

## CHAPTER 2

### Literature Review

#### 2.1 Tangerines

The tangerine (*Citrus reticulata* Blanco cv. Sai Nam Pung) is an orange-colored citrus fruit that is closely related to a type of mandarin orange. The name was first used for fruit coming from Tangiers, Morocco, described as a mandarin variety. Tangerine is most grown in Asia. The important production areas are Japan, South Korea, People's Republic of China, India, Australia and Mediteranean.

##### 2.1.1 Nutrition value of tangerines

Tangerine consists of several nutrition which are sufficient to maintain life, growth and body functions (Davis and Albrigo, 1994). The high nutrition value of tangerines is due to the existing nutrients in Table 2.1. Tangerine contains 0.31 g of fat. Another factor of tangerine nutrition is water. Since human body needs the fresh supply of fresh water every day, the water rich fruit as tangerine could help retaining well-balanced nutrition. Water content of tangerine is 85.17 g/100 g of tangerine. Carbohydrates provide the energy to human body for the daily activities. Carbohydrates content in tangerine 100 g is 13.34 g that is the main fuel source of human body. Hence, carbohydrate in tangerine nutrition has a very important function. It benefits for health by sustaining the level of cholesterol. Sugar constituent in tangerine is responsible in metabolic activities by 10.58 g per tangerine 100 g. There is no cholesterol and low calories levels in tangerine nutrition, thus it's good for diet conscious people. Fiber builds up the nutrition level in tangerines. It contains 1.8 g of fiber per 100 g of tangerine. The fiber is indigestible and passes through the intestinal tract, so it is beneficial for excretory system. Protein is necessary for human body in building and repairing tissues such as bones, muscles, cartilage, blood and skin. Tangerine nutrition contains protein 0.81% (w/w). Protein content in tangerine help in sustaining low blood pressure and enhancing brain function. The other minerals which help in operating various systems of human body.

The nutrition of tangerine is derived from several macro- and micro- nutrients association. Macronutrients i.e. carbohydrates, proteins and fats are voluminous body required. On the other hand, micronutrient such as vitamins and minerals are required in a little bit amounts. Although it's tiny amounts requirement but it also reaches to many health problems without them, because of their vital functions. Some essential vitamin as shown below (Table 2.1) from tangerine improved the valuable nutrition of tangerine.

**Table 2.1** The nutrition value of tangerines at the serving size 100 g

<b>Tangerine Serving Size</b>	<b>100 g</b>
Carbohydrate	13.34 g
Fiber	1.8 g
Sugar	10.58 g
Protein	0.81 g
Protein to Carbohydrate Ratio	0.06
<b>Vitamins</b>	
Vitamin A : Retinol	34.0 µg
Vitamin B1 : Thiamin	0.058 mg
Vitamin B2 : Riboflavin	0.036 mg
Vitamin B3 : Niacin, Nicotine Acid	0.376 mg
Vitamin B5 : Pantothenic Acid	0.216 mg
Vitamin B6 : Pyridoxin	0.078 mg
Vitamin B9 : Folic acid, Folate	16.0 µg
Vitamin B12 : Cobalamin	0.0 µg
Vitamin C : Ascorbic Acid	26.7 mg
Vitamin E (Tocopherole)	0.2 mg
Vitamin K (Phyllochinone)	0.0 µg
Lycopene	0.0 µg
Lutein + Zeaxanthin	138.0 µg
Choline	10.2 mg
Fat	0.31 gm

**Table 2.1** (Continued)

<b>Minerals</b>	
Potassium	166.0 mg
Iron	0.15 mg
Sodium	0.00 mg
Calcium	37.0 mg
Magnesium	12.0 mg
Zinc	0.07 mg
Phosphorus	20.0 mg
Manganese	0.039 mg
Copper	0.042 mg
Selenium	0.1 µg
<b>Fatty Acids</b>	
Omega 3s	18.0 mg
Omega 6s	48.0 mg
<b>Sterol</b>	
Cholesterol	0.0 mg
Phytosterol	Not Available
Water	85.17 g
Ash	0.38 g

(FAO, 1997)

**2.1.2 Tangerine characteristics**

Rind of tangerine is orange to reddish-orange when ripe and peel off easily, a medium-thick and loosely adherent rind. Fruit globose to oblate; 5-8 cm in diameter; found on pale collar ; glabrous with slightly rough with sunken oil glands in exocarp; 10-17 locules; axis large and hollow. Juice sacs flesh, juicy and orange in color. Seed 3-7 in each locule.

## 1) Tangerine anatomy

Tangerine fruit develops from fertilized ovary and composed of 8-16 fused carpels with axile placentation (Morton,1987). Each carpel forms the locule or segment containing seed around the core and juice sacs (vesicles) inside. Pericarp develops from ovary wall and is divided into of exocarp or *flavedo* that containing oil glands and covered with cuticle. The other segment is mesocarp or *albedo* with colorless or white color. (Figure 2.1 and Figure 2.2)

The *flavedo* is composed of epicarp, hypodermis, outer layer of mesocarp, and oil glands. Epicarp is cover with multilayered cuticle which consists of cutin, a heteropolymer derived from fatty acids and cellulose , in outer and inner layer of cuticle. (Baker *et al.*, 1975).

The cutin mold consist of cutin, wax and cell-wall body. Wax is accumulated continues as fruit grows. The wax hardens and develops breaks spontaneously. Epidermis synthesise lipids and waxes and depositing on the cutin layer (Figure 2.3).

