular level. In sericulture, various types of pesticide are used in order to obtain optimized food crop (*i.e*., mulberry leaf) productivity for feeding the silkworms. A vast amount of research work done on various pesticides and their effect in silkworm productivity clearly reveals that the silkworms cannot evade the residual effects of pesticide that have been applied on their food plants, mulberry.

Several model systems were utilized to understand the toxic and mutagenic nature of this pesticide covering wide range of mammalian and sub-mammalian system. Silkworm is an excellent laboratory tool for genetic studies [128]. By virtue of several advantages it confers, silkworm *Bombyx mori* has been chosen as an appropriate test system to evaluate the toxicity mutagenicity and damage to the genomic level.

Silkworm is a ideal bioassay material for toxicological studies (Kashi, 1971). Apart from routine test systems used for mutagenicity studies, silkworm *Bombyx mori* has been proved to be one of the effective test system to study the mutagenic effects of pesticides or other chemicals (Murato and Murakami, 1977; Tazima, 1980; Murakami, 1981a) [183]. In addition, it can detect a wide spectrum of genetical damages either at genetic or chromosomal level (Murakami, 1976) [129]. Mutagenicity studies using silkworm reveals that various chemicals like Formaldehyde (Tazima, 1961), EMS (Tazima and Onimaru, 1966), Acridine orange (Murakami, 1972), Mercury and cadmium compounds (Tazima and Fukase, 1974), 5-bromouraciland5-bromodeoxyuridine (Tazima,1974), have been successfully test edas mutagenic compounds.

Mulberry fields are contaminated with several kinds of pesticides and these pesticides have resulted in the intoxification of silkworm when contaminated leaves are fed to silkworm larvae (Tsuita1957, Kuwana *et al.*, 1967) [105]. Kuwana *et al.*, (1968) [106] assed the comparative toxicology of 22 kinds of insecticides using larval mortality as a major parameter. Commonly used pesticides to control pests in

mulberry garden are dimethoate, dichlorvos, ddvp, monocrotophos, phosphamidon, malathion, vijay neem, acetamiprid, dimethoate, aldrin, quinalphos *etc.*

Effect of pesticides on economic characteristics of Silkworm *Bombyx mori*

There are few studies that have focused on the effect of insecticides on *Bombyx mori* deal with toxicity, retardation of development and growth, fecundity, mortality, food utilization and economic parameters (Kuribayashi, 1988; Kumar *et al.,* 1992; Maria Vassarmidaki *et al.,* 2000; Vyjayinthi and Subramanyam 2002, Datta *et al.,* 2003) [103, 114, 197, 41]. Suhas *et al*., (1985) [180] tested the impact of 10 insecticides (endosulfan, Dichlorvos and carbyxl, monocrotophos and demeton methyl, Malathion and cypermethrin, and its effect on economic parameters where it reduce the shell weight. Residual toxicity of Ekalux EC-25 on biochemical constituents of fat body and cocoon weight was tested by Bhosale *et al.,* (1985, 1988) [17, 16]. Bashamohideen and Ameen (1998) [14] studied the effect of Dichlorvos on total protein content in the Haemolymph and fat body of the silkworm, *Bombyx mori*Effect of some pesticides monocrotophos, Acephate and Dichlorvos and Botanicals (neem pesticides) caused highly significance decline in the cocoon characters of silkworm was also studied by Bandyopadhyay *et al.,* (2005) [11]. Naseema Begam *et al.,* (2003) [132] studied the effect of sublethal dosage of endosulfan along with heptachlor Bromosphos, Larvae feeding on mulberry leaves contaminated with carbendazim residue showed a decrease in economic and biological characteristics of silkworm (Bizhannia *et al*., (2005) [19]. Raghuveer *et al.,* (2006) [151] investigated the effect of Dichlorvos on economic traits of silkworm races.

