REVIEW OF LITERATURE
2.1 PESTICIDES:

Pesticides are used widely in agriculture to maintain and increase crop yields. Pesticides used in agriculture are designed to protect crops against unwanted species, such as weeds, insects and fungus. These chemicals may be extracted from plants or may be synthetic (WHO, 1990). According to the study made by the US Geological Survey (USGS), 90% of streams and 50% of wells tested ground and surface water were positive for at least one pesticide monitors among 76 pesticides and seven pesticide breakdown products. There are many groups of chemicals used as pesticides. A great potential for adverse effects of pesticides is through contamination of the hydrological system, which supports human life, aquatic life and related food-chains.

![Pathways of pesticide movement in the hydrologic cycle.](image)

**Courtesy: U.S. Geological Survey, Fact Sheet FS-152-95**

Fig. 2.1: Pathways of pesticide movement in the hydrologic cycle.

The contamination of the hydrologic system results in the adverse effect of pesticides. Water is one of the primary means by which pesticides are transported from their application areas to other parts of the environment. Thus, there is potential for movement of pesticides into and through all components of the hydrologic cycle which supports not only human life, but aquatic life and related food chains as well.
There are hundreds of different active principles or main ingredients of pesticide groups (e.g. approximately 300 in Uruguay and 900 in the USA).

![Pesticides Classification](image_url)

**Fig. 2.2: Pesticides Classification**

In India, the production of pesticides started in 1952 with the establishment of a plant for the production of BHC near Calcutta. Since then, its production and usage increased with a greater pace as a result of which in 1996–97 the demand for pesticides in terms of value was estimated to be around Rs. 22 billion (USD 0.5 billion), which is about 2% of the total world market. The pattern of pesticide usage in India is different from that for the world in general.
2.2. PESTICIDES EXPOSURE:

Different groups of population are exposed to pesticides in different ways and in different degrees. Individuals are frequently exposed to many different pesticides or mixtures of pesticides, either simultaneously or serially. These exposures are often highly correlated, particularly within functional or chemical groups, making it difficult to identify effects of particular agents. Exposure is acute if exposed to a large amount of pesticide once. A spill on the body is one example. It’s usually easy to identify acute exposure. Exposure is chronic when having a low-level exposure over and over. Chronic exposure may be hard to tell. Either kind of exposure is dangerous.

There are four ways a person can be exposed to pesticides:

- **Oral exposure** — swallowing pesticide
- **Dermal exposure** — getting pesticide on your skin, the most common type
- **Inhalation exposure** — breathing in pesticide
- **Ocular exposure** — getting pesticide in your eye.
There are different causes of each type of exposure as summarized in Table 2.1.

<table>
<thead>
<tr>
<th>Type of Exposure</th>
<th>Cause of Exposure</th>
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<tbody>
<tr>
<td>Oral exposure</td>
<td>• Not washing hands before eating, drinking, using tobacco.</td>
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<td></td>
<td>• Eating or drinking a pesticide by mistake.</td>
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<td>• Getting pesticide on food.</td>
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<td>• Splashing pesticide into the mouth.</td>
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<td>• Blowing out plugged nozzles with the mouth.</td>
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<td>Dermal exposure</td>
<td>• Getting pesticides on bare skin.</td>
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<td>• Applying pesticides in windy weather.</td>
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<td>• Wearing inadequate PPE.</td>
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<td>Inhalation exposure</td>
<td>• Prolonged contact in poorly ventilated areas.</td>
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<td></td>
<td>• Not using proper PPE.</td>
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<td></td>
<td>• Breathing vapors after application.</td>
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<td></td>
<td>• Using the wrong respirator.</td>
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<td></td>
<td>• Using an improperly fitted respirator.</td>
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<td></td>
<td>• Using tainted filters, cartridges, or canisters.</td>
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<tr>
<td>Ocular exposure</td>
<td>• Getting pesticides in the eyes.</td>
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<td></td>
<td>• Not using proper eye cover when:</td>
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<td></td>
<td>• Spraying pesticide</td>
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<td></td>
<td>• Handling pesticide</td>
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<tr>
<td></td>
<td>• Rubbing the eye with tainted gloves or hands.</td>
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2.3 PROS AND CONS OF PESTICIDES