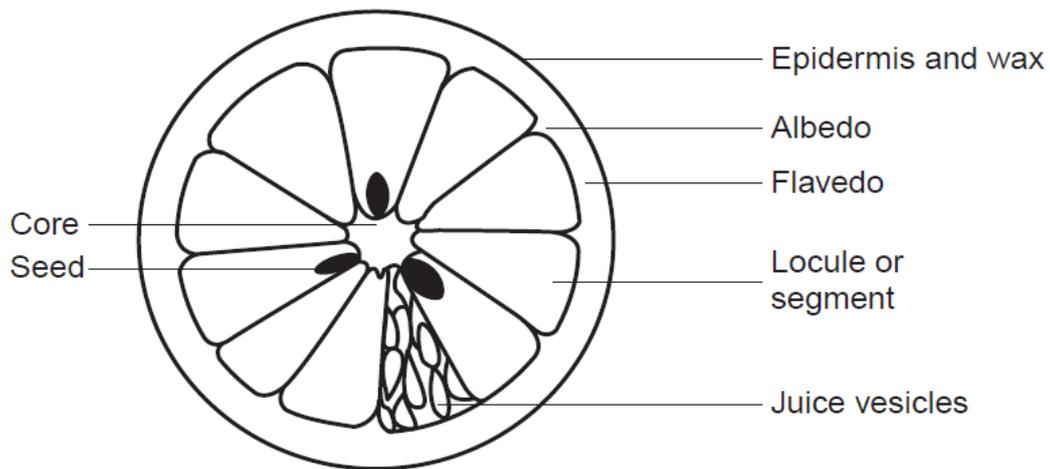
Wax is formed in various shapes such as platelets, rods and others accumulated inside and above cuticle in form of several derivatives i.e. alcohol-, aldehyde-, ketone-, paraffin derivatives and so on. Wax accumulation forming several layers of wax. It is amorphous in the beginning and then there are some projection and splitting part separated vertically (Albrigo, 1972a; Freeman, 1978 and Freeman *et al.*, 1979). Whole surfaces finally split and lift up forming large, flat, irregular plates. These can be wipe tenderly to make polish shining surface. The cuticle are mainly responsible for maintaining water within tissue which is essential for basic metabolism. It reduces the evaporation rate from 3.6 to 0.14 mg/cm<sup>2</sup>/Pa/h. in living plant cells. The evaporation rate is depend on fruit type or commodity, anatomy and environmental circumstance. The coefficient of transpiration is 42 and 7400 for apple lettuce, respectively (Wills *et al.*, 1998). Furthermore , number of stomata on epidermis have an effect on evaporation rate also (Turrell and Klotz, 1940). There are more stomata number on upper hemisphere of the fruit than the stalk end. Whereas the number of stomata is fewer or none around the stalk and calyx (Albrigo, 1972b). Stomata in matures fruit are blocked

by the wax nevertheless stomatal function remain despite the harvest. Most exogenous coated PGRs absorption occurs thru cracks in wax layers and cutin more than the stomata. Wax layer of young fruits do not fully complete thus the absorption of coated PGRs is rather large amount.

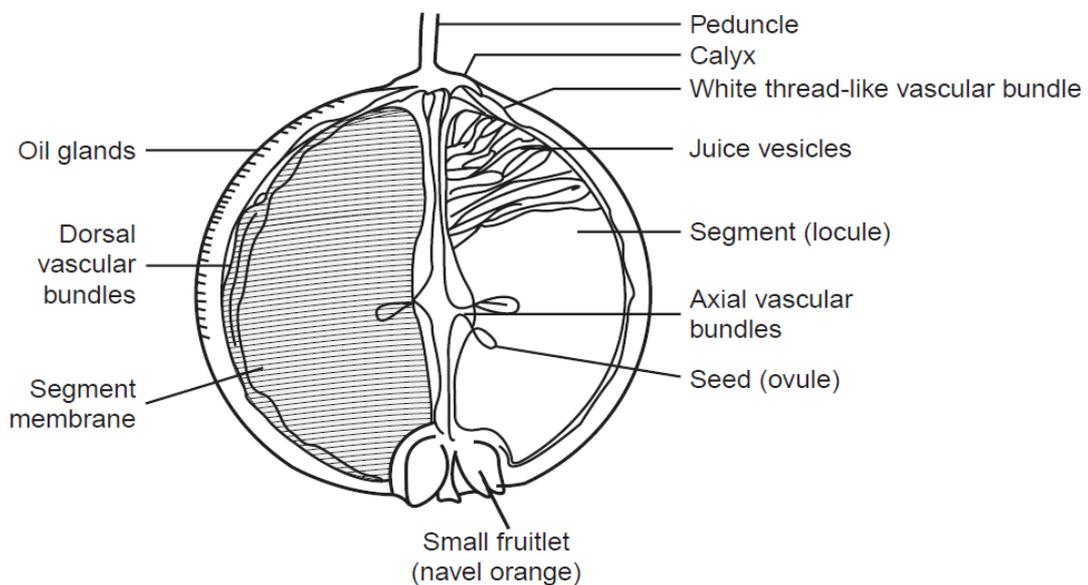
Plastids in exocarp are slowly change from chloroplasts into chromoplast in ripening fruit make fruit changes in color (Thomson, 1969). The color turns from green to yellow and back to green again in re-greening phase. Photosynthesis is occur in young green fruits and re-greened fruit also. Chlorophyll is infinitesimal remain in yellow fruit. Chloroplasts especially transform into globose chromoplast and vice versa make the color changing (Ljubescic, 1984).

Hypodermis and outer mesocarp, achromatic cells, which are located underneath epicarp and contain oil glands (10-100 $\mu$ m or more). The characteristic aroma and flavor are in consideration of terpenes ( mostly *d*-limonene) and sesquiterpine of essential oil in different citrus species. In case of glands rupture by crash , oleocellosis develops – a characteristic injury on the peel make fruit an unpleasant appearance, because the oil damages other cells of peel (Wardowski *et al.*, 1976). Most citrus rind is not edible because of the oil. Nevertheless, kumquat's rind is sweet and edible company with the flesh. Albedo compose of inner mesocarp which consist of large air chamber parenchyma (Scott and Baker, 1947). It could be protect fruit from pressure and crash. The thickness of albedo is 1-2 mm, 2-5 mm and up to 200 mm in lime and tangerine, sweet orange and pomelo, respectively. The outer side of albedo attached to flavedo and inner side adnate to segment. The exocarp (flavedo) and mesocarp (white puff and soft tissue or albedo) are mixed to each other. The endocarp (segment) is located at the innermost of the peel and parted into locular membrane. The entire exocarp (flavedo) and mesocarp are removed when rind peeled off. Edible part are the segments surrounding the central axis. Each segment enclosed by continuous endocarp lamina containing compacted juice sac. Juice sacs are multicellular vesicle and club-shape which fill the segment and attached to laminar segments. There is a tiny oil gland in the center of the juice sac. Seeds attach to the wall of segment to the core of fruit , namely axile placentation. The core consists of pale or tinted, loose, spongy mesh of cells as albedo. Laminar between each segment connect the core and abedo. The Core of all

