CHAPTER 2
LITERATURE REVIEWS

2.1 Bird chilli (*Capsicum frutescens* Linn.) (Owens, 2003)

Name: capsicum, bird eye chilli, bird chilli, chilli

Origin: Tropical Central America

Distribution: Grown commercially in all tropical and sub-tropical countries

Historians agree on new world origin of *Capsicum.* Originated in South America, the crop was taken to old world by early explorers since 1585. Bird chilli is widely produced in India, Thailand, Vietnam and Malaysia. Bird chilli has a very high capsicum content, which is 100,000 to 225,000 Scoville Heat Units, and therefore yields a high market price in export markets and experiences less price volatility (Matthew, 2009).

Botanical family of chilli is Solanaceae. Indispensible throughout tropical Asia today, the chilli is not a native but was introduced from the Americas by the Portuguese and Spanish. Before the advent of chilli, black pepper was used to give a pungent flavor to food. During the past four centuries, the chilli has flourished and today is found in an almost bewildering variety of shapes sizes and pungencies throughout the region.

Generally speaking, the two most common varieties found in Asia are the finger-length chilli (*C. annuum* cv. group *longum*), sold either green (unripe) or red (ripe), and the fiery little bird chilli (*C. frutescens*). There are also mild, fat, long chilli; small round chilli; yellow or creamy white chilli; arrow-shaped chilli, pale orange chillies-the range seems endless. Parts of bird chilli are shown in Figure 2.1.

The hottest part of the chilli is the seeds; to reduce the pungency of a dish without losing the chilli flavor, discard some or all of the seeds are discarded before using. One should take care when preparing chilli as the juice sensitive areas (Hutton, 1998).

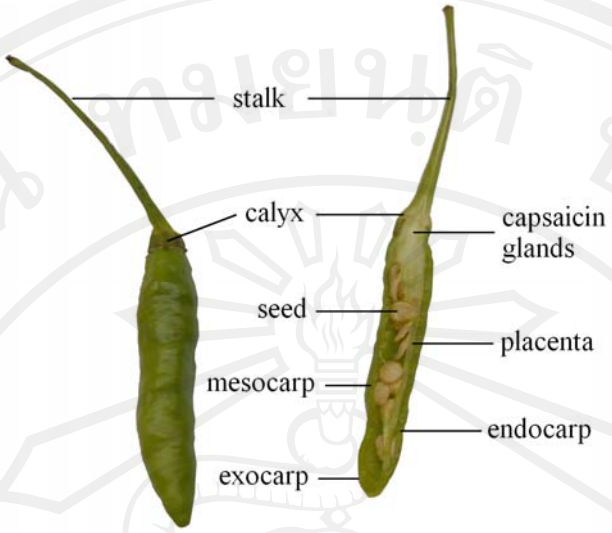

Figure 2.1 Parts of bird chilli.

Climate

Chilli is a crop of warm humid tropics or subtropics and is able to grow from sea level to 600 m above sea level. The crop cannot tolerate frost. Compared to tomato and brinjal, chilli can tolerate shade to some extent. Medium pungent chilli, cultivated for green chilli and dry chilli purpose, prefers a temperature of 20 - 30 °C for growth. Low pungent capsicum prefers a lower temperature of 17 - 23 °C. High pungent *C. frutescens* and *C. chinense* come up well in high rainfall. Though the crop prefers high humidity for its growth, fruit set and yield, incidence of fungal diseases particularly mildews and anthracnose is more under high humid conditions (Owens, 2003).

2.1.1 Nutritive value and medicinal use of chilli

Chilli is one of richest sources of vitamin C and its content is more than of tomato. Fruits accumulate maximum ascorbic acid when it turns to maturity and it ranges from 100 to 320 mg/100 g of fruits. Active principle for pungency is capsaicin (8-methyl-N-vanillyl-6-nonenamide) and its content in Indian varieties ranges from 0.002 to 1.86 %. Capsaicin is a country irritant. The principal coloring pigment of dried chilli is a carotenoid pigment such as capsanthin (Gopalakrishnan, 2007). Chilli nutrition is shown in Table 2.1.

Table 2.1 Bird chilli nutrition (Amusa *et al*., 2004).

Composition	Content (per 100 g fresh weight)
Calorie (Unit)	94.00
Moisture (%)	74.00
Protein (g)	4.10
Fat (g)	2.30
Carbohydrate (g)	18.00
Fiber (g)	6.00
Phosphorus (mg)	101.00
Calcium (mg)	58.00
Iron (mg)	9.00
β-carotene (mg)	7.14

Culinary use

Capsicums are a popular vegetable eaten raw in salads or cooked in many Asian dishes. Chillies are eaten raw or used in cooking as a condiment for flavorings. They are also used as herbal medicine to treat poor circulation, fever and colds, and digestive disorders. Chillies have also been used as an insecticidal spray (Owens, 2003).

2.1.2 Diseases and pests of chilli

The most common diseases and pests to chilli plants are listed below, along with symptoms to look out for and the best ways of ridding your chillies of the diseases (Table 2.2) and pests (Table 2.3).

Table 2.2 Chilli diseases (Department of Agriculture, 2008).

Disease	Cause	Symptoms	Control
Anthracnose (Figure 2.2)	*Colletotrichum capsicii* and *Colletotrichum piperatum*	Initially small water soaked spots appear on fruits which later become sunken and dark in color with numerous acervulus in concentric rings. Affected fruits rot and fall down. In dieback, braches show necrosis from top to down.	Benomyl 50%WP 0.5 - 1 g/l or Prochloraz 50%WP 0.5 - 1 g/l of water at 7 days (Than *et al*., 2008)
Powdery mildew	*Oidiopsis sicula* and *Leveillula taurica*	Disease first shows on older leaves. Chlorosis, necrotic, brown spots on upper leaf surface. Leaves curl upward. Premature defoliation. Sun scald as a result of leaf drop.	Flusilazole 47%EC 0.5 ml/l of water at 7 days
Phytophthora Root rot (chilli wilt)	*Phytophthora capsici*	Fruit become infected when wet humid conditions persist for several days	Avoid poorly drained, heavy soils
Leaf spot (Frog eye)	*Cercospora capsicii* and *Alternaria* sp.	Small brown circular leaf lesions that have a watery appearance. Excessive leaf drop may occur in common infestations.	Bavistin 1 - 1.5 g/l Mancozeb 80%WP 1.5 - 2.5 g/l Benomyl 50 %WP 0.5 - 1 g/l carbendazim 50%WP 0.5 - 1 g/l of water

Table 2.3 Chilli pests (Department of Agriculture, 2002).

Pest	Insecticides			Ratio
	Common name	LD_{50}	Commercial name	
Caterpillar (*Spodoptera mauritia*) (Figure 2.3)	Deltamethrin	135	Decis 3%EC	1 g/l of water
Chilli thrips (*Scirtothtips dorsalis*) (Figure 2.4)	Carbaral	300	Sevin 85%WP	1 - 1.5 g/l of water
	Prothiofos	925	Tokuthion 50% EC	1 - 1.5 g/l of water
	Methiocarb	100	Mesurol 50%WP	1 - 1.5 g/l of water
Chilli broad mite (*Polyphaotarsonemus latus*)	Wettable sulfur	3,000	Thiovit 80%WP	3 - 4 g/l of water
			Ecosulf 80%WP	3 - 4 g/l of water
	Abamectin	10	Vertimec 1.8%EC	1 - 1.5 g /l of water
Cotton bollworm (*Helicoverpa armigera*)	Cypermethrin	472	Parzon 6.25%/ 22.5%EC	2.5 ml/l of water

Figure 2.2 Anthracnose symptoms on chilli fruits (Than *et al*., 2008).

Figure 2.3 Caterpillar and symptoms of chilli (World of chillies, 2009).

Figure 2.4 Chilli thrip (Ludwig and Bográn, 2007).