Crops are affected by different pests and by competition from weeds. Several insects and other arthropods, fungi, mollusks and bacteria attack crops and result in quantitative and qualitative losses and the degree of damage varies greatly in different climatic and agricultural regions. During last three decades, chemical control of pests and weeds aimed at minimizing losses has been introduced throughout the world. Pesticides have been an integral part of the process by reducing losses from weeds, diseases and insect pests that can markedly reduce the amount of harvestable produce (Aktar et al., 2009).

If the credits of pesticides include enhanced economic potential in terms of increased production of food and amelioration of vector borne diseases, then their debits have resulted in serious health implications to man and his environment. These
chemicals produce a potential risk to humans and other life forms and unwanted side effects to the environment (Forget, 1993; Igbedioh, 1991). The world wide deaths and chronic diseases conditions due to pesticide exposure number about 1million per year (Environews Forum, 1999). The high risk groups exposed to pesticides include production workers, formulators, sprayers, mixers, loaders and agricultural farm workers. In industrial settings, workers are at increased risk since they handle various toxic chemicals including pesticides, raw materials, toxic solvents etc. Various pesticides in industrial settings of the unorganized sector revealed a high occurrence of generalized symptoms (headache, nausea, vomiting, fatigue, irritation of skin and eyes) besides psychological, neurological and gastrointestinal symptoms coupled with low plasma cholinesterase (ChE) activity (Gupta et al., 1984).

2.4 PESTICIDES AND NEUROTOXICITY

Many pesticides target the nervous system of insect pests. Because of the similarity of neurochemical processes, these compounds are also likely to be neurotoxic to humans. This concern is of particular relevance to the developing human brain, which is inherently much more vulnerable to injury caused by toxic agents than the brain of adults (Dobling, 1968). Most types of pesticides, including organophosphates (OPs), carbamate, and organochlorine insecticides as well as fungicides and fumigants, can be neurotoxic, but only OPs have been studied in detail (Keifer and Mahurin, 1997). The response to OPs can occur within minutes. Less severe cases of OP poisoning display symptoms including headache, dizziness, nausea, vomiting, pupillary constriction, and excessive sweating, tearing, and salivation. More severe cases develop muscle weakness and twitches, bronchospasm, and changes in heart rate and can progress to convulsions and coma. Studies have also shown that prenatal exposure to a OPs reduces child IQ (Bouchard et al. 2011), impairs mental
development (Engel et al., 2011), and causes cognitive deficits (Eaton et al., 2008; Bouchard et al., 2010; Rauh et al., 2011). Furthermore, exposure to cholinesterase-inhibiting compounds including OPs, pesticides, pyridostigmine bromide (PB, an anti-nerve agent medication), and low-level sarin nerve gas is linked epidemiologically with a chronic multi-symptom illness in veterans from the Persian Gulf War (Staines, 2005; Thomas et al., 2006; Haley et al., 2009).