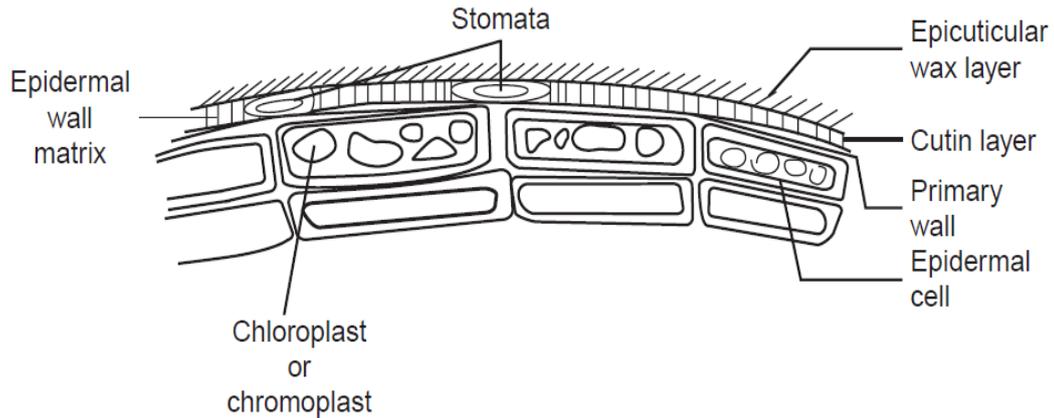
citrus is rigid in immature phase of development and become hollow in mature fruit, especially sweet orange, mandarin, pomelo, grapefruit, lemon and lime. When peeling off, the white fibrous vascular bundle nucleate the connection in albedo and continue along the axis of fruit outside of the segment are also removed.



**Figure 2.1** Cross sectional of tangerine fruits presenting various anatomical parts of tangerine (Ladaniya, 2008)



**Figure 2.2** Long sectional presenting vascular bundles and fruitlets of navel fruit (Ladaniya, 2008)



**Figure 2.3** Schematic diagram : epidermal layer, cutin, and wax on peel of citrus (Ladaniya, 2008)

## 2) Tangerine fruit physiology

There are several physiological changes occur in citrus fruit development. The changes in growing age occurs due to association of various functions in organelles inside cells which lead to changes in tissues, organs, and finally the whole system.

### 2.1) Respiratory activity

There are two types fruit ripening i.e. climacteric and non-climacteric. Climacteric fruit such as mango or banana change from mature fruit to senescence can be induced by ethylene, which make several changes in biochemistry and increasing respiration, eventually lead to spontaneous ripening after harvest (Biale, 1950; Rhodes, 1980). On the other hand, non-climacteric fruit does not produce ethylene and increase respiration rate to exhibit the maturity and ripening stage. Citrus and tangerine are members of the non-climacteric fruit so there have no biochemistry activities in harvested fruit (Boonyakiat *et al.*, 2011). It does not ripen after take away from the tree. It will be best practice to get the best quality citrus and tangerine by pick off the fruits on optimum mature stage (Medeira *et al.*, 1999).

## 2.2) Biochemistry of respiration

After harvest, the available amount of sugar and organic acid of citrus fruit is gently changed and release CO<sub>2</sub>, H<sub>2</sub>O and heat because of respiration rate quite very low and decrease constantly. The sweetness is stable since there is no starch. But it could be raise a bit sweet by total soluble solid slightly increase by hydrolytic enzymes activities, or fast water loss in dry air storage lead to slightly high concentration. Furthermore, organic acid i.e. citric acid will be decreased in high temperature (Gloria *et al.*, 2010).

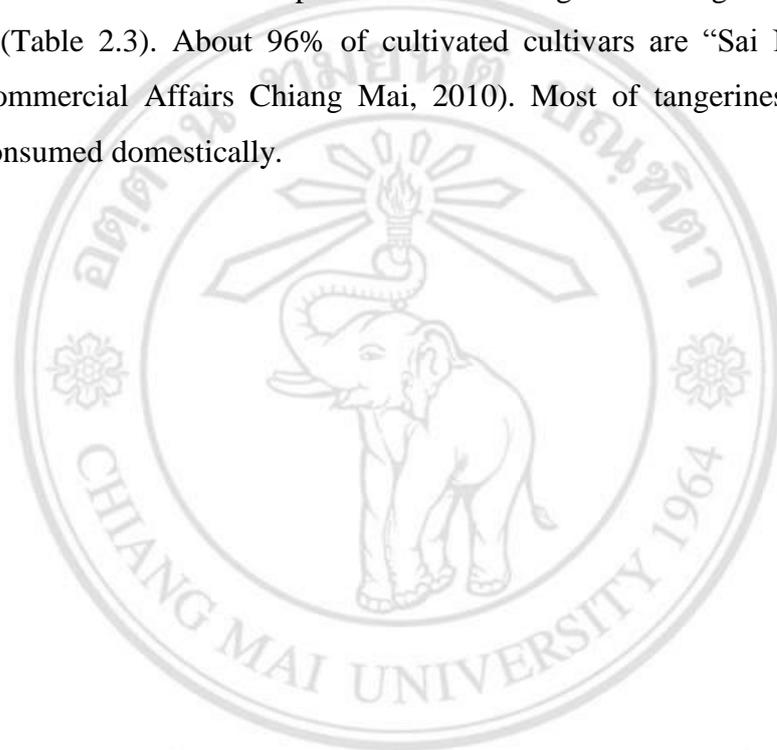
## 2.3) Transpiration

Transpiration is the loss of water of plant. Cuticle and waxed covered stomata on epidermal cells depress the transpiration. The platelets of wax on fruit are overlap accumulate and insoluble. Inter platelet space is fill up by the air. The transpiration is limited by soft wax which derived from alcohol-, aldehydes, ester and fatter acid molecule. Water constituent of tangerine fruits is 80-85%. Water losses in citrus rind mostly occur at stalk end that is 4-6 times more than other region left on the peel (Petracek, 1996). In low humidity storage, transpiration is take place at peel more than the pulp, and then the peel starts to dry out. Therefore, fresh weight basis recorded by means of fruit juice percentage increases (Siddiq, 2012).

Peel softness cause by pectin hydrolysis from intercellular space in long time chilling. Dry air containing lead to low level of humidity make the peel turning firm and coriaceous contribute gas exchange to more difficult. This induces an anaerobic condition occur inside the fruit, alcoholic compound will be uplifted. The transpiration and decomposition limitation can expand storage time of citrus fruit. Natural wax on the peel is insufficient to decrease transpiration because its pits and crack spaces due to stomatal opening and wax cracks, respectively. The high-density polyethylene film at 10 µm thick coated on each fruit can reduce the transpiration and inhibit gas exchange of citrus fruit (Ben-Yehoshua *et al.*, 1983).