2.1.4 Harvesting and yield

Chilli can be grown all year. Harvesting capsicums can be picked green as soon as they are a suitable size (about 12 weeks) or they are left to mature and turn red. Chillies are picked at the required color stage.

Chilli is harvested by hand picking and harvesting period extends up to two months. Farmers usually take one or two harvest for green chilli purpose even if crop is raised for dry chilli purpose. Yield of fresh green chilli is 3 - 4 times more than that of fresh red ripe chilli and 6 - 10 times than that of dry chilli.

Quality and pungency are determined by oleoresin and capsaicin contents. Inner pericarp contains 90 % pungency. Yield of oleoresin in chilli varieties ranges from 8.0 to 17.5 %.

Varieties with good pungency, bright red color, good flavor, medium size fruits with thin pericarp, smooth and glossy surface, a few seeds in fruit and tight pedicel are ideal for dry chilli purpose. Fruits after harvesting are heaped in shade for 2 - 3 days and later dried to less than 10 % moisture level under direct sunlight for 5 - 15 days, depending on temperature and humidity.

2.1.5 Storage conditions (Owens, 2003)

Capsicums and chilli should not be stored for long periods, or with fruit such as tomatoes that produce ethylene. Storage conditions should be 7 - 13 ºC at 90 - 95 % relative humidity (RH) for a maximum period of two to three weeks.

2.2 Principles of insect-pest control (Krishiworld, 2012)

Once it is established that an insect is causing economic losses, it becomes necessary to control it.

The first principle underlying the control of an insect is its correct identification. When it is correctly identified, we can refer to the available information on the biology and the habits of the insect and determine its most vulnerable stage, the appropriate time and the most suitable method or methods to control it.

The choice of the proper method or methods of control becomes easier, when the above mentioned information becomes available in respect of a particular pest. Some of the important methods for control of insect pests are discussed below.

2.2.1 Physical and mechanical control

This is one of the oldest methods, and has been in use since time immemorial. It includes measures like, collection of egg masses and other inactive stages, the

removal of infested parts or whole plants, the beating of drums or tins or trenching. This method can prove to be effective during the initial stage of the pest incidence and when practiced as a concerted effort by a large number of farmers in a particular area. Physical control methods can be classified as passive methods (e.g. trenches, fences, organic mulch, mulches from artificial materials, particle films, inert dusts, trapping, oils and surfactants and soaps) and active methods (mechanical, thermal, electromagnetic radiation, miscellaneous treatments and combination of methods) (Vincent *et al*., 2003).

2.2.2 Cultural control

This is a preventive method which is inexpensive and may prove more effective and efficient, if employed after acquiring a thorough knowledge of the life history and habits of a pest. Deep plugging after harvesting the crop (to expose the hiding and resting insects), the removing and destroying of the suitable and other trash, adjusting the time of sowing (to avoid the peak incidence period), clean cultivation, the removal of alternative wild hosts, catch crops and suitable rotations are some of the important measures included under this method of control.

2.2.3 Host plant resistance

Host plant resistance has generally been considered one of the components of cultural method, but because of its importance, it deserves an independent status as a major method of protecting crops against insect damage. The method involves the utilization of the inherent property of certain strains or varieties of crops, of being less infected or less damaged than other strains or varieties of same crop. Enormous economic benefits have been achieved by using this method in the case of crops, e.g. wheat, maize, cotton and alfalfa. It is realized that the success of this method requires the close co-operation of entomologists, geneticists and plant-breeders.

2.2.4 Biological control

Practically every crops pest has its natural enemies in the form of parasites, predators and disease causing organisms. The biological control involves a large scale multiplication of and liberation of such agents, or creating conditions under which the

naturally occurring agents can act effectively. This type of control cannot be undertaken by individual farmers and has necessarily to be carried out by specialized agencies. Some very outstanding successes have been achieved by using this method, but the method suffered a set-back owing to the large-scale and in discriminated use of insecticides. The approach at present is to evolve methods by which the biological and chemical methods can be integrated, so that the harmful effects of insecticides do not interfere with the activities of the natural enemies.

2.2.5 Legislative or regulatory method

Legislative or regulatory method is a method mainly employed to prevent the introduction of pests from other countries or to prevent the spread of a pest from one area to another. The method is operated through specific regulations known as plant-quarantine laws.

2.2.6 Chemical control

The method has become so popular that most of the cultivators and extension workers speak only about insecticides, whenever the question of insect control is raised. The main reason for its popularity is the spectacular and immediate results obtained by the use of such chemicals. Hundreds of insecticides are one available to control different insect pests. These are mainly used as dusts, sprays or granules on the crops; dust and granules can also be incorporated into the soil for the control of soil inhabiting insects. In recent years, the soil application of some of the systemic insecticides has proved to be effective in controlling insect attacking crops in the early stages of their growth.

Some of the serious limitations of the insecticides have been highlighted in recent years. Out of these, the problems of insecticides residues on crops and other products used as food and fodder and on pollution of environment have been agitating the minds of people in developed and developing countries of the world. This realization has resulted in certain extremist quarters demanding the banning the use of the most of the insecticides. However the sensible approach suggested and generally accepted is for a judicious and restricted use of insecticides. It is strongly felt that in

the near future, there is no possibility of replacing the chemical method of controlling insects entirely by any other method.

National Bureau of Agricultural Commodity and Food Standard (2008) reported the maximum residue limits (MRLs) of pesticide residue in chilli (Table 2.4).

Table 2.4 Pesticide residue in chilli (National Bureau of Agricultural Commodity and Food Standard, 2008).

Pesticide	Maximum residue limits: MRLs (mg/kg)
Chlorpyrifos	0.5
Carbaryl	0.5
Carbendazim/benomyl	2
Carbosulfan	0.5
Cypermethrin	1
Deltamethrin	0.5
Dithiocarbamates	2
Prothiofos	3
Profenofos	5
Phosalone	1
Malathion	0.1
Methomyl	0.7
Abamectin	0.02
Ethion	3

2.3 Concerns about pesticides

Concerns raised for the last 35 years have been of acute toxicity and chronic health problems. For years we have known that pesticides kill and permanently harm people who work directly with them. The acute effects of many pesticides are well documented; impacting the liver, kidneys, lungs, skin, eyes and brain. Long-term chronic effects on humans include a whole series of cancers, liver and kidney failure, sterility, neurological disorders and birth defects. We have known that pesticides

which take a long time to break down in the environment accumulate in organism as they move up to the food chain (Egendorf, 2000).

2.4 Chlorpyrifos

Chlorpyrifos has been used for chilli production by reducing insect's populations. While the inherent toxicological properties of pesticides allow them to control pests in agriculture, there is significant concern about the potential risks posed by chlorpyrifos to the environment and humans. Thai Agricultural Commodity and Food Standard (2006) reported that imported countries detected chlorpyrifos in chilli over MRLs was rejected. Chlorpyrifos is used in a formulated form as a broad spectrum insecticide (organophosphorus; OPs) for the control of *Coleoptera*, *Diptera*, *Homoptera* and *Lepidoptera* in soil or on foliage in a wide range of crops. Chlorpyrifos use for crop protection is extensive covering a wide variety of horticultural and field crops. The mode of chlorpyrifos action is non-systemic, and exposure of insects to the active (via contact, ingestion and/or inhalation) affects the nervous system by inhibiting the activity of acetyl cholinesterase (National Registration Authority for Agricultural and Veterinary Chemicals, 2000).

2.4.1 Chemical and properties of chlorpyrifos

Chlorpyrifos degrades a variety of compounds. A primary metabolite (breakdown product) in water, air, soil, plants and animals is 3,5,6-trichloro-2-pyridily taken up by plants than is chlorpyrifos.

The chemical structures (Figure 2.5) and physico-chemical properties (Table 2.5) of chlorpyrifos are shown below.