Individual response to pesticide exposure may be affected by polymorphisms in genes affecting pesticide metabolism. The best-known example is paraoxonase, an enzyme that hydrolyzes active metabolites of OPs (Costa et al., 2003). In humans, paraoxonase polymorphisms affect the relationship of OP exposure to both erythrocyte acetylcholinesterase (AChE) inhibition and symptom prevalence (Lee et al., 2003; Leng and Lewalter, 1999; Mackness et al., 2003; Sozmen et al., 2002). An extensive literature suggests that pesticide exposure may increase risk of Parkinson disease (Le Couteur et al., 1999). Many studies have found an association of Parkinson disease risk with living in rural areas, drinking well water, and farming as an occupation (Priyadarshi et al., 2001). More specifically, case–control studies have observed that pesticide exposure is associated with increased Parkinson disease risk. Most studies of pesticide exposure and Parkinson disease risk have been unable to implicate specific pesticides. Several studies found increased risk associated with exposure to either insecticides or herbicides (Butterfield et al., 1993; Gorell et al., 1998; Semchuk et al., 1992), and one study indicated that risk was elevated by exposure to organochlorines, OPs, or carbamates (Seidler et al., 1996). Several studies have implicated the herbicide paraquat (Hertzman et al., 1990; Liou et al., 1997), which produces selective degeneration of neurons involved in Parkinson disease (McCormack et al., 2002). Case reports have described Parkinson disease in
individuals exposed to OPs (Bhatt et al., 1999; Davis et al., 1978); to herbicides including glyphosate (Barbosa et al., 2001), paraquat (Sanchez-Ramon et al., 1987), and Diquat (Sechi et al., 1992); and to fungicides including maneb (Meco et al., 1994) and other dithiocarbamates (Hoogenraad, 1988). Information on pesticide exposure and other neurologic diseases is more limited. Numerous studies were located that provided information on the neurological effects of exposure of humans to organophosphate pesticides in air, but few evaluated exposure specifically to malathion.

2.4.1 MALATHION

Malathion is a pesticide used for agricultural and non-agricultural purposes. The CAS nomenclature of malathion is Diethyl[(dimethoxyphosphino-thiyl)thio] butanedioate. The empirical formula is C_{10}H_{19}O_{6}PS_{2} and its structural formula is Butanedioic acid, [(dimethoxyphosphinothiyl)thio]-, diethyl ester.

![Fig. 2.4: Structure of Malathion](image)

It is released to the environment primarily through spraying on agricultural crops and at agricultural sites. Once it is introduced into the environment, it may be activated by atmospheric photo-oxidation or degraded by hydrolysis or biodegradation mediated by micro organisms found in most sediment, soils and water. The general population is not likely to be exposed to large amounts of malathion. Some exposure to residues of malathion is possible, however as many studies show that malathion has been detected in foods and atmosphere samples (US Department of
Health & Human Services, 2003). Malathion is toxic via skin contact, ingestion and inhalation exposure (Tomlin, 2006). Malathion has been shown to be mutagenic, a possible carcinogen implicated in vision loss, causing myriad of negative health effects in human and animal studies damaging non-target organisms and has a legacy of serious problems (Cantor et al., 1992; Balaji and Sasikala, 1993). It has many structural similarities with naturally occurring compounds and the primary target of action in insects is the nervous system; it also inhibit the release of the acetylcholinesterase at the synaptic junction (Cabello et al., 2001). Several studies showed that malathion induced various physiological, biochemical, immunological and histological changes in experimental animals (Bhatia, 1996; Rezg, 2006 & 2007; Saadi, 2008). Malathion toxicity is compounded by its metabolites and contaminants. Malaoxon, the metabolites produced by malathion oxidation in insects, mammals and plants is a preliminary reason of malathion toxicity and it is 40 times more toxic than malathion (Aldridge et al., 1979). Malathion intoxication causes cholinergic stimulation acutely, but delayed neuropsychiatric sequel may also result in a sub acute paralytic syndrome known as the intermediate syndrome. Intermediate syndrome is characterized by deterioration of muscle strength and mental status, headache, confusion, insomnia and decreased rate of respiration (Choi, 1998). Malathion exposure can cause blood pressure changes with either rapid or decreased heart rate.

### 2.4.1.1 MODE OF ACTION OF MALATHION

Malathion is converted inside animals into malaoxon, a chemical relative that inhibits an important central nervous system enzyme i.e. AChE which is involved with the transmission of nerve impulses (US EPA, 2000). When this enzyme is inhibited, the transmission system “jams,” resulting in restlessness, hyperexcitability, convulsions, paralysis, and death. In mammals, malaoxon has similar effects on AChE
However, in mammals AChE is not used in the central nervous system, but rather in nerves that connect with muscles. This means that symptoms in mammals are different than those in insects (Ware, 2000).