### 2.1.3 The Status of tangerines production in Thailand

Tangerine is an economically important fruit in Thailand. It is widely grown in northern Thailand, especially in Chiang Mai. It is contributing 40% of citrus planted area and 76% of total citrus production. In 2013, the harvested area of tangerine in Chiang Mai was about 27,164 hectare (Table 2.2). Most of the planting areas are in Fang , Chiang Dao and Mae Ai districts. The total production in Chiang Mai during 2013 was 19,733 metric tones (Table 2.3). About 96% of cultivated cultivars are “Sai Nam Pheung” (Office of Commercial Affairs Chiang Mai, 2010). Most of tangerines produced in Thailand is consumed domestically.



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**Table 2.2** The areas (hectare) of tangerine production in Chiang Mai Province during 2008-2013

Districts	2008	2009	2010	2011	2012	2013
<b>Mueang Chiang Mai</b>	-	-	-	-	-	-
<b>Chaing Dao</b>	2,924	2,924	2,122	2,122	2,122	2,122
<b>Chom Thong</b>	56	56	45	-	-	-
<b>Doi Saket</b>	181	181	25	25	25	-
<b>Fang</b>	40,873	40,873	14,350	16,529	13,784	13,784
<b>Hang Dong</b>	19	19	-	-	-	-
<b>Hot</b>	15	15	5	3	-	-
<b>Mae Chaem</b>	42	42	0	0	34	34
<b>Mae Rim</b>	172	172	173	193	193	193
<b>Mae Taeng</b>	1,950.50	1,951	313	842	517	97
<b>Omkoï</b>	76	76	76	76	76	20
<b>Phrao</b>	764	764	19	262	157	55
<b>Samoeng</b>	30	30	61	61	61	61
<b>San Kamphaeng</b>	-	-	-	-	-	-
<b>San Pa Tong</b>	0	-	-	-	-	-
<b>San Sai</b>	69.25	69	23	18	5	5
<b>Saraphi</b>	54	54	4	4	-	-
<b>Mae Ai</b>	25,749	25,749	9,110	8,040	9,971	9,971
<b>Doi Tao</b>	-	-	-	-	-	-
<b>Wiang Haeng</b>	240	240	63	71	71	95
<b>Chai Prakan</b>	1,903	1,903	1,253	695	695	695
<b>Mae Wang</b>	85	85	23	99	99	30
<b>Mae On</b>	33	33	25	3	-	-
<b>Doi Lo</b>	14	14	17	-	2	2
<b>Kanlayanawatthana</b>	-	-	-	-	-	-
<b>Total</b>	<b>75,249.75</b>	<b>75,250</b>	<b>27,707</b>	<b>29,043</b>	<b>27,812</b>	<b>27,164</b>

Source : Office of Chiang Mai Agriculture, 2014

**Table 2.3** The quantity of tangerine production (tonnes) in Chiang Mai province during 2008-2013

Districts	2008	2009	2010	2011	2012	2013
<b>Mueang Chiang Mai</b>	-	-	-	-	-	-
<b>Chaing Dao</b>	2,924	2,924	4,594	7,885	2,434	3,183
<b>Chom Thong</b>	142	142	158	-	-	-
<b>Doi Saket</b>	489	489	25	50	38	-
<b>Fang</b>	183,929	183,929	59,151	38,926	46,636	8,422
<b>Hang Dong</b>	76	76	-	-	-	-
<b>Hot</b>	14	14	8	2	-	-
<b>Mae Chaem</b>	84	84	0	0	91	24
<b>Mae Rim</b>	112	112	322	338	354	232
<b>Mae Taeng</b>	3,316	3,316	278	2,526	316	126
<b>Omkoï</b>	33	33	128	53	10	18
<b>Phrao</b>	1,681	1,681	57	262	162	55
<b>Samoeng</b>	90	90	15	70	46	31
<b>San Kamphaeng</b>	-	-	-	-	-	-
<b>San Pa Tong</b>	0	-	-	-	-	-
<b>San Sai</b>	201	201	36	27	6	5
<b>Saraphi</b>	65	65	4	4	-	-
<b>Mae Ai</b>	82,397	82,397	19,131	18,444	11,436	6,980
<b>Doi Tao</b>	-	-	-	-	-	-
<b>Wiang Haeng</b>	600	600	104	23	27	124
<b>Chai Prakan</b>	1,903	1,903	1,002	2,085	483	512
<b>Mae Wang</b>	76	76	102	120	91	21
<b>Mae On</b>	50	50	35	5	-	-
<b>Doi Lo</b>	14	14	70	-	2	2
<b>Kanlayaniwatthana</b>	-	-	-	-	-	-
<b>Total</b>	<b>278,194</b>	<b>278,194</b>	<b>85,218</b>	<b>70,820</b>	<b>62,132</b>	<b>19,733</b>

Source : Office of Chiang Mai Agriculture, 2014

#### **2.1.4 Problems involve tangerine production in Thailand**

Tangerine production in Thailand environment has both advantages and disadvantages (Sethpakdee, 1996). The main advantage is the fast growth of both vegetation and fruit, as a result of the warm climate and ample supply of water. The disadvantages of this kind of climate are the poor fruit color, and the diseases and insect pests which attack throughout the year. Hence large quantities of pesticides are generally used (Sethpakdee, 1996). The important diseases and pests of tangerines are as follow:

##### **1) Disease**

In the past 20 years, Asian citrus production has been damage by plant diseases which have brought amount product and quality loss. Thus, plant diseases have become a main problem to the region's tangerine cultivation.

Greening is the most devastating disease for tangerines in Thailand. Infected trees, especially Som Kiew Waan and Shogun, have reduced life-span as well as lower yields, A disease eradication program aimed at providing growers with healthy disease-free planting materials has been carried out for many years.

##### **2) Pests**

There are a number of common insect pests, including thrips, citrus leaf miner, boll worm, psyllids, Oriental fruit fly, fruit moth, scale insect, citrus rind borer and mites. There are two kinds of mite common in tangerine plantations are the Oriental red mite and the citrus rust mite.