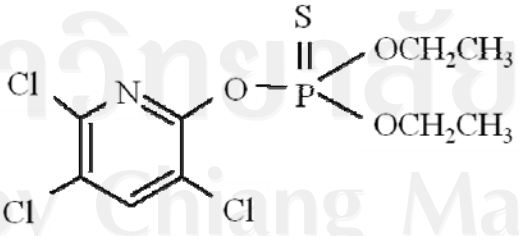

Figure 2.5 Chlorpyrifos structure**.**

Table 2.5 Physico-chemical properties of chlorpyrifos (Odenkirchen and Eisler, 1998).

Variable	**Data**
Common name	Chlorpyrifos
Chemical name	Phosphorothioic acid O, O-diethyl O- (3,5,6 - trichloro-2-pyridinyl) ester
Alternate names	CAS 2921-88-2; Dursban; Lorsban; Dowco 179; ENT 27311; Trichlorpyrphos; Brodan; Eradex; Killmaster; Pyrinex; Chlorpyriphos-ethyl
Primary uses	Insecticide, acaricide
Empirical formula	$C_9H_{11}Cl_3NO_3PS$
Molecular weight	350.57
Physical state at 25 °C	White granular crystalline solid
Melting point	41.5 - 43.5 °C
Vapor pressure at 25 °C	1.87×10^{-5} mm Hg
at 35 °C	8.87×10^{-5} mm Hg
Heat of sublimation	26,800 cal/mol
Solubility in water, 23 - 25 °C	0.4 - 2.0 mg/l
in methanol, 25 °C	450.0 g/kg (Hartley and Kidd, 1983)
in isooctane, 25 °C	790.0 g/kg (Hartley and Kidd, 1983)
in acetone, 25 °C	6,500 g/kg (Hartley and Kidd, 1983)
Log n-octanol/water partition coefficient	5.2

2.4.2 Mode of action

Chlorpyrifos is organophosphate insecticide. It is directly toxic to the nervous system. It is caused by the inhibition of enzyme acetylcholinesterase which results in the accumulation of the neurotransmitter, acetylcholine, at the nerve endings. This results in excessive transmission of nerve impulses, which causes mortality in the target pest. This reaction is also the mechanism by which high levels of organophosphate insecticides can produce toxic effects in mammals (Dow AgroSciences, 2003).

2.4.3 Acute toxicity

Symptoms of acute chlorpyrifos poisoning in humans include headache. nausea, dizziness, muscle twitching, weakness, increased sweating and salivation and fluid filled lungs (Cox, 1994). Chlorpyrifos toxicity and its categories are shown in Table 2.6 and 2.7.

Table 2.6 Chlorpyrifos properties and toxicity (Pesticide Properties Database, 2011).

Variable	**Data**
Soil organic carbon/water partition coefficient	13,600
Half life in soil	30 days (Vogue *et al*., 1994)
in plant foliage	less than 1 - 7 days (Ware *et al*., 1993)
Toxicity	
Acute oral LD_{50} in rat	64 mg/kg (High)
Dermal LD_{50} in rat	more than 1,250 mg/kg
Inhalation LC_{50} in rat	0.1 mg/l
WHO Classification	II (Moderately Hazardous)
US EPA Classification (formulation)	II (Warning - Moderately toxic)
ADI - Acceptable Daily Intake (mice)	0.01 mg/kg of body weight/day
AOEL - Acceptable Operator Exposure Level - Systemic (rat)	0.01 mg/kg of body weight/day

Table 2.7 Chlorpyrifos toxicity categories (*Signal Word*) (U.S. Environmental Protection Agency, 1997).

Category	High toxicity (Danger)	Moderate toxicity (Warning)	Low toxicity (Caution)	Very low toxicity (Caution)
Oral LD_{50}	Less than 50 mg/kg	50 -500 mg/kg	500 -5000 mg/kg	Greater than 5000 mg/kg
Inhalation LC_{50}	Less than 0.2 mg/l	0.2 - 2 mg/l	2 - 20 mg/l	Greater than 20 mg/l
Dermal LD_{50}	Less than 200 mg/kg	200 -2000 mg/kg	2000 -5000 mg/kg	Greater than 5000 mg/kg
Eye effects	Corrosive	Irritation persisting for 7 days	Irritation reversible within 7 days	No irritation
Skin Effects	Corrosive	Severe irritation at 72 h	Moderate irritation at 72 h	Mild or slight irritation at 72 h

2.4.4 Environmental levels and human exposure

1) Water

Chlorpyrifos was not detected in surveys of municipal and private drinking-water supplies in Canada between 1971 and 1986 (Health Canada, 1989). U.S. Environmental Protection Agency (1998) has reported detecting chlorpyrifos in surface waters, with the majority of results being less than 0.1 μg/l and with a maximum reported concentration of 0.4 μg/l. It was detected in groundwater in less than 1 % of the wells tested, with the majority of measurements being less than 0.01 μg/l.

2) Food

Chlorpyrifos was detected in only 49 of 6391 domestic food samples in the USA, 94 % of which had concentrations below 2.0 mg/kg (Hundley *et al*., 1988). Based on a US market basket survey, the average daily intake of chlorpyrifos was estimated to be 0.241 μg (Gartrell *et al*., 1986). Estimated theoretical maximum daily

intakes for the 5 food regional diets, based on existing maximum residue limits, were in the range of 6 - 30 % of the acceptable daily intake (ADI) (FAO/WHO, 2000).

2.4.5 Kinetics and metabolism in laboratory animals and humans

After oral administration to rats, radiolabelled chlorpyrifos was rapidly and extensively absorbed (up to about 90 % of the dose) and eliminated, predominantly in urine (68 - 93 %) and faeces (6 - 15 % of the dose), within about 72 h of administration. The urinary metabolites included the glucuronide (about 80 %) and sulfate (about 5 %) conjugates of chlorpyrifos and 3,5,6-trichloro-2-pyridyl phosphate (TCP; about 12 %). The tissue concentrations of residues of chlorpyrifos were very low (generally less than 1 mg/kg) within 72 h of dosing. The longest half-time of residues in rats was 62 h in fat, and low levels were also detected in the fat of several other species and in the milk of goats. In humans who were poisoned with chlorpyrifos formulations, diethylphosphorus metabolites were excreted in the urine by first-order kinetics, with an average elimination half-time of 6.1 ± 2.2 h in the fast phase and of 80 ± 26 h in the slow phase. In volunteers, the time to maximal concentration of TCP in the blood was 0.5 h after oral dosing and 22 h after dermal treatment, but the elimination half-time by both routes was 27 h, and the percentage of the administered dose recovered from the urine was 70 % after oral dosing and 1.3 % after dermal administration. Chlorpyrifos is rapidly metabolized by mixed-function oxidases to the highly reactive chlorpyrifos oxon by oxidative desulfuration. The oxon can be deactivated by hydrolysis to diethylphosphate and 3,5,6-trichloropyridinol, while a minor reaction pathway is hydrolysis to monoethyl 3,5,6-trichloro-2-pyridinyl phosphorothioate (FAO/WHO, 2000).

2.4.6 Effects on laboratory animals and *in vitro* test systems

The lowest oral lethal dose fifty (LD_{50}) value was 96 mg/kg of body weight (range 96 - 475 mg/kg of body weight) in rats and 100 mg/kg of body weight (range 100 - 150 mg/kg of body weight) in mice. Female rats were generally more sensitive to the acute effects of chlorpyrifos than males. The signs of acute intoxication with chlorpyrifos were consistent with cholinesterase inhibition. The acute dermal LD_{50} of

chlorpyrifos was more than 2000 mg/kg of body weight in rats and more than 1200 mg/kg of body weight in rabbits (FAO/WHO, 2000).