2.4.1.2 MALATHION AND NEUROTOXICITY

Exposure to organophosphorous pesticides is also a potential cause of longer-term damage to the nervous system, with reports of poor mental health and deficits in memory and concentrations (Davis, 1991; Mason, 2000; Nigg and Knaak, 2000). Effects on the central nervous system can also include headache, confusion, insomnia, decreased rate of respiration and coma (Reigart and Roberts, 1999; Wagner, 1997). At high dose, Malathion may cause intermediate syndrome in humans (Lee and Tai, 2001). In an experimental study, five volunteers ingested malathion at up to 16mg/day (0.23mg/kg/person/day) for 47 days and displayed no significant reduction in cholinesterase activity, when consuming 24mg/day (0.34mg/kg/day) for 56 days, five volunteers displayed reduced cholinesterase activity two weeks after dosing began. A maximum cholinesterase inhibition of 25% was observed three weeks after the end of the dosing period where four male volunteers reported to inhale malathion products at 5.3, 21.0 or 85.0mg/m$^3$ for one hour per exposure, two exposures per day for 42 consecutive days (Moeller and Rider, 1962). It concluded that no known effects on cholinesterase activity was observed but noted that one subject in each of the two highest dose groups exhibited reduced plasma cholinesterase activity (Golz, 1959). Studies in which workers were exposed to a combination of pesticides (not limited to organophosphates) that included malathion showed either a decrease of 26% in RBC cholinesterase in 85 workers exposed from 0.1 to 29 years compared with the same number of unexposed individuals (Hermanowicz and Kossman, 1984) or no significant difference among 11 pesticide applicators when comparing exposure...
periods with periods during the year of no exposure (Stdlberg et al., 1978). Some studies also examined the possible association between changes in cholinesterase levels and the presence or absence of clinical signs of cholinergic stimulation. For example, Peedicayil et al., (1991) found that plasma cholinesterase activity in workers who exhibited cholinergic signs and symptoms was 17% lower than in workers without such signs or symptoms. A significantly higher percentage of peripheral neuropathies (evaluated by electromyograph [EMG] recordings) were observed among pesticide workers than in controls in a study by Ernest et al., (1995). The study by Kahn et al., (1992) which described adverse respiratory effects on residents from an urban area where aerial spraying with malathion was conducted, did not find a significant increase in visits to hospital emergency departments for the category or anxiety, following the spraying. In fact, after the spraying, there was a decrease in anxiety-related symptoms. Exposure of rabbits to 123 mg malathion/m³ as an aerosol generated from a technical malathion formulation (95% pure) for 6 hours inhibited plasma cholinesterase activity by 37% at 24 hours post exposure and 41% at 72 hours post-exposure (Weeks et al., 1977).

In a 13-week study, Sprague-Dawley rats given a whole body exposure for up to 2,010 mg/m³ of malathion (96.4% pure) 6 hours/day, 5 days/week (Beattie, 1994). At termination, a dose dependent effect on cholinesterase activity was observed. Another finding showed that any effect was more pronounced in females as compared to males. Plasma cholinesterase activity was decreased 30% at 450 mg/m³ and 70% at 2,010 mg/m³, respectively in females. RBC cholinesterase activity was decreased 22 and 27% at 450 mg/m³ in males and females, respectively, and 43 and 44% at 2,010 mg/m³ in males and females, respectively. Brain cholinesterase activity was decreased 41% at 2,010 mg/m³ in females. Excess salivation was seen mostly in rats from the
high-exposure group, although it occurred sporadically in other exposed groups also (Beattie, 1994). Kumar et al., (1995) observed increased failure of eggs to hatch after untreated females were mated with treated males, presumably due to dominant lethal mutations. The study also found increased sex-linked recessive lethal mutations. Another study, however, showed no differences in sex-linked recessive lethal mutations, although this test used a *Drosophila* strain selected for increased malathion resistance (Velázquez et al., 1987). Results of the wing spot test, which can test genotoxic activity without exogenous metabolic activation, were negative (Osaba et al., 1999).