Since Thailand is located in the tropics, insects readily attack the trees throughout the year. If growers delay apply insecticide, this may result in the loss of the whole crop (Sethpakdee, 1996). Laohasakparsit (2006) found that there were a variety of pests attacked tangerine in stage during the growth development such as Chilli thrips (*Scirtothrips dorsalis* Hood), Citrus leafminer (*Phyllocnistis citrella* Stainton), Oriental citrus jumping plant lice (*Diaphorina citri* Kuwayama) and African mite (*Eutetranychus africanus* (Tucker)) causing unacceptable products. So that growers

applied a lot of chemical pesticides in the whole fruit development cycle of tangerines repeatedly resulted in over-use of pesticides.

In 2008-2009, the Office of Agricultural Research and Development, Region 1 (OARD1) investigated pesticide residues in tangerines in Chiang Mai, Lampang and Lamphun provinces. It was found that the whole tangerine fruits were contaminated with organophosphate, organochlorine and pyrethroid. While there were merely organophosphate in tangerine pulps. Moreover, the analyses the whole tangerine fruits indicated that ethion residue was the highest (1.14 mg kg<sup>-1</sup>, the standard MRLs of ACFS not exceed 1 mg kg<sup>-1</sup>) among other pesticides. In tangerine pulps, ethion residues was found 0.1 mg kg<sup>-1</sup> (Table 2.4). In 2010, tangerine samples from tangerine orchards, there were 4 samples found ethion exceeding MRLs (Table 2.5).

**Table 2.4** Chemical residues contaminated tangerine produce during November 2008 – December 2009, in Chiang Mai, Lampang and Lamphun provinces

Tangerine	Chemical groups	Chemical name	Quantity of chemical residues (mg kg <sup>-1</sup> )	CODEXMRLs (mg kg <sup>-1</sup> )	ACFS MRLs (mg/kg)	No. of samples contaminated with chemical residues (%)		
Whole fruits	Organophosphate	Chlopyrifos	0.09	1	1	40		
		Profenofos	0.41	-	0.1	46		
		Ethion	1.14	-	1	92		
		Malathion	0.12	-	1	6		
		Fenitrothion	0.06	-	-	2		
		Pirimifos-methyl	0.87	-	-	8		
		EPN	0.56	-	-	16		
		Diazinon	0.04	-	-	4		
		triazofos	0.63	-	-	4		
		Organochlorine	organochlorine	0	-	-	0	
		Pyrethroid	Cyhalothrin(Lamdacyhalothrin)	Cyhalothrin	0.05	0.5	-	42
				Cypermethrin	0.21	2.0	-	84
				permethrin	0.1	-	-	2
				ethion	0.01	-	-	50
Pulp	Organophosphate	ethion	0.01	-	-	50		
	Organochlorine	organochlorine	0	-	-	0		
	Pyrethroid	cypermethrin	0	-	-	0		

**Source :** Olsen *et al.*, 2012 ; National Bureau of Agricultural Commodity and Food Standard (2008)

**Table 2.5** Chemical residues contaminated in tangerine fruits, water resources and soils within tangerine orchards in Chiang Mai Province, 2010

Chemical residues	CODE	ACF	Exceed MRL	Peels	Pulps	Juice	MRL (g•L <sup>-1</sup> )	>MR L	Soils	MRL (mg•kg <sup>-1</sup> )	>MR L
<b>Organophosphorus group</b>											
Chlopyrifos	1.0	-	1	21	-	-	-	-	7	.03	3
profenofos	-	0.1	7	1	-	-	-	-	1	.05	-
Pirimifos-ethyl	-	-	-	15	-	-	-	-	-	0	-
Ethion	-	1.0	4	1	5	-	-	-	13	.05	8
Dimethoate	-	5.0	-	15	-	-	-	-	-	-	-
Triazophos	-	-	-	-	-	-	-	-	-	-	-
Fenitrothion	-	-	-	-	-	-	-	-	-	-	-
Diazinon	-	-	-	-	-	3	0.5	-	1	0.05	-
<b>Organochlorine group</b>											
Endosulfan	-	-	-	-	-	1	-	-	-	-	-
<b>Pyrethroid group</b>											
Cypermethrin	2.0	2.0	-	3	3	3	-	-	4	-	-
L-cyhalothrin	-	-	-	-	-	-	-	-	4	-	-

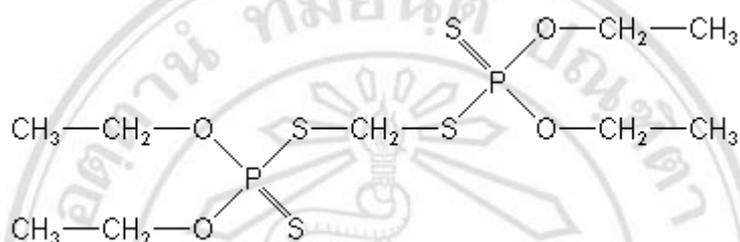
**Source :** Olsen *et al.*, 2012

The chemical residues using in tangerine orchards were discharged to water resources. Surface water are the accumulation place for these residue. While surface water is used for water source of drinking water, widespread of agricultural residues in surface water may cause a problem to water quality.

## 2.2 Ethion

Ethion [O,O,O',O'-tetraethyl S,S'-methylene bis (phosphorodithioate) or C<sub>9</sub>H<sub>22</sub>O<sub>4</sub>P<sub>2</sub>S<sub>4</sub> (Figure 2.4) belongs to organophosphate group (Hartley and Kidd, 1983) and was first registered in the United States in the 1950's. It is an essentially non-systemic pesticide and acaricide in pre-harvest application on various kinds of crops, particularly citrus, to control aphids, scales, mites, leaf miners and leaf hoppers (Fenik *et al.*,2011). It was

formulated in WP, EC or GR and applied from one to four times at a rate of 0.36-2.3 kg ai/ha. For responding to risk concerns, mitigation was decided to reduce in the maximum rate of application and a amount of applications on citrus (FAO/WHO, 2000) forbid of aerial application, control direction of airblast applications, and stop of non-commercial ethion uses in 1998. It was suggested to apply ethion in tangerine plantation of the spray concentration rate 0.05-0.1 kg ai/ha, with the PHI (Pre Harvest Interval) 20 days. In USA (1984), it was found that residues of ethion in tangerine which had 3 times application with 3.4 kg ai/ha the quantity of ethion started from 2.6 mg kg<sup>-1</sup> on PHI 0 day - 1.0 mg kg<sup>-1</sup> on PHI 21 days.