WHO (1999) has classified chlorpyrifos as “moderately hazardous”. Chlorpyrifos was irritating to the eye and skin of rabbits, but it did not sensitize the skin of guinea-pigs in Magnusson-Kligman maximization or Buehler tests. In short-term studies, the no observed adverse effect level (NOAEL) for inhibition of erythrocyte cholinesterase activity was 0.03 mg/kg of body weight/day in dogs and 0.1 mg/kg of body weight/day in rats. The NOAEL for inhibition of brain cholinesterase activity was 1 mg/kg of body weight/ day in dogs and rats. The signs of toxicity were largely limited to cholinergic signs and decreased body weights and/or food consumption. The NOAEL for these effects in short-term studies was 1 mg/kg of body weight per day in rats, and the NOAEL for clinical signs was 3 mg/kg of body weight per day in dogs. In rats, the NOAEL for increased fatty vacuolation of the adrenal zonal fasciculata and changes in haematological and clinical chemical parameters was 5 mg/kg of body weight/day. When rats received chlorpyrifos dermally for 21 days, the NOAEL for inhibition of cholinesterase activity in erythrocytes and brain was 5 mg/kg of body weight per day. In long-term studies, inhibition of cholinesterase activity was again the main toxicological finding in all species. In rats, the NOAEL was 0.1 mg/kg of body weight/day for inhibition of erythrocyte acetylcholinesterase activity and 1 mg/kg of body weight per day for inhibition of brain acetylcholinesterase activity, but clinical signs were not seen at doses up to 10 mg/kg of body weight/day, and the NOAEL for reduction in body weight was 1 mg/kg of body weight/day. In mice, erythrocyte and brain acetylcholinesterase activities were inhibited at 50 mg/kg, equal to 6.1 mg/kg of body weight/day, and the NOAEL was 5 mg/kg, equal to 0.7 mg/kg of body weight/day. Cholinergic signs and reductions in body weight were reported only at the highest dietary concentration of 250 mg/kg (equal to 32 mg/kg of body weight/day). Other treatment-related findings included effects on the liver in mice, with a NOAEL of 50 mg/kg (equal to 6.6 mg/kg of body weight/day), and increased adrenal weight in rats, with a NOAEL of 1 mg/kg of body weight/day. There was no treatment-related increase in the incidence of neoplastic lesions in any of the long-term studies. The meeting concluded that chlorpyrifos is unlikely to pose a carcinogenic risk to humans.

Chlorpyrifos was not genotoxic in an adequate range of studies *in vitro* and *in vivo.* The Meeting concluded that chlorpyrifos is not genotoxic. In multigeneration studies of reproductive toxicity in rats, the treatment-related effects of chlorpyrifos were limited to inhibition of cholinesterase activity, consistent with that seen in other short- and long-term studies, and cytoxicity, characterized by reduced pup viability, body weights and survival. No significant treatment-related clinical signs were reported. The NOAEL for inhibition of maternal acetylcholinesterase activity was 0.1 mg/kg of body weight/day for erythrocytes and 1 mg/kg of body weight/day for brain. The NOAEL for developmental toxicity was 1 mg/kg of body weight/day. No effects on reproductive parameters were observed at the highest dose tested, 5 mg/kg of body weight/day. In studies of developmental toxicity in mice, rats and rabbits, the maternal effects included inhibition of erythrocyte and/or brain acetylcholinesterase activity and cholinergic signs (lowest NOAEL, 1 mg/kg of body weight/day in rats and mice) and reductions in body weight and food consumption (lowest NOAEL, 2.5 mg/kg of body weight/day in rats). The observed fetal toxicity (lowest NOAEL, 2.5 mg/kg of body weight/day in rats) and developmental toxicity (NOAEL, 1 mg/kg of body weight/day in rats) were consistent with treatment-related maternal toxicity; there was no evidence of treatment-related malformations in any of the studies. There was no effect on cognitive function (learning, memory and habituation) in pups exposed to chlorpyrifos *in vitro* and for a period postpartum at doses up to and including the highest dose of 5 mg/kg of body weight/day, while inhibition of cholinesterase activity, decreased brain weight and delayed development were seen at lower doses, consistent with findings in other studies. In studies of delayed neurotoxicity, chlorpyrifos was given to chickens as either single or repeated doses. Significant inhibition of both cholinesterase and neuropathy target esterase activity was observed, and mild delayed neuropathy was seen in a number of studies; aggressive antidotal therapy was always necessary to allow at least some of the treated birds to survive. Despite the marked cholinergic toxicity of chlorpyrifos, there was no evidence that it caused delayed neurotoxicity, and there was no increase in the incidence of hisopathological lesions in the nerve tissues of birds treated at doses up to 10 mg/kg of body weight per day for up to 91 days. In a number of studies in rats given single doses of up to 100 mg/kg of body weight, repeated doses of up to 10

mg/kg of body weight/day for 4 weeks or repeated doses of up to 15 mg/kg of body weight/day for 13 weeks, there were no treatment-related neurological lesions or effects on cognition and no inhibition of neuropathy target esterase activity, although significant inhibition of erythrocyte, brain and peripheral tissue cholinesterase activity was seen at some doses. In a study that included a functional observational battery of tests, clinical signs of intoxication were observed after a single dose only when brain acetylcholinesterase activity was inhibited by more than 60 % or when whole-blood cholinesterase activity was inhibited by more than 80 %.

2.4.7 Effect on humans (FAO/WHO, 2000)

When chlorpyrifos was applied as a single dose of up to 5 mg/kg of body weight to the skin of volunteers for 12 h, erythrocyte cholinesterase activity was not significantly inhibited. Plasma cholinesterase activity was inhibited after 12 - 20 h dermal exposures to 5 mg/kg of body weight/day over 4 weeks or after three daily 12 h exposures to 25 mg/kg of body weight/day on consecutive days, but erythrocyte cholinesterase activity was not inhibited under any treatment regimen. A single oral dose of up to 1 mg/kg of body weight or repeated doses of up to 0.1 mg/kg of body weight/day for 9 days did not significantly inhibit erythrocyte acetylcholinesterase activity in volunteers. No clinical signs were observed in these studies. Inhibition of erythrocyte acetylcholinesterase activity was observed in a single female volunteer (of a group of six men and six women) given a single oral dose of 2 mg/kg of body weight. In a case of human poisoning with chlorpyrifos at an estimated dose of 300 - 400 mg/kg of body weight, significant inhibition of neuropathy target esterase in lymphocytes and of plasma and erythrocyte acetylcholinesterase activity was reported, with severe cholinergic signs, which required aggressive, extensive antidote therapy and artificial ventilation. Mild distal axonopathy consistent with organophosphate-induced delayed polyneuropathy was reported some weeks after the poisoning incident.

2.5 Strategies for pesticides removal

2.5.1 Washing with salt

The first step in the removal of pesticide residues from the food products is washing. Washing with 2 % of salt water will remove most of the contact pesticide residues that normally appear on the surface of the vegetables and fruits. About 75 - 80 % of pesticide reduces are removed by cold water washing. The pesticide residues that are on the surface of fruits like grapes, apples, guava, plums, mangoes, peaches and pears and fruity vegetables like tomatoes, brinjal and okra require two to three washings. The green leafy vegetables must be washed thoroughly. The pesticide residues from green leafy vegetables are removed satisfactorily by normal processing such as washing blanching and cooking (Krishnaswamy and Sesikeran, 2010).

2.5.2 Soaking in potassium permanganate

Potassium permanganate solutions effectively remove a variety of chemical pesticide residues from fruits and vegetables, making them safer to consume. Dilute solutions will degrade chlorinated solvents, polyaromatic hydrocarbons, phenolics, organo-pesticides and substituted aromatics. As one example, the International Information System for the Agricultural Sciences and Technology, an information management program started by the Food and Agriculture Organization of the United Nations (FAO) reported in 2010, that Leafy Chinese-Kale treated with a 0.001 % potassium permanganate solution effectively removed pesticide residues. Washing vegetables in potassium permanganate removed more pesticide residue than washing in water alone (Klinhom *et al.*, 2008).

2.5.3 Washing with detergent

Agricultural pesticides do not come off with water alone or farmers would not use them. Luckily, just adding washing-up liquid (detergent) to water and generously swishing the fruit or vegetables around for a couple of minutes can often lift off much of the pesticide residue. This can test by dipping organic grapes in water, and comparing this with dipping pesticide-laden grapes in water, and then in soapy water. The pesticide content is immediately obvious (Tomley, 2009).