Several investigators have extensively used larval mortality as a role parameter to assess the toxicity of chemical or physical agents in Silkworm. The chemicals which do not cause the death in larval stage do cause pupil death because of the physiological disturbances experienced during the larval stages. Yamonai, 1984 and Sugiyama 1980 [179] conducted investigations utilizing survival rate (larval and pupal mortality) and cocoon characters as parameters to assess the toxic effect of EDB. Pai *et al*., (1991) [136] found that out of eight economic traits assessed, there were significant variations in six traits, when PABA is orally administered to *Bombyx mori*. Sugiyama and Emori (1980) [179] have reported the significant reduction in cocoon weight when silkworm larvae were fed with MEP and MPP treated leaves a result confirmed in this study. Kuroda and Gamo (1978) have shown that herbicide poisoned V instar larvae of silkworm manifested a significant reduction in free amino acids in haemolymph, weight of silk gland, as well as reduction in the values of cocoon characters. Carbaryl treatment reduces cocoon characters (Venkata Reddy *et al*., 1989, and Venkata reddy, 1984). Mutagens (DES) at higher doses were deleterious in their effect on larval characters and economic characters. Silkworm treated with insecticides showed larval, pupal; mortality and altered sex ratio (Naseema Begum *et al.,* 2003) [132]. In both Carbamate and Organophosphorus insecticide treated larvae, significant differences in cocooning, pupation, sex-ratio and egg lying were observed. Administration of Albendazole through the diet of *Bombyx mori* decreased larval weight and yield of cocoon at higher concentrations (Prakash *et al.,* 2006) [148]. At higher concentration Albendazole were toxic to synthesis of Protein and alter cellular components and decreased the body weight of mice (Devries, 2002) [46]. Higher concentration of Dichlorvos 20ppm and 40ppm showed a significant effect on the larval mortality and other economic traits analysed in both the races multivoltine pure Mysore race and bivoltine NB4D2 race (Raghuveer *et al.,* 2006) [151].

Screening of Environmental Mutagenic Compounds in *Bombyx mori*

Using the silkworm oocytes system, screening of mutagenic compounds has been carried by Tazima *et al*., 1980 [183]. The chemicals tested were mostly known carcinogens and their allied compounds. The chemicals screened are

1. Aziridines and derivatives
2. Mustards
3. Nitrosamines and nitrosamines
4. Amines and amides
5. Carboxylic acids (and their derivatives)
6. Epoxides, aldehydes, and lactones
7. Allyl Sulfides and alkane sulfonic esters
8. Heterocyclic compounds
9. Acridines and pyridinium salts
10. Nitrofurans
11. Polycyclic hydrocarbons
12. Azo,azoxy, and hydrazo compounds
13. Mycotoxins
14. Pyrrolizidine alkaloids
15. Base analogues
16. Others (Sodium nitrite, Potassium bromated *etc*.)

Genotoxic effects of various chemicals in *Bombyxmori*

Apholate induces embryonic mortality by causing embryonic lethality in the progeny of the treated male silkworm (Sugiyama, 1966). Acridine orange was mutagenic for meiotic spermatocytes, (Murakami, 1972). Ethylhexyl Sulfonate (EMS) is a powerful mutagen for pre-meiotic oocytes in silkworm pupae (Murakami, 1972). EMS induces predominant number of single strand breaks (or nicks) of DNA (Aricra and Murakami, 1975). Murakami (1975) has shown in *Bombyx mori* EMS is strongly mutagenic when administered to pre meiotic oocytes at the pupal stage. Dutta *et al.,* (1978) have indicated that sensitivity of silkworm to EMS is lower in spermatocytes than in spermatids and spermatozoa. Murota and Murakami (1975) have shown that no dominant lethality as observed in sperm of mid-pupae with caffeine or phloxine. Diethylsulphate induced egg colour mutation in mature sperm due to delayed mutagenic effect (Murakami, 1975). Diazepam was found to induce an increase in the number of egg colour specific locus mutations in the pupal oocytes of silkworm female germ cells (Murakami *et al.,* 1979). Pyrrolizidine alkaloids with otonecine as the necine base were mutagenic to silkworm germ cells. (Murakami *et al.,* 1980). Mitotoyin C induced mutation in spermatocytes of silkworm (Murakami, 1982). N-ethyl-N-nitrosourea increases the mutation frequency in spermatocytes, meiotic spermatocytes of larvae and spermatozoa and oocytes in Pupae of silkworm (Murakami, 1982). Benalate induces dominant lethal in old pupae (Krishnamurthy *et al.,* 1985). Dominant lethal mutations in larva and pupa were induced by diethyl sulphate (Boopathy and Muthukrishna, 1985). Apron 35 SD induced dominant lethal in early male germinal cells (Pai *et al*., 1985). Metabolic changes in the nerve tissue of the silkworm were affected by endosulfan (Jadav and Kallapur, 1988) [16]. Diethane M-45 induced

significant dominant lethal in silkworm (Vasudev *et al.*, 1994) [193]. Captan is non mutagenic to silkworm and Methyl parathion has induced acute genotoxic effects through dominant lethal test in silkworm (Deepak and Subramanya, 2000).