In a study of acute neurotoxicity in rats receiving doses of 0, 500, 1000, or 2000mg/kg body weight (bw), an NOAEL was not identified, as clinical signs were present at all doses. In a 13-week study of neurotoxicity, also in rats, at dietary concentrations of 0, 50, 5000, or 20,000 parts per million (ppm), the NOAEL was 5000 ppm, equal to 350 mg/kg bw per day, using inhibition of brain acetylcholinesterase at the highest dose as a marker. Two studies on the neurotoxicity of malathion in hens were reviewed. Neither produced any evidence that malathion can cause delayed neuropathy, however some inhibition of neuropathy target esterase was found in the brain at 2000 mg/kg bw (Pesticide Residues in Food-1997: Report). Exposure to some organophosphorous insecticides and other organophosphorous compounds causes delayed neurotoxicity, often referred to as organophosphorous induced delayed neurotoxicity (OPIDN). OPIDN involves the inhibition of neuropathy target esterases (NTE) and is a neurological effect which is totally different from AChE inhibition (Abou-Donia, 1995; De Bleecker, 1995; Ecobichon 1994; Jamal, 1997).
A tentative diagnosis of delayed neurotoxicity was proposed in one incident of human poisoning (Dive et al., 1994). In a human neuroblastoma cell line (SHSY-5Y), however neither malathion nor malaxon were found to inhibit NTE. This result is consistent with failure of malathion (88% solution) to induce delayed neurotoxicity in adult hens after oral doses of 75, 150 or 300mg/kg (Ehrich et al., 1995). In this study malathion inhibited both brain NTE and brain AChE; however brain AChE was inhibited to a greater extent than was brain NTE. Lotti and Moretto, (2005) reviewed numerous case reports and toxicity studies and concluded that the available information does not demonstrate an association between malathion exposure and delayed polyneuropathy. Effects on the nervous system may be reflected in behavioural changes. Uppal et al., 1983 reported that malathion could impair conditional responses in rats. Kurtz (1997) reported that malathion doses (50mg/kg after intraperitoneal) did not reduce AChE activity substantially but resulted in impaired avoidance performance in rats. Similarly Abdel-Rehman et al., 2004 noted impaired sensory motor performance in rats after a 30days dermal exposure to 44.4mg/kg/day malathion in the absence of significant changes in ChE and BChE activities measured in different brain regions and in plasma.

2.4.2 PARAQUAT

Paraquat is highly toxic herbicide to be marketed over the last 60 years. Paraquat is an acutely toxic herbicide and it enters the body mainly by oral or dermal route. The IUPAC nomenclature of paraquat is \(N,N'\)-dimethyl-4,4'-bipyridinium dichloride. The empirical formula of paraquat is \(C_{12}H_{14}Cl_2N_2\).
Paraquat was first synthesized in 1882. Its herbicidal properties were discovered in 1955 by ICI and registered in England in 1962 (US EPA, 1997). The WHO classifies paraquat as Class II, moderately toxic; but Pesticide Action Network (PAN) believes it should be reclassified as class I because of its acute toxicity, delayed effects and lack of antidote. Paraquat may damage the lungs, heart, kidney, adrenal glands, CNS, liver, muscles and spleen causing multiorgan failure as well as damaging the skin and eyes. Paraquat is used as a herbicide, desiccant defoliant and plant growth regulator (US, EPA., 1997). It has been reported that nitric oxide, a highly reactive, short-lived radical species which plays an important role in many physiological mechanisms but with a cytotoxic potential when produced in excessive concentrations (Moncada et al., 1991), is also involved in the production of lung tissue injury induced by paraquat (Berisha et al., 1994).

2.4.2.1 MODE OF ACTION OF PARAQUAT

The mechanism of the paraquat toxicity involves cyclic reduction/reoxidation of the herbicide with consequent NADPH consumption and production of oxygen free radicals (Farrington et al., 1973) which react with membrane phospholipids, leading to peroxidation and irreversible cell damage (Plaa and Witschi, 1976; Melchiorri et al., 1996). Based upon structural similarity with MPP+, paraquat is commonly believed to act through a similar mechanism of action. There has been speculation that paraquat may be actively transported into dopaminergic neurons by dopamine
transporter (DAT) (Shimizu et al., 2001, 2003) and inhibit complex I (Fukushima et al., 1994; Tawara et al., 1996). However, the role of the DAT and complex I in paraquat neurotoxicity remains to be established.