2.5.4 Washing with vinegar

Some people swear by vinegar, and use one part vinegar to three parts water. This is great for removing bacteria, and may help break down wax, too. The editors of Cooks Illustrated magazine tested this theory by using four different methods to clean pears and apples: a vinegar and water solution (3 : 1, water : vinegar), antibacterial liquid soap, scrubbing with a stiff brush, and just using plain water. Not only did the vinegar mixture work the best, it was far, far better when measured for bacteria - it removed 98 % of bacteria, compared to just under 85 % for scrubbing. The quickest way to do this at home is to keep a bottle of vinegar with a spray-top the fruit or vegetables with vinegar, then rinse under a tap. Leave fruit or vegetables soaking for 10 - 20 min in a vinegar/water solution, then rinse (Tomley, 2009).

2.5.5 Using a commercial fruit cleaner

There are many commercial fruit cleaners available on the market, some of which are made up of 100 % natural produce - normally some form of citric acid. These claim to remove wax, pesticides and 99.9 % of bacteria (including *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, etc.) (Tomley, 2009).

2.5.6 Fruit and vegetable super-cleaner

A simple detergent or vinegar-based wash (see above), or make a super-wash, using either of the following mixes (Tomley, 2009)

1) 1 tablespoon of lemon juice, 2 tablespoons of baking soda, 1 cup (250 ml) of water. Put the mixture in a spray-topped bottle. Spray the fruit or vegetables, leave to sit for 5 - 10 min, then rinse well.

2) 1 tablespoon lemon juice, 2 tablespoons white vinegar (distilled works best), 1 cup (250 ml) water in a spray-topped bottle. Spray the fruit or vegetables, wipe and eat.

2.5.7 Rinsing with tap water

Rinsing fruits and vegetable prior to consumption reduces the amounts of pesticide residues. A short rinse in tap water reduces pesticide residues on many types of produce. The water solubility of pesticides does not play a significant role in the

observed decrease. The majority of pesticide residue appears to reside on the surface of produce where it is removed by the mechanical action of rinsing (Krol *et al*., 2000).

2.5.8 Blanching

A short treatment in hot water or steam applied to most of the vegetables. Certain pesticide residues can effectively be removed by blanching. But before blanching it is very important to thoroughly pre-wash the vegetables and fruits (Krishnaswamy and Sesikeran, 2010).

These strategies to reduce residual pesticides include washing with water, potassium permanganate and detergents etc. But this method causes water pollution and has a high cost with limited effectiveness. Nowadays, a person interested about green technology washing to reduce chemicals residue without requiring chemical input.

2.6 Advanced oxidation processes

Advanced oxidation processes (AOPs) are defined as processes that can generate radicals in a sufficient quantity to be able to oxidize majority the complex chemicals present in the effluent water. Some oxidation-reduction potential (ORP) of species used in water treatment is show in Table 2.8.

AOPs can be broadly defined as aqueous phase oxidation methods based on the intermediacy of highly reactive species such as (primarily but not exclusively) hydroxyl radicals ($^{\bullet}OH$) in the mechanism leading to the destruction of the target pollutant. Over the past 30 years, research and development concerning AOPs has been immense particularly for two reasons, namely (a) the diversity of technologies involved and (b) the area of potential application (Klavarioti *et al*., 2009).

Hydroxyl radicals are powerful oxidizing regents with an oxidation potential of 2.8 V. In addition, they react with most organic and many inorganic compounds, exhibiting faster rates of oxidation reaction as compared to that conventional oxidants (Gogate *et al*., 2002). Table lists the oxidation-reduction potential of the most used oxidant species in water treatment. Afterwards, a short explanation of the most important advanced oxidation process is given along with a detailed description of the ozonation process.

Table 2.8 Oxidation-reduction potential of chemical species used in water treatment (Hunsberger, 1977).

Chemical specie	Oxidation-reduction potential, E°(V)
Fluorine	3.0
Hydroxyl radical	2.8
Ozone	2.1
Hydrogen peroxide	1.8
Potassium permanganate	1.7
Hypochlorite acid	1.5
Chlorine dioxide	1.5
Chlorine	1.4
Oxygen	1.2

2.7 Ultrasonication (Suslick, 1994)

Ultrasound is simply sound pitched above human hearing. It has found many uses in many areas. At home, we use ultrasound for dog whistles, burglar alarms, and jewelry cleaners. In hospitals, doctors use ultrasound to remove kidney stones without surgery, to treat cartilage injuries (such as "tennis elbow"), and to image fetal development during pregnancy. In industry, ultrasound is important for emulsifying cosmetics and foods, welding plastics, cutting alloys and large-scale cleaning. None of these applications, however, take advantage of the effects that ultrasound can have on chemical reactivity.

Ultrasound has wavelengths between succession compression waves measuring roughly 10 cm to 10^{-3} cm. These are not comparable to molecular dimensions. Because of this mismatch, the chemical effects of ultrasound cannot result from a direct interaction of sound with molecular species. Nonetheless, the ultrasonic irradiation of liquids does produce a plethora of high energy chemical reactions. This occurs because ultrasound causes other physical phenomena in liquids that create the conditions necessary to drive chemical reactions. The most important of these is cavitations, the formation, growth, and implosive collapse of bubbles in a liquid. The dynamics of cavity growth and collapse are strikingly dependent on the local environment. Cavity collapse in a homogeneous liquid is very different from

cavitations near a liquid-solid interface, which will be considered later. As ultrasound passes through a liquid, the expansion cycles exert negative pressure on the liquid, pulling the molecules away from one another. If the ultrasound is sufficiently intense, the expansion cycle can create cavities in the liquid. This will occur when the negative pressure exceeds the local tensile strength of the liquid, which varies according to the type and purity of liquid. (Tensile strength is the maximum stress that a material can withstand from a stretching load without tearing).

Acoustic cavitations (tiny micro bubbles) are created when it reaches rarefaction cycle where a negative acoustic pressure is sufficiently large to pull the water molecules from each other (the critical molecular distance, radius for water molecules is 10^{-8} m). As a result, 'voids' are created in the liquid. On the other hand, the acoustic pressure is positive during compression cycle of ultrasonic wave to push molecules apart. Cavitation bubbles will grow over a few cycles by entrapping most of the vapor from the medium to reach a critical size before the implosion of the bubbles occurs (Figure 2.6). The radius of the bubble before collapsing when irradiated at 20 kHz is estimated to be in the order of several hundred micrometers. The time scale for the collapse of bubbles is less than 100 ns. The effective lifetime is less than 2 μs after which the start to collapse. In addition, the critical size of cavitation bubbles formed in water is inversely proportional to the frequency of the ultrasound. For instance, it was reported that the size of the cavities were within 100-170 μm when 20 kHz of ultrasonic irradiation was used while at 1 MHz, it was about 3.3 μm. In summary, the phenomenon of cavitation consists of the repetitive and distinct three steps: formation (nucleation), rapid growth (expansion) during the compression/rarefaction cycles until they finally reach a critical size. After that, they start to undergo violent collapse (implosion) in the liquid (Pang *et al*., 2011).

Cavitation is a phenomenon of formation, growth and collapse of microbubbles within a liquid that promotes the formation of $^{\bullet}$OH radical. Acoustic cavitation (ultrasonication) occurs by means of high frequency sound waves, while hydrodynamic cavitations take place due to pressure variations in the flowing liquid promoted by a change in the geometry of the flowing system. The mean advantage of the cavitation use is the non-introduction of chemicals in the medium. Moreover, no toxic byproduct is formed as in the case with chemical treatment using chlorine. The

suitability of this process could be real if the raw water supply is available with a considerable high pressure and its pressure needs to be reduced using pressure reducer (Jyoti and Pandit, 2001).