Fixing the LC_{50} value:
The concentration of the chemical in air that kills 50% of the test animals in a given time (usually four hours) is the LC_{50} value. Sub-lethal concentrations of ¼, $^1/_2$, and ¾ of LC_{50} shall be calculated and used to study toxicity parameters in silkworm *Bombyx mori*. After determining the sub-lethal concentration, they will be administered by oral, topical and subcutaneous injection method. The LC_{50} values are obtained from the percent mortality versus dosage curves. The mortality observed for each concentration will be converted as percent mortality value from it. The probit mortality is derived as described by Finney (1952) [57].The LD50 valued of few insecticides commonly utilized in sericulture are presented (Kuribayashi, 1988) [103].

Procedure for Administration of Chemicals in silkworm
The study of chemical mutagenesis, special techniques required for the administration of chemicals in accordance with their toxicity, permeability, metabolic characteristic, and other properties. The three commonly used methods of pesticide administration namely, oral topical and subcutaneous injection methods (Kuwana *et al.,* 1967) [105].

Oral Administration
The most natural way of administering chemical compounds is feeding. Usually, the chemicals are given to the larvae by painting or spraying them on the mulberry leaf surface. However, there are many points to be considered. First, there is the difficulty in controlling the administered dose, which depends mainly on the amount of food ingested. The dose varies with the appetite and preference of the test insect. *Bombyx* larvae are attracted to sweets but dislike bitter taste. Accordingly, a limit is set on the administer dose according to the properties of the chemical compounds. Second, extra measurement of the administered dose is difficult when the larvae are at the younger stage it can be measured on an average by weighing the increment of the body weight after feeding. After the second moult weighing an individual a basis becomes feasible. Third, interaction between the test compound and several components of the food leaves must be taken in to account. For instance, the possibility of mutation induction by base substitution of DNA was investigated by administering a base analog, bromouridine or bromodeoxyuridine with mulberry leaves but the result were inconclusive. This is not surprising because precursors of thymidine are contain in quantity in mulberry leaves. This experimental disturbance can be excluded if we use synthetic food from which the chemical component in question is removed. Semi synthetic food has therefore been developed. By oral administration, a positive mutagenic response has been obtained for MMC and Panfuran. The chemicals were given to larvae just after the second moult by painting of mulberry leaves.

Topical Method: Topical application is mainly used in toxicity tests because of its convenience. It is easily done by dropping or painting a definite volume of insecticide solution on the body surface of the larvae. Various sub-lethal concentrations shall be prepared and applied to the anterior abdominal region of the each larva in batches using a micro syringe. The mortality percentage is recorded after 24 hours of treatment.

Sub-Cutaneous Injection Method: Administration of chemical compounds by injection is unnatural, but has been conveniently practiced in several experiments. Chemical are dissolved in physiological saline or 0.85% aqueous saline solution, and these are injected into the hemoceole Compounds insoluble are hardly soluble in water are dissolve first in alcohol and acetone, and these are then dissolve in water. Dimethyl sulfoxide emulsion can also be injected. The quantity to be injected varies according to the development stage of the silkworm, from 0.001ml IV-3(Fourth instar Day 3) 0.05ml at the most advanced larval stage just before cocoon spinning. The administered doses became gradually more accurate with the progress of the developmental stage. Fluctuation occurred in administered dose due to the leakage of hemolymph after injection, but it can be reduced to a minimum if the injection is practiced in the pupal stage through the wing bud.

Toxic symptoms in treated silkworm *Bombyx mori* (Kuwana *et al.,* 1967) [105]
The primary step in assessing the toxicity of a chemical substance is to observe the physical and behavioural responses of the poisoned animal. When the silkworms were treated with chemical substances the following toxic systems are generally observed.

Slight excitement: raising the head at regular intervals,

Swinging: swinging of the anterior half of the body,
Vomiting: vomiting of digestive juice,

Lying on the side: inelasticity of the body and lying on the side,

Body shortening: shortening of the body due to loss of digestive juice, muscle contraction due to contraction the body shrinks,

Paralysis: legs loose clasping power and effect of nervous system,

Irregular moulters: retarded and irregular growth at moulting stage,

Non-exuviated: The faecal matter remains in the system,
Atony: Larva looks inactive, does not feed or move about, and stretches the body and Death.

Measurement of Mutation Frequency
Several methods have been utilized for the quantitative assessment of radiation-induced mutation frequency, namely the dominant lethal method, specific loci method, and autosomal recessive lethal method. The most convenient may be the specific loci method. Mutations for dominant traits are inconvenient for the purpose of quantitative study.