2.4.2.2 PARAOQUAT AND NEUROTOXICITY

Paraquat which is widely used as a cationic nonselective bipyridyl herbicide to control weeds and grasses in many agricultural areas, has emerged as a putative risk factor on the basis of its structural homology to 1-methyl-4-phenyl-pyridine (MPP\textsuperscript+), the active metabolite of 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP), a neurotoxicant that induces Parkinson’s-like features in rodents, nonhuman primates, and humans (Langston and Ballard, 1984; Tanner and Ben Shlomo, 1999). There is considerable evidence that paraquat may cause the onset or accelerate the development of Parkinson’s disease (Hatcher et al., 2008). The evidence showed that the longer exposure extends the risk of development of the disease that may depend upon the time between exposure and development of symptoms. The California Environmental Protection Agency states that paraquat can penetrate the nervous system are a neurotoxicant and impacts brain functions. Exposure to paraquat, even in relatively low doses, during critical periods in childhood may adversely affect the development of brain functions. Paraquat causes extensive damage to the mitochondria of cells through the production of free radicals and oxidative stress, resulting in the interruption of important biochemical processes, cell death and multiorgan failures (Suntres, 2002; Mohammadi-Bardbari and Chazi-Khansari, 2008). Effects have been measured in rats in the mitochondria of brain cells (Castello et al., 2007; Dreschel and Patel, 2009), in brain neurons (Yang and Tiffany-Castiglioni, 2005; Zaidi et al., 2009), in blood liver, lung and kidney cells (Ray et al., 2007) and in the hippocampus of the mice brain (Chen et al., 2010a).
The US EPA did not require neurotoxicity tests, 1997 because of the chemical nature of paraquat and the fact that it did not inhibit cholinesterase or damage the structure of the nervous system. As far back as 1984, it was known that, at high doses paraquat produced symptoms of neurological disturbance in rats, including decreased motor activity, lack of co-ordination, ataxia and dragging of the hind limbs (IPCS, 1984). A number of studies have shown that exposure of laboratory animals to paraquat causes reductions in neurotransmitters in the brain (Endo et al., 1988; Miranda Contereras et al., 2005) resulting in significantly disturbed or reduced motor activity including walking, drinking, rearing and rational activity (Chanyachukul et al., 2004) and increased anxiety (Little john et al., 2008). Paraquat also kills neurons in the brain- both mature and immature cerebellar granule neurons (Stelmashook et al., 2007) and damages the hippocampus region of the brain, reducing learning and memory (Chen et al., 2010a).

California Environmental Protection Agency (Cal EPA, 2010) has raised real concerns about the effects of paraquat on the developing brains of children. It was concluded that “Paraquat is a neurotoxicant and impacts brain functions”. Paraquat may affect different systems of the brain including the nigrostriatal dopaminergic system. Animal studies have shown to cause dose-dependent loss of dopamine neurons and degeneration of the nigrostriatal dopamine system; aggregation of alpha-synuclein protein and formation of Lewy bodies; and decreased or altered locomotion activity (Liou et al., 1996; Brooks et al., 1999; Uversky et al., 2001; McCormack et al., 2002; Mollace et al., 2003 and Li et al., 2005). Paraquat has the ability to cross blood-brain barrier (Shimizu et al., 2001) and enter the brain (Lee, 2008a). The uptake of paraquat into the brain is age dependent with higher concentrations found in very young and very old animal studies (Thiruchelvan et al., 2002). The effect of
paraquat in inducing Parkinson’s disease or symptoms is heightened by synergistic interaction with the fungicide maneb (Thiruchelvan et al., 2000a, 2000b; Thrash et al., 2007). There is growing evidence that paraquat has chronic effects on the brain. In Taiwan the risk of Parkinson’s disease among farmers was greater for subjects who had used paraquat and other herbicides/pesticides than those who had used herbicides/pesticides other than paraquat (Lion et al., 1997). Parkinsonism has been linked to insufficient levels of dopamine in the brain. Paraquat was found to be toxic to dopamine producing nerve cells in animals studies (Li et al., 2005, McCormack et al., 2005).