**Figure 2.4** The chemical structure of ethion (Wood, 2009)

### 2.2.1 Characteristics and properties of ethion

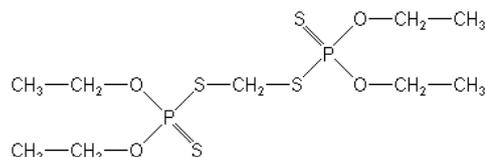
The chemical and physical properties of ethion was shown in Table 2.6

**Table 2.6** The chemical and physical properties of ethion

Properties	Details	Reference
<b>Chemical name</b>	Ethion	EPA( 1998) HSD B 1998
<b>CAS Number</b>	563-12-2	EXTOXNET (1996)
<b>Chemical Class</b>	organophosphate	EXTOXNET (1996)
<b>Commercial names</b>	Acithion, Aqua Ethion, Ethanox, Ethiol, Hylmox, Nialate, Rhodiacide, Rhodocide, RP-Thion, Tafethion, Vegfru Fosmite.	EXTOXNET (1996)
<b>Synonym(s)</b>	O,O,O',O'-tetraethyl ester; ethyl methylene phosphorodithioate; O,O,O',O'-tetraethyl S,S'-	Merck (1989) Vogue (1994)

methylenediphosphorodithioate;

**Chemical structure**



Merck (1989)

**Molecular weight**

384.48

Merck (1989)

**Color**

Pale or white to amber-color

Tomlin (1994)

**Physical state**

Liquid

Tomlin (1994)

**Formulation**

Available in dust, emulsifiable concentrate, emulsifiable solution, granular, and wettable powder.

EXTOXNET, (1996)

**Melting point**

-12 to -13 °C

Merck (1989)

**Boiling point**

164–165 °C at 0.3 mm Hg

EPA (1989b)

**Density at 19 EC**

1.220 at 20 EC

Merck (1989)

**EC**

**Odor**

pure form is unscented and unpleasant odor in technical product.

HSDB (1998)

ATSDR (2011)

**Odor threshold:**

**Water**

No data

**Air**

No data

**Solubility:**

**Fresh water at 25 EC**

0.6 mg/L

Sharom *et al.*

(1980a,1980b)

**Salt water at 25 EC**

No data

**Organic solvent(s)**

acetone, benzene, chloroform, kerosene plus 1% methyl ethyl ketone, Xylene

Merck (1989)

Table 2.6 (Continued)

Properties	Details	Reference
<b>Hydrolysis half-life</b>		
<b>30 EC (sterile water)</b>		Dierberg and Pfeuffer (1983)
<b>30 EC (non-sterile drainage water)</b>	pH 4 and 7 in 146 days pH 8 in 62 days	
<b>25 EC (Water with 1% ethanol)</b>	pH 10 in 1 day and 26 days 26 days	Chapman and Cole (1982)
	pH 4.5 in 99 weeks pH 5 in 63 weeks pH 6 in 58 weeks pH 7 in 25 weeks pH 8 in 8.4 weeks	
<b>Autoignition temperature</b>	No data	
<b>Flashpoint</b>	No data	
<b>Flammability limits at 25 EC</b>	Burnable but not kindle	HSDB (1998)
<b>Reactivity</b>	- Hydrolysable in acid and alkaline solution - Sulfur oxides and phosphorous oxides released (T\$150 EC)	Tomlin (1994) Sax (1984) Tomlin (1994)
<b>Conversion factors (25 EC)</b>	1 ppm = 15.7 mg/m <sup>3</sup> 1 mg/m <sup>3</sup> = 0.064 ppm	Calculated
<b>Explosive limits</b>	May decomposable with violence above 150 EC	HSDB (1998)
<b>Regulatory Status</b>	Moderate to high toxicity	ATSDR (2000); WHO (1999)

### 2.2.2 Mode of action, classification and utilization

Ethion degradation on oranges may be attributable to environmental factors rather than metabolism. In ethion degradation, ethion was the predominant product in the ethyl acetate fractions (90%) of the organosoluble residue). Ethion mono-oxon and ethion dioxon was identified as the products increased from 1% and <1% respectively at day 0 to 11% and 4% of the total residue by day 90. The degradation of the initial compound occurred on leaf and fruit surface. The breakdown of ethion on citrus leaves was faster than on fruit.

Moreover, it was found that residues of ethion in oranges and orange processing fractions such as orange juice (Iñigo-Nuñez *et.al.*, 2010) from a processing trial in USA, 1984 (Witkonton and Arabinick, 1985). Ethion (4EC) was applied once to Valencia orange trees at 5 kg ai/ha at a spray concentration of 0.05 kg ai/ha. Oranges were harvested 7 days later and processed. It was found that the ethion residues were contaminated in unwashed fruits, washed fruits, juice, syrup, dry pulp, finisher pulp and oil vary from 0.76, 0.64, <0.02, 0.21, 3.3, 0.02 and 7.7 mg kg<sup>-1</sup> respectively.

Ethion residues in citrus fruits are quite constant and small differences in the PHI have little effect on residue levels. The residue in the whole fruit was concentrated primarily in orange oil (11 times) and dry pulp (4.3 times). The residue was reduced in syrup (0.25 times that in the fruits) and finisher pulp (0.02 times). No residues of ethion or its oxygen analogues were found in orange juice (<0.02 mg kg<sup>-1</sup>) (U.S.EPA, 1997).

### 2.2.3 Acute and chronic toxicity

The acute toxicity of ethion was shown in Table 2.7

**Table 2.7** Acute and chronic toxicity of ethion

<b>Toxicological Effects</b>	<b>Acute toxicity:</b> <u>Oral route</u> - Moderate to high toxicity (LD <sub>50</sub> values in rat are 208 mg kg <sup>-1</sup> and 21-191 mg kg <sup>-1</sup> for pure ethion and technical ethion respectively. <u>Inhalation</u> - Moderate toxicity (4-hour inhalation present LC50 value at 0.864 mg L <sup>-1</sup> in rats). <u>Dermal route</u> - Moderate to high toxicity (LD <sub>50</sub> value is 62 mg kg <sup>-1</sup> in rats)  Toxicity is cause by the amount and concentration of organophosphate uptake. They may bring about nauseate, abdomen pain, diarrhea, immoderately h, indistinct eye sight, headache, tiredness, having tight chest, irregular heart beating and breathing, impaired balance, writhe, trauma and death. It might be burn on skin. Ethion respiratory uptake generates cholinesterase inhibitors, abnormal cholinesterase processing, or breakdown liver function. The toxicity would be increase by high temperature condition or ethion exposure to white light or ultra-violet.	EXTOXNET, ( 1996) ; U.S. Department of Health Services (2000)
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**Table 2.7 ( Continued )**