Specific Locus Method
The specific locus test procedure described by Murakami (1976) [129]. This test is a method for detecting and measuring frequencies of mutation at a given locus. The

method consists essentially of mating treated wild-type silkworm, either male or female, to a marker strain of homozygous for recessive genes such as egg-colour mutant (pe, re, and w-2) and a body colour mutant of newly hatched larvae (ch). These recessive genes are readily expressed as visible phenotype in the homozygous state. Each F_1 egg receives one chromosome 5 from the untreated marker strain and another from treated wild-type strain. If deletions or mutations occur at any of recessive pe or re locus in the treated wild-type strain, these can easily be detected as either yellowish-white or red whole- or fractional-body mutants detected in F_1 eggs is recorded separately for each locus. The overall frequency of each class mutation for each locus is calculated by dividing the number of mutants by the total number of fertilized eggs and 95% confidence limits are calculated.

Dominant Lethal Test

Dominant lethal mutation is defined as a mutation that kills zygotes even in heterozygous conditions in the immediate filial generation (Tazima, 1978) [181]. The primary advantage of the dominant lethal test is that fewer insects are required to obtain a statistically significant result (Murota and Murakami, 1975). These classes of mutation are mainly characterized by early embryonic death or reduction in hatchability in fertilized eggs. It has been be lived that in the silkworm this biological effect is strongly associated with structural or numerical chromosomal anomalies as observed in other organisms.

Although the dominant lethal test may be conducted in males or females, mainly silkworm male pupae or sperm have been used for this test, since there are a number of non genetic causes in the treated female germ-cells which might prevent the egg from hatching after treatment of males with mutagens, they were mated to non-treated females. The actual scoring of the number of hatched and non-hatched embryos (or eggs) are carried out the frequency of dominant lethal mutations is obtained by dividing the number of dead embryos (eggs) by the total number of fertilized eggs, and multiplying this value by a hundred:

$$\text{Relative dominant lethality} = \frac{\%\text{ of hatching in treated series}}{\%\text{ of hatching in control series}}$$

Sex Linked Recessive Lethal Mutation Test

Because of the presence of a single Z chromosome in the silkworm females the origin of sex linked recessive lethal mutations can be scored in female. Mating system used to detect recessive lethal on the z chromosome. The treated wild-type (W^{pe+}/sch^+;pe/pe) parental females mated to marker males(sch/sch;pe/pe) may, as a result of chemically-induced mutations, produced many kinds of Z chromosomes(sch^+) in her ova, some of which bear recessive lethal genes. If the sons of treated wild-type females does not carry a chemically-induced mutation in their Z chromosomes, an F_2,as well as male, may produce chocolate brown and wild-type black larvae in equal proportions. If the Z chromosome (sch^+) carried by F_1 bears a chemically-induced recessive lethal mutation.

Conclusion

Tremendous advancement made in the field of science and technology has provided a means of development on one hand and concomitant problems on the other. One such advancement is introduction of environmental chemicals through pesticides and as a result present world is confronted with the indiscriminate use of the several environmental chemicals in agriculture, livestock production, conservation of plants and animals, industrialization and biodiversity. These environmental chemicals are known to affect not only the target organisms and non target organisms endangering the homeostasis mechanism but also causing severe damage to ecosystem because of long lasting residual effects. It is well known that several of them polluting the atmosphere causing "Global warming". These environmental chemicals since they are extensively used, need be screened both at stomatic and gematic system in the interest of human health, welfare and posterity. A battery of test systems is available in order to analyse the genotoxic effects of the environmental agents. The information developed utilizing the laboratory models from prokaryotes to eukaryotes have richly contributed to diversify the knowledge of genotoxicity. It is in this context the silkworm *Bombyx mori* offers as one of the best and convenient laboratory model because of its holometabolous type of metamorphosis(precise life cycle) and many of the important physiological, genetical mechanism simulates higher eukaryotic system. The methodology developed to study the genotoxic effects of environmental chemicals/ pesticides utilizing silkworm *Bombyx mori* includes dominant lethals, specific locus and sex linked recessive test. These tests are accepted as standard protocol to evaluate the environmental chemicals/ agents. Hence using silkworm it is possible to understand the toxic and mutagenic potential of many hazardous pesticides if any. It is with this in view the present documentation work was undertaken is presented keeping in view of the utility of the silkworm *Bombyx mori* as one of the best laboratory model organism for genotoxicity.

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