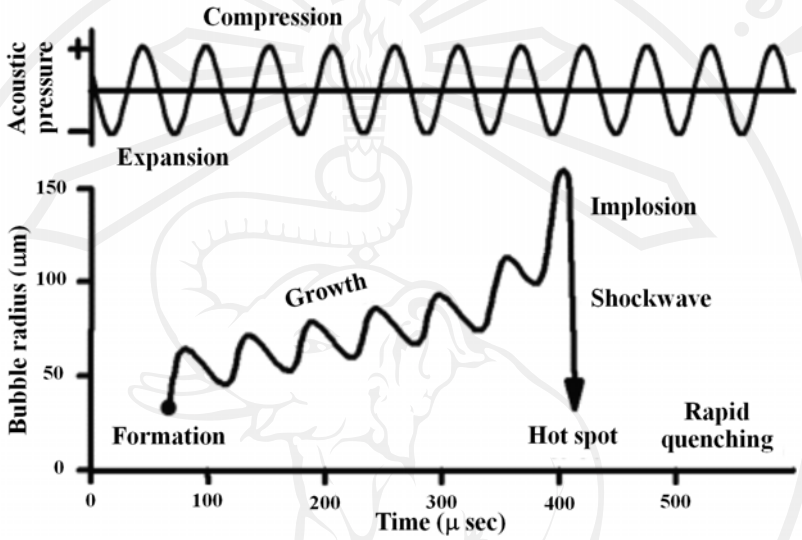

Figure 2.6 The origin of sonochemistry (Jyoti and Pandit, 2001).

2.7.1 The sonochemical hot-spot

Compression of a gas generates heat. On a macroscopic scale, one can feel this when pumping a bicycle tire, the mechanical energy of pumping is converted into heat as the tire is pressurized. The compression of cavities when they implode in irradiated liquids is so rapid than little heat can escape from the cavity during collapse. The surrounding liquid, however, is still cold and will quickly quench the heated cavity. Thus, one generates a short-lived, localized hot spot in an otherwise cold liquid. Such a hot spot is the source of homogeneous sonochemistry, it has a temperature of roughly 5,000 °C (9,000 °F), a pressure of about 1,000 atmospheres, a lifetime considerably less than a microsecond, and heating and cooling rates above 10 billion °C/s (Suslick, 1994). Cavitation bubble formation and collapse is shown in Figure 2.7.

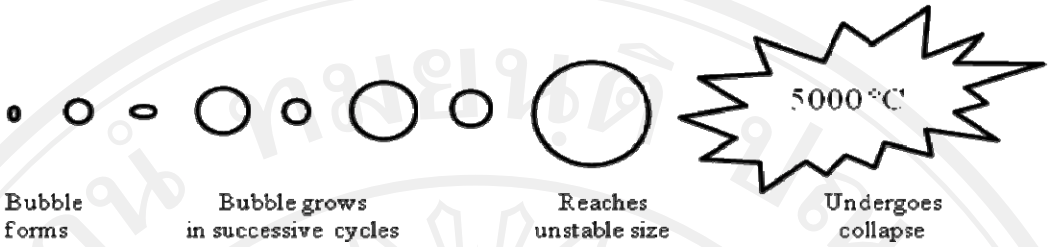

Figure 2.7 Cavitation bubble formation and collapse (Chowdhury and Viraraghavan, 2009).

2.7.2 Sonochemistry in homogeneous liquids

The chemical effects of ultrasound on aqueous solutions have been studied for many years. The primary products are molecular hydrogen (H_2) and hydrogen peroxide (H_2O_2). Other high-energy intermediates may include $^{\bullet}H$ (atomic hydrogen), $^{\bullet}OH$ (hydroxyl radical), $^{\bullet}OOH$ (hydroperoxyl radical) and e-(aq) (solvated electron).

The sonolysis of simple hydrocarbons creates the same kinds of products associated with very high temperature pyrolysis. Most of these products - H_2, CH_4 (methane), and the smaller 1-alkenes, derive from a well-understood radical chain mechanism. Relatively large amounts of acetylene (C_2H_2) are also produced, which is explained by the stability of this gas at very high temperatures (Suslick, 1994).

2.7.3 Ultrasonication for chemical degradation

Ultrasonication technology has been reported to be effective in reducing a variety of organic and inorganic contaminants (Weavers *et al.*, 1998). Its acoustic cavitations generate many hot spots with locally high temperatures and pressures and give rise to the sonolysis of H_2O molecules. Consequently, radical species (H, $^{\bullet}OH$, $^{\bullet}OOH$) are formed which lead to a direct destruction of chemicals in aqueous solution. Hoffmann *et al*. (1996) reported that ultrasonication at the frequency of 20 to 500 kHz was effective in rapid destruction of organic contaminants in water. Pétrier *et al.* (1998) found that the sonolysis at 20 and 500 kHz destructs volatile chloroaromatic hydrocarbons (1,2-dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, 1,3,5-trichlorobenzene, 1-chloronaphthalene). In addition, Wang *et al*. (2006) indicated that methyl parathion could be degraded by using the

ultrasonication at a frequency of 40 kHz. Song *et al*. (2005) also found that ultrasonication was an effective and practical method for the detoxification of microcystins in drinking water. Song and O'Shea (2007) reported that ultrasonication at 640 kHz effectively and rapidly degrade 2-methylisoborneol (MIB) and geosmin (GSM). Their study revealed that hydroxyl radical scavenger plays a major role in the degradation. Moreover, Matouq *et al.* (2008) used a high frequency of ultrasound wave at 1.7 MHz to decrease the concentration of diazinon in water.

Schramm and Hua (2001) studied ultrasonic irradiation of dichlorvos decomposition mechanism. It was found dichlorvos quickly decomposes when exposed to ultrasonic irradiation. In addition, the reaction intermediates and products including dimethyl phosphate, formate, carbon dioxide, chloride and phosphate. Chloride ion of dichlorvos rapidly accumulates for the O_2 and Ar/O_2 sparge gases, nearly reaching the maximum theoretical concentration of 1.0×10^{-3} M after 1 h of sonication at 161 W and 500 kHz. Tiehm and Neis (2005) observed sonochemical degradation at 3.2 MHz was increasing 2,3,5-trichlorophenol (TCP) decomposition corresponded to an increasing formation of chloride. Similarly, the chloride ions liberated after chlorpyrifos degradation under different types of radiation source and in dark were determined quantitatively (Muhamad, 2010). According to Yao *et al.* (2010) observed the anions from parathion degradation under ultrasonic irradiation, the sulphate concentration rapidly increased in the first 45 min and then stabilized, which corresponds to the sonochemical degradation curve of parathion. At the same reaction, an amount of nitrate was also detected. The concentration of nitrate increased steadily and reached a concentration after 120 min. The formation of nitrate was much slower than those of other anions possibly because of the sequential oxidation of nitrogen-containing species. Sonochemical degradation of chlorophenols is more efficient at low pH (Ku *et al*., 1997; Pétrier *et al*., 1998). Because at acidic pH diffuse more easily into the interfacial region of the cavitation bubble where the concentration of radicals is high (Tiehm and Neis, 2005).

2.8 Ozonation

2.8.1 Chemical and physical properties of ozone (Dantas, 2007)

Ozone (O_3) is, at ambient pressure and temperature, an irritating pale blue gas, heavier than air, very reactive and unstable. Moreover, at some conditions the ozone can be explosive and toxic. It can be neither stored nor transported, so it has to be generated in situ. The structure of molecular ozone is presents in Figure 2.8, where its two extreme forms of resonance are presented. Besides, the physical and properties of the ozone are presented in Table 2.9.

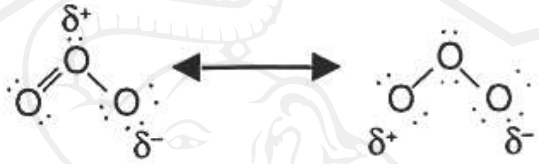

Figure 2.8 Molecular structure of ozone.