Several animal studies reveal postnatal exposure to paraquat (Fredriksson et al., 1993), indicating that previous exposure to paraquat enhances vulnerability to neurotoxins, and that there is progressive neurotoxicity with continuing exposure (Thiruchelvam et al., 2002). Kriscenski-Perry et al., (2002) demonstrated that thermal stress and paraquat have a synergistic effect in damaging spinal motorneurons. A Taiwanese study reported that paraquat exposure for more than 20 years was associated with Parkinson’s disease (Liou et al., 1997). Accumulating evidence strongly points to environmental toxins as feasible triggers of neurodegeneration of nigrostriatal dopaminergic neurons, and the common use of pesticides in rural life has been correlated to Parkinsonism in humans (Di Monte et al., 2000).

Occupational paraquat exposures have been associated with Parkinsonism (Hertzman et al., 1990; Liou et al., 1997), although the mechanism of paraquat toxicity is yet poorly understood. Several studies have suggested the involvement of reactive oxygen species (ROS) in its effect (Gonzalez-Polo et al., 2004; Mollace et al., 2003). Some other studies have demonstrated that free radicals play an active role in the paraquat induced apoptotic events that culminate with cell death (Gonzalez-Polo
et al., 2004). The mechanism of action of paraquat is as yet poorly understood. However, it is well known that paraquat acts as a reactive oxygen species (ROS) producer (Bus et al., 1984; Suntres, 2002). Several studies have suggested the involvement of reactive oxygen species (ROS) in the toxicity induced by paraquat (Cappelletti et al., 1998, Bus et al., 1984; Gonzalez-Polo et al., 2004, Mollace et al., 2003). Recent studies have shown that paraquat is not a substrate for dopamine transporter (DAT), but when converted to the monovalentcation paraquat by either a reducing agent or NADPH oxidase on microglia, it becomes a substrate for dopamine transporter (DAT) and is accumulated in dopamine neurons, where it induces oxidative stress and cytotoxicity (Rappold et al., 2011). Paraquat is more toxic and induces synucleinopathy and tauopathy in striata of mice via its inhibitory effects on proteasomes and autophagy, which lead to accumulation of α-Syn and p-Tau (Wills et al., 2012).