<p><b>Chronic toxicity:</b></p> <ul style="list-style-type: none"> <li>- Oral uptake high dose ethion in rat; 0.1, 0.2 or 2 mg kg<sup>-1</sup> daily for 18 months can drop the level of cholinesterase.</li> <li>- Nontoxic</li> <li>- re-exposure or long time uptake of organophosphate may cause acute toxicity effect, delayed symptoms in addition.</li> </ul>	<p>EXTOXNET, (1996)</p>
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#### 2.2.4 Impact on living creatures

The impacts on living creatures were shown in Table 2.8

**Table 2.8** Impacts of ethion on living creatures

Impacts	Details	References
<b>Reproductive effects</b>	Nontoxic	EXTOXNET (1996)
<b>Teratogenic effects</b>	Postponement pubic bones hardening in pregnant rats appears on day 6-15 by doses of 0.2, 0.6 or 2.5 mg of ethion per kilogram of body weight per day.	EXTOXNET (1996)
<b>Mutagenic effects</b>	Ethion is not mutagenic.	EXTOXNET (1996)
<b>Carcinogenic effects</b>	Nontoxic in rodents.	EXTOXNET (1996)
<b>Organ toxicity</b>	Primary influence nervous system by inhibiting cholinesterase activity which directly affect the function of nerve.	EXTOXNET (1996)
<b>Fate in humans and animals</b>	Ethion could be excreted through kidney system in 1 or 2 days.	EXTOXNET (1996)

### 2.2.5 Impact on environment

The impacts of ethion on environment were shown in Table 2.9

**Table 2.9** Impacts of ethion on environment

Impact	Details	References
<b>Ecological Effects</b>	<u>Birds</u> : depending on the species.	EXTOXNET (1996)
	- Practically nontoxic to highly toxic, <u>Aquatic organisms</u> : <i>Freshwater and marine fish</i> - Highly toxic <i>Freshwater wirbellose</i> - Ethion accumulation occur in fish tissues.	EXTOXNET (1996)
	<u>Other organisms</u> : honeybees - Nontoxic (LD <sub>50</sub> is 20.55 µg per bee)	EXTOXNET (1996)
<b>Environmental Fate</b>	<u>Degrade in soil and subterranean water</u> - Moderate to high persistence. <u>Laboratory conditions</u> -Ethion half-life in soil is 1.3-8 weeks <u>greenhouse organic soil</u> - Ethion half-life in soil is 16-49 weeks - Increasing in soil when used repeatedly. Ethion is insoluble so it highly adsorbs to the soil particles. Thus subterranean water could be contaminated and microorganism hazardous. The hydrolysis of ethion could be generated in base condition (pH>9).	EXTOXNET (1996) , ATSDR (2000); Faust and Gomma (1972) ; Foster <i>et al.</i> (2004)

**Table 2.9** ( Continued)

Impact	Details	References
<u>Degrade in water</u>	- Rather water insoluble.	EXTOXNET (1996) ; Dierberg
<u>In open waters</u>	- It adsorbs to suspended matter and sediment. Ethion half-life is varied with 4-22 week under laboratory condition from 3 different sources of open water. Ethion degradation generates unhurriedly in irrigation canal with 26 days of half-life. The hydrolysis of ethion half-life are 63, 58 and 8.4 weeks with pH value 5,6,7 and 8 under 25 °C temperature condition. The half-life omit to one day with pH value 10 and under 30°C temperature condition. Microorganism cannot be degraded by ethion in open water. The evaporation affect in shallow-water and rapid moved stream. Photo-oxidation happen under sunlight. Ethion bioconcentration may effective.	and Pfeuffer (1983); Graves and Strom (1995); Tomakiewicz <i>et al.</i> , (2010)
<b>Exposure Guidelines</b>	<b>ADI:</b> 0.002 mg per kilogram of body weight per day	EXTOXNET (1996)

### 2.3 Principles of insect-pest control

For preventing of economic losses in agriculture farming, insect-pest control is necessary. First of all, the insect was identified correctly then determine the weakness stage of insect. This information becomes available to choose the method of control on suitable time (Krishiworld, 2012). The appropriate methods for pest control are discussed below.

### **2.3.1 Physical and mechanical control**

These are the common methods, and have been used for ancient time. For example; collection infested parts and other inactive stages, the removal of egg masses or whole plants, the beating of drums or tins or trenching. Physical and mechanical control could be effectively during the first stage of the pest infection. These methods can be grouped as active methods such as engineered, warm, electromagnetic emission, numerous treatments and combination of methods and passive methods such as trenches, pallisade, living materials mulch, artificial materials mulches, particle films, sediment dusts, trapping, oils, detergents and soap (Vincent *et al.*, 2003).

### **2.3.2 Cultural control**

This method is precautionary which is not cost and may established more efficient and effective, if applied after acquiring a thorough knowledge of the life circle of a pest. Deep plugging after harvesting the product (to expose the hiding and resting insects), the removing and destroying of other trash, sowing with suitable time to avoid the peak incidence stages, clean farm, eliminate of alternative hosts, intercropping and suitable crop rotations are the important actions included under these methods of control.

### **2.3.3 Host plant resistance**

Plant resistance varieties have been considered as one of cultural methods of protecting crops against insect damage. It involves the utilization of the existing property of plant's strains, cause less infected or damaged than other strains of same crop or plant. Large amount of benefits have been achieved by this method in case of crops, e.g. alfalfa, cotton, maize and wheat. This method realized that the requirement of co-operation of plant breeders, entomologists, plant pathologist and geneticists .

### **2.3.4 Biological control**

Pests have enemies in the forms of parasites predators and disease causing organisms. This control involves producing and releasing of agents, or originating conditions which

the agents can operate effectively. Biological control should be carried out by specialized staff in discriminated use of chemical agents. At present the appropriate method is to integrate chemical and biological methods that insecticides do not effect on natural enemies

### **2.3.5 Legislative or regulatory method**

The objective of this method is mainly preventing the pests from abroad or preventing outbreak of pests. The method is operated under plant-quarantine laws by Department of Agriculture.

### **2.3.6 Chemical control**

The most popular method of pest control is using insecticides, while the insect are still increasing. Hundreds chemical agents are available to control pests, in form of dusts, sprays or granules. For soil inhabiting insects, dust and granules are applied by systemic insecticides which would be affected through insect in early stages of next cultivation. The serious problems of insecticides residues on agricultural product used as food and feed, and on pollution of environment have been agitating the minds of people in developed and developing countries of the world.