Table 2.9 Physico-chemical properties of ozone (Beltrán, 2004).

Property	Value
Molar mass	47.998 g/mol
Solubility in water	0.105 g/100 ml at 0 °C
Melting point	-251 °C
Boiling point	-112 °C
Critical pressures	54.62 atm
Critical temperature	-12.1 °C
Specific gravity	1.658 higher than air
	1.71 g/cm^3 at -183 °C
Density (1 atm and 0 °C)	2.144 g/l
Critical density	436 kg/m^3
Heat of vaporization	2,980 cal/mol[a]
Heat of formation	33,880 cal/mol[b]
Free energy of formation	38,860 cal/mol[b]
Oxidation potential	2.07 V[c]

[a]At the boling point temperature, [b]At 1 atm and 25 °C, [c]At pH = 0.

2.8.2 Ozone generation (Dantas, 2007)

Nowadays, numerous methods to produce ozone are known and a large amount of patents have been published. Nevertheless, three categories of ozone production stand out as the most important. Electrochemical, UV and discharge methods are those most used. Among them, the last one appears as the most common in water treatment.

In the discharge method, a continuous flow of oxygen or oxygen containing-gas (usually air) passes through the space between two electrodes separated by a dielectric material which is habitually glass made. When a voltage is supplied to the electrodes, a corona discharge is formed between the two electrodes, and the oxygen in the discharge gap is converted to ozone (Figure 2.9). So, oxygen molecules (O_2) are split into oxygen atoms (O) and then oxygen atoms combine with remaining oxygen molecules to form ozone.

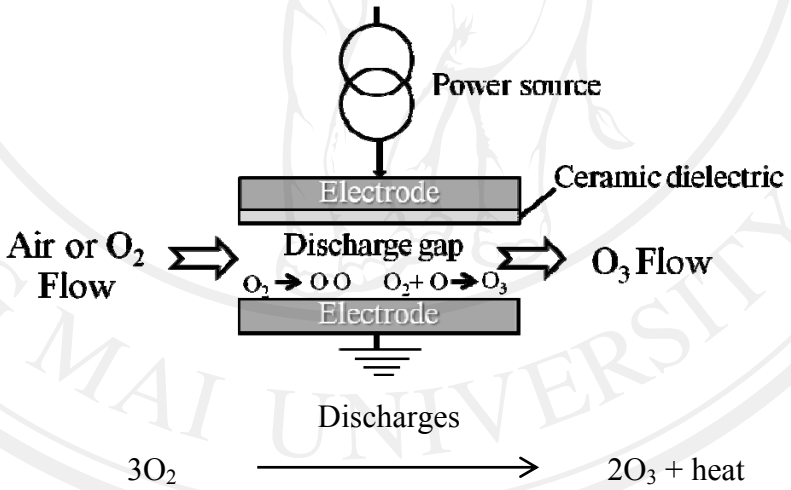

Figure 2.9 Ozone generator by corona discharge.

This type of ozone generation is based on a reversible reaction. As a result, the inverse reaction increases when temperature rise. Consequently, more oxygen molecules are formed.

2.8.3 Ozone decomposition in water (Dantas, 2007)

During ozonation, if the ozone is responsible for the substrate oxidation, disinfection and oxidation can be achieved simultaneously. However, if some compounds resistant to ozone are present in the medium, ozone has to be converted into $^{\bullet}OH$ radicals in order to achieve accepted levels of substrate removal. When the ozonation is carried out by means of the radical pathway, a reduction of disinfection power occurs (Gunten, 2003). Thus, a careful evaluation of the overall process is essential to optimize the system. With regard to better control of the ozonation process, the understandings of the diverse reactions that have influence on the ozonation mechanism constitute an important tool for a proper operation.

In aqueous medium, the anion OH^- promotes the decomposition of ozone with subsequent reaction chains that lead to the formation of the $^{\bullet}OH$ radical. The reactions involved in the ozone decomposition pathway are complex and can suffer influence of several experimental factors and concentration of present species. An explanation, step by step, of the ozonation reactions (decomposition in water) is attempted as follows:

The reaction between ozone and the hydroxide ion produces the ion superoxide radical and the hydroperoxide radical.

$$O_3 + OH^- \longrightarrow O_2^{\bullet-} + HO_2^{\bullet} \qquad k = 70\ M^{-1}\ s^{-1} \qquad \text{equation 1}$$

The ozone reacts with the hydroperoxide radical producing hydroxyl radical. In addition, the anion O_3^- formed by the reaction between the ozone and the anion superoxide radical decomposes quickly in hydroxyl radicals.

$$O_3 + HO_2^{\bullet} \longrightarrow OH^{\bullet} + O_2^{\bullet-} + O_2 \qquad k = 2.8\ 10^6\ M^{-1}\ s^{-1} \qquad \text{equation 2}$$

$$O_3 + O_2^{\bullet-} \longrightarrow O_3^{\bullet-} + O_2 \qquad k = 1.6\ 10^9\ M^{-1}\ s^{-1} \qquad \text{equation 3}$$

For $pH \leq 8$

$$O_3^{\bullet-} + H^+ \longleftrightarrow HO_3^{\bullet-} \qquad K^+ = 5\ 10^{10}\ M^{-1}\ s^{-1} \qquad \text{equation 4}$$

$$K^- = 3.3\ 10^2\ s^{-1}$$

$HO_3^{\bullet} \longrightarrow OH^{\bullet} + O_2$ $k = 1.4\ 10^5\ s^{-1}$ equation 5

For pH ≥ 8

$O_3^{\bullet -} \longleftrightarrow O^{\bullet -} + O_2$ $K^+ = 2.1\ 10^3\ s^{-1}$ equation 6

$K^- = 3.3\ 10^9\ s^{-1}$

$O^{\bullet -} + H_2O \longrightarrow OH^{\bullet} + OH^-$ $k = 10^5\ s^{-1}$ equation 7

Additionally, the ozone can react with hydroxyl radicals.

$O_3 + OH^{\bullet} \longrightarrow OH_2^{\bullet} + O_2$ $k = 10^5\ s^{-1}$ equation 8

Although ozonation constitutes a chemical oxidation, it exhibits lower rate of degradation when compared to the processes based on free radicals (Arslan and Balcioglu, 2001; Gogate *et al*., 2002). However, free radicals are generated when ozone is used in combination with hydrogen peroxide, UV/sun light or ultrasound (Weavers *et al*., 2000; Fung *et al*., 2000; Gogate *et al*., 2002). Another fact that be taken into account is the decomposition of ozone in water, which induces the formation of $^{\bullet}$OH. This decomposition is catalyzed by OH^- species, making the pH of the medium an important variable in the determination of kinetics and reaction pathways. The two agents involved in the process, play different parts in the process of process of ozonation. While disinfection is made by the molecular ozone, both agents are responsible for the oxidation process. The ozone is a selective oxidant, while the $^{\bullet}$OH radical is not selective and it is very important for the oxidation of compounds that have resistant to ozone. The pH of the medium affects the double action of ozone on the organic matter, that may be a direct or an indirect (free radical) ozonation pathway. As commented before, these different reaction pathways lead to different oxidation products and are controlled by different kinetic models. At low pH, ozone mainly reacts with compounds with specific functional groups through selective reactions, such as electrophilic, nucleophilic or dipolar addition reactions (direct pathway). On the other hand, at basic conditions, ozone decomposes yielding hydroxyl radicals, which are high oxidizing species that react in a non-selectively way

with a wide range of organic and inorganic compounds in water (indirect ozonation) (Dantas, 2007).