The behavioral and neuropathological effects of both systemic and intra-hippocampal injections of paraquat dichloride in rats had been studied by Bagetta et al., (1992). Paraquat (0.1–1.0mmol), injected into the dorsal hippocampus, produced seizures within a few minutes of injection, and caused neuronal damage in the CA1 and CA3 pyramidal cell layers, pyriform cortex, dentate granule cell layer and in the hilus fascia dentata at 24 h (n= 9 rats). A smaller dose of paraquat (10nmol) was ineffective. The effects of intrahippocampal injections of paraquat (1mmol) were prevented by coadministration with atropine (50 nmol). Systemic injections of paraquat (20–100mg/kg bw) produced forelimb clonus and rearing in 10 out of 15 animals. Neuronal cell death was found 24h later in nine of these rats and was restricted to the pyriform cortex, this being the region of the brain with the highest concentrations of paraquat. Atropine (at a dose of 150mg/kg bw given intraperitoneal
60min before paraquat) completely prevented the motor seizures, but cell death still occurred in two of the six animals tested. The effects of paraquat (1–5mg) on behavior, morphology and neurochemistry were investigated in male Wistar rats treated by unilateral injection into the substantia nigra. There was vigorous contralateral rotational behavior in response to administration of apomorphine. The animals were killed 2 weeks after dosing. Morphologically, there was loss of Nissl substance, glial reaction and loss of neurones in the substantia nigra, and neurochemically, there was dopamine depletion (Liou et al., 1996). In a study of the pathological effects of paraquat when administered directly into different parts of the rat brain, the micro infusion of paraquat (3.2, 16, 32 or 160 nmol) into the pars compacta of the substantia nigra produced neuropathological changes culminating in neuronal necrosis. A particular feature of paraquat neurotoxicity after its micro infusion into the substantia nigra (3.2 mmol/l at 1ml/min for 1 min) or into the ventral tegmental area (1.6 mmol/l at 1ml/min for 1 min), but not into other areas of the brain, was selective vulnerability of hippocampal CA3 neurons. This initially comprised a decrease in dendritic spines, which was followed by neuronal degeneration and cell loss. No damage was reported after micro infusion of paraquat into other areas of the brain near or distant from the infusion sites. In addition, similar neuropathological alterations occurred in other nondopaminergic areas. The authors concluded that paraquat possesses marked neurotoxicity that is not selective for dopaminergic neurons (Calò et al., 1990). In a study of neurotoxic effects after neonatal exposure to paraquat and MPTP, groups of mice (aged 10 or 11 days) were given vehicle (water), paraquat, or MPTP by orally; MPTP was administered at a dose of 0.3 or 20mg/kg bw, and paraquat at a dose of 0.07 or 0.36mg/kg bw. Neonatal spontaneous motor activity was tested on day 18 in mice given paraquat at 0.36mg/kg bw. Adult spontaneous
motor activity was tested at ages 60 and 120 days. On day 125, the mice were decapitated and the contents of dopamine and serotonin and metabolites in striatum were analyzed. Acute toxicity was not observed in any of the groups. No respiratory distress or motor performance dysfunction was seen on day 18 in mice given paraquat at 0.36mg/kg bw. The results of behavioral tests carried out at age 60 days showed a marked hypoactive condition in the mice given paraquat (at both doses) or MPTP (at both doses). At age 120 days, the hypo activity persisted and appeared even more pronounced. Reduced striatal content of dopamine and metabolites was seen in the striatum with both compounds, but concentrations of serotonin were unaffected. The effect was greater at the higher doses (Fredriksson et al., 1993).

2.5 Drosophila AS A MODEL OF NEUROTOXICITY

Different animal models from yeast to C. elegans have been used to identify the factors that influence risk and reproduced the key pathological features of the major neurodegenerative disease and neurotoxicity (Jason and Greenamyre, 2011). In the last decade, a suitable animal model of dopaminergic neuronal damage caused by pesticides such as paraquat, has been identified in the form of D.melanogaster (Coulom et al., 2004). In fact, the fly shows parkinsonian symptoms when exposed to paraquat and other neurotoxicants (Coulom et al., 2004). Locomotor activity in D. melanogaster has been recorded by many methods and in different contexts. To mention just a few, in an early study, spontaneous walking activity in an open field apparatus was used to select for active/inactive Drosophila strains (Connolly 1966, 1967). Later on, this method was used for single gene mutants with aberrant locomotor activity (Meehan and Wilson, 1987; Burnet et al., 1988; O’Dell and Burnet, 1988). In an object fixation task (Buridan’s paradigm) developed by Go¨tz, (1980), single flies with clipped wings are allowed to walk freely on a platform between two
opposing inaccessible landmarks (vertical black stripes). Different parameters, such as walking speed, straightness of walk, walking activity, and time course of activity are extracted from a video recording (Goetz, 1989; Strauss et al., 1992; Strauss and Heisenberg, 1993). Locomotor activity of flies has also been extensively analyzed with respect to circadian rhythms. Walking activity in these recordings is monitored in the range of hours to days and is evaluated for circadian and ultradian oscillations (Konopka and Benzer, 1971). Foraging is another example of locomotion. In this case, the distance covered by a larva or adult fly away from feeding site in a certain amount of time is the parameter of interest (Sokolowski, 1980; de Belle and Sokolowski, 1987; Pereira and Sokolowski, 1993).

On the basis of the above articles reviewed and reported observations experimental studies of paraquat and malathion in the current proposal were done. Clinical and experimental reports had increased the interest that environmental toxin, “Paraquat and organophosphate Malathion” appears to be a promising tool to study the neuronal cell death in vivo and in vitro in D.melanogaster.