2.8.4 Ozone for chemical degradation

The FDA considers ozone with the status of "Generally Recognized As Safe" (GRAS), as a sanitizer and disinfectant for food (Graham, 2001). Ong *et al*. (1996) found that chlorine and ozone treatment decreased both captan and formetanate hydrochloride which were rapidly degraded in 50 and 500 mg/l chlorine solutions at pH 7 and 10.7 respectively and ozonation also effects the degradation of the pesticides generally which increases at higher pH and temperature on fresh apple. Ku *et al*. (1998) investigated the decomposition of diazinon in aqueous solution by ozonation at various pH values, gaseous ozone dosages, gaseous pressure, alkalinity levels and solution temperatures. They found that nearly complete decomposition of diazinon could be achieved within 1 h. It was found that the formation of sulfate ions was stoichiometrically detected and was accomplished at a very early period of the decomposition of diazinon as same chlorpyrifos structure, then the formation of the phosphate ion (PO_4^{3-}), and nitrate ions (NO_3^-) was to be proceeded sequentially after about 30 min of reaction time. The sulfur groups are easily detached from diazinon molecules and are oxidized to sulfate ions (SO_4^{2-}). Besides, Tango and Gagnon (2003) reported that ozonated water showed reduction of 15 % total organic carbon and less than 25 μg/l bromate concentration. Chitsamphandhvej *et al*. (2003) reported that the use of ozone was also able to reduce malathion and methyl parathion residues in cabbage. Whangchai *et al.* (2004b) suggested that ozonation technology was beneficial for the destruction of toxic metabolites in shrimp grow-out ponds by decreasing nitrite and H^+ from nitrite oxidation. Moreover, ozonation was a promising method for the reduction of sulfite residues in pericarp and aril of fresh longan (Whangchai *et al.*, 2004a). Furthermore, the effect on aflatoxin reduction was also reported by Inan *et al*. (2007) who found that aflatoxin B_1 content was reduced in flaked and chopped red peppers (*Capsicum annuum*) by 80 and 93 % at the rate of 33 and 66 mg/l for 60 min, respectively after ozone exposures. Wu *et al*. (2007) demonstrated that using low level of dissolved ozone (1.4 mg/l) effected 60 - 99 % oxidation of methyl-parathion, cypermethrin, parathion and diazinon in aqueous

solution forms in 30 min. Whangchai *et al*. (2009) also reported that the content of aflatoxin in senna (*Cassia angustifolia*) was reduced after exposure to ozone at a concentration of 100 mg/l for 120 min. Coelho *et al*. (2009) studied intermediates of diclofenac during degradation by ozonation. They found that ozonation is an efficient treatment for diclofenac degradation. In addition chlorine ions and nitrogen as ammonia were released into the aqueous solution during diclofenac degradation. Similarly, the sulfur group of diazinon was released from the cyclic structure early during the decomposition reaction by the direct oxidation of ozone molecules (Ku *et al*., 1998).

2.9 Combination of ultrasonication and ozonation for chemical degradation

The main advantage of ultrasonication combined with ozonation is the increased mass transfer of ozone from the gas phase to the bulk solution to react with substrate by mechanical effects of ultrasound. It has been reported that cavitation bubbles can more readily induce O_3 decomposition under mild conditions. Decomposition of O_3 yields molecular O_2 and triplet atomic oxygen ($^{\bullet}O$) (reaction [1]). They can also contribute to increase the formation of $^{\bullet}OH$ (reaction [2]) (Pang e*t al*., 2011).

$$O_3 \xrightarrow{\text{ultrasonication}} O_2 + {}^{\bullet}O \qquad [1]$$

$$^{\bullet}O + H_2O \longrightarrow 2{}^{\bullet}OH \qquad [2]$$

Therefore, combined ultrasonication and ozonation is an effective oxidation method compared to its individual oxidation methods as two •OH are formed for every O_3 molecule consumed (reaction [2]). These reactive radicals may react with the target substrates and their initial degradation by-products. Hence, major portion of the dissolved O_3 is efficiently decomposed by ultrasonic irradiation.

In the combined system, ultrasonication and ozonation were believed to enhance each other in the oxidation of organic pollutants (Xiong *et al*., 2011). A synergetic effect of ozone and ultrasonic radiation on degradation of chitosan was found. It is able to enhance the production of hydroxyl radical generated by the

radiolysis of ozone in the presence of ultrasonic radiation (Yue *et al*., 2008). Ultrasonic/O_3 combined process was employed to pretreat heterocyclic pesticide wastewater for increasing biodegradability and reducing biological toxicity (Xiong *et al*., 2011). Weavers *et al*. (1998) pointed out the importance of the rapid degradation of organic contaminants by using ozone and ultrasonication (20 kHz) with an increase in hydroxyl radicals. Ince and Tezcanlí (2001) found that the degradation of a reactive dye was affected by the combination of sonolysis (520 kHz) and ozonation.

Many researches were explained intermediates and by-products of chemicals during ultrasonication and ozonation process. Toxicity of chemical was very importance to confirm the safe technology. Conventional toxicity to estimated chemicals toxic is bioassay methods.

2.10 Bioassay systems and techniques (Bheen, 2012)

The bioassay systems vary based on the biological system used like animals (mouse, rat, guinea pig and rabbits etc.), plant bioassay (using plant constituents to evaluate a sample like (haemolytic activity) microbiological or cell based assay (using microbes like bacteria, fungi or cultured cells for anti biotic compound screening etc.). Based on techniques they can be differentiated into three broad types like;

2.10.1 *in vivo* techniques

These techniques employ a living animal recommended for the purpose of assay. The technique aims to study the biological effect or response of the compound under screening in a living system directly, use of rodents and rabbits etc.

2.10.2 *in vitro* techniques

These techniques employ a cell culture of recommended biological system to study the effect of compound under standard condition not similar to that of living environment. Here the cell culture survives by utilization of the nutrition in the media, use of stem cells, cell culture and microbes etc.

2.10.3 *ex vivo* techniques

These techniques employ a tissue or cells of recommended living system to study the effect of compound under test in suitable conditions within the stipulated time of organ survival outside the body. The methods described in the videos employ a living tissue of an animal in an apparatus to study the contractile effect of drugs.

Use of any isolated organ from animals in a glass ware to study the effect of compound within the period of its survival outside the living body with provision of only oxygen, glucose and isotonic salts to maintain cell integrity.

2.11 Brine shrimp bioassay for chemical toxicity test

Artemia salina L. (Artemiidae), the brine shrimp, is an invertebrate component of the fauna of saline aquatic and marine ecosystems. It plays an important role in the energy flow of the food chain (Lewan *et al*., 1992) and it can be used in a laboratory bioassay in order to determine toxicity by the estimation of the medium lethal concentration. Brine shrimp (*Artemia salina* L.) is one of the tiny aquatic animals that lives in hypersaline environments. Cysts are dormant brine shrimp babies, and nauplii are the first stage in the life cycle of brine shrimp after they have been hatched from eggs. Brine shrimp are well adapted to the high salinity and play an important role in its ecosystem. Brine shrimp feeds on microorganisms such as algae and bacteria and helps rid the lake water of contaminates such as phosphorus and nitrogen. Under optimal conditions, brine shrimp can live up to three months or more. However, due to changes such as temperature and food supply in the lake, the average life cycle is closer to 1.5 months (Kanwar, 2007).

Brine shrimp can be used in a laboratory bioassay in order to determine toxicity by the estimation of the medium lethal concentration which has been reported for a series of toxins, pesticides and other contaminants (Kanwar, 2007). Varó *et al.* (1998) studied on acute lethal toxicity of the organophosphorus pesticide chlorpyrifos to different species and strains of *Artemia.* It was found that the bisexual strains *A. salina* (*A. tunisiana*) was the most sensitive to chlorpyrifos and the mean LC_{50} values for bisexual populations were 0.95 mg/l significantly different from other strains. In addition, Sánchez-Fortún and Barahona (2009) studied on toxicity and characterization of cholinesterase-inhibition induced by diisopropyl fluorophosphatein

Artemia salina larvae. They were found that the acute toxicity of diisopropyl fluorophosphates on three age classes of *A. salina* was evaluated. An increase in toxicity of this organophosphorous compound was found following longer development of *A. salina* larvae.