## **2.4 Pesticides removal techniques**

Nowadays, people are interested in green products and healthy life. They realize on pesticide residues which cause of health problems directly for farmers and also to consumers more than 35 years. Pesticide acute affects on many organs e.g. liver, kidneys, lungs, skin, eyes and brain. Long-term chronic toxicity affects on humans included a whole series of cancers, liver and kidney failure, sterility, neurological disorders and birth defects. It takes a long time to break down pesticide in food chain or in environment (Egendorf, 2000). Hence, the agricultural products should be clean enough for consuming. Varieties of chemical removal were proposed as the followings:

### **2.4.1 Rinsing with tap water**

Fruits and vegetables were rinsed with water to reduce the amounts of pesticide residues before consumption. A short time of rinsing in tap water reduces many types pesticide residues but not 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).

### **2.4.2 Blanching**

Vegetables were heated with short time by hot water or steaming and pesticide can be removed effectively. But it is necessary to wash vegetables and fruits with water before blanching. (Krishnaswamy and Sesikeran, 2010). These strategies are to reduce residual pesticides included a washing with water, potassium permanganate and detergents etc. But this method causes water pollution.

### **2.4.3 Washing with salt**

Washing fruits and vegetables with salt solution (2%) can lift off most pesticide residues on contacted surface. In cold water washing, pesticide about 75-80% are removed. On fruits surface such as grapes, apples, guava, plums, mangoes, peaches and pears and fruity vegetables like tomatoes, eggplant and okra require twice to three times of washings to remove pesticide residues (Krishnaswamy and Sesikeran, 2010).

### **2.4.4 Soaking in potassium permanganate**

Potassium permanganate solutions is safe for consumption. This solution can remove a variety of chemical pesticide residues effectively from fruits and vegetables more than washing or soaking in water. Potassium permanganate will degrade chlorinated solvents, polyaromatic hydrocarbons, phenolics, organo-pesticides and substituted aromatics. For example, the International Information System for the Agricultural

Sciences and Technology (AGRIS), an information management program started by the Food and Agriculture Organization of the United Nations (FAO) reported in 2010, that 0.001% potassium permanganate solution effectively removed pesticide residues from leafy Chinese-kale (Klinhom *et al.*, 2008).

#### **2.4.5 Washing with vinegar**

Using one part of vinegar to three parts of water that can leach bacteria and remove wax together. From the experiment of 4 methods of washing pears and apples by the editor of Cooks Illustrated magazine found that the mixture of vinegar and water (3:1) was better than others ways (antibacterial liquid soap, scrubbing with brush, and using water) when measure amount of bacteria. The mixture of vinegar removed bacteria for 98% . The easy way to do that is to spray vinegar on fruit or vegetables then rinse under tap water. Soaking fruit or vegetables for 10 - 20 min in a vinegar/water solution, then rinse with water (Tomley, 2009).

#### **2.4.6 Washing with detergent**

Washing fruits or vegetables with water do not lift off all of agricultural pesticides. By adding detergent (washing liquid) to water and generously swishing for a few minutes can remove much of the pesticide residue (Tomley, 2009).

#### **2.4.7 Using a commercial fruit cleaner**

There are many kinds of fruit cleaner products in commercial. Lots of cleaners made from natural product e.g.citric acid. These can lift off pesticides 99.9%, cuticle wax of fruit and bacterias (including *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, etc.) (Tomley, 2009).

#### **2.4.8 Fruit and vegetable super cleaner**

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

1) Consist of

- lemon juice           1     tablespoon
- baking soda           2     tablespoons
- water                 250    ml (1 cup)

Mix in a sprayed bottle. Spray on fruit or vegetables and leave for 5 - 10 minutes then rinse with water.

2) Consist of

- lemon juice           1     tablespoon
- white vinegar        2     tablespoons (distilled works best)
- water                 250    ml (1 cup)

Mix in a sprayed bottle. Spray on fruit or vegetables and wipe before eating.

## 2.5 Advanced Oxidation Processes (AOPs)

Nowadays, people are interested in green technology washing which reduce chemical residue without requiring chemical input (Munter,2001). Advanced Oxidation Processes (AOPs) produced hydroxyl radical (OH<sup>-</sup>) by means of chemical, photochemical, photocatalytic, or electrochemical reactions ( Alberici.*et.al*,2013) . It is the effective treatment for wastewater containing high pollutants ( Zapata *et al.*,2010). Photocatalytic processes make use of various oxides (TiO<sub>2</sub>, ZnO, ZrO<sub>2</sub>, CeO<sub>2</sub> etc.) or sulphides of CdS, ZnS etc. as photocatalyst (Rajeswari and Kanmani, 2009). The surface area and number of active sites offered by catalyst for adsorption of pollutant plays an important role in deciding overall rates of degradation ( Ameta *et al.*,2013). Among many catalysts, TiO<sub>2</sub> seems to have the most interesting attributes (Coleman et al.,2000; Daghrir *et al.*,2013) (high stability, good performance and low cost).

Advanced oxidation processes (AOPs) are defined as aqueous phase oxidation processes that can generate hydroxyl radicals (<sup>•</sup>OH) in a sufficient quantity to be able to oxidize majority the complex chemicals ( Arslan and Balcioglic, 2011) present in the target pollutant (Klavarioti *et al.*, 2009). Some oxidation-reduction potential (ORP) of species used in water treatment is shown in Table 2.10.

Hydroxyl radicals are powerful oxidizing reagents 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.

## 2.6 Titanium dioxide photocatalysis

Photocatalytic oxidation process, can be defined as a "catalytic reaction involving the production of a catalyst by absorption of light, is an interesting treatment to decompose toxic ( Linklater, 2008) and hazardous pollutants in the very small quantity (Konstantinou and Albanis, 2002). Photocatalytic processes make use of various oxides (TiO<sub>2</sub>, ZnO, ZrO<sub>2</sub>, CeO<sub>2</sub> etc.) (Martiney *et al.*,2011) or sulphides of CdS, ZnS etc as photocatalyst (Rajeswari and Kanmani, 2009). The overall rates of degradation depend upon many factors, especially surface area and number of active sites of catalyst for adsorption of contaminant. Among the catalysts, TiO<sub>2</sub> shows the most interesting attributes (high stability, good performance, safe for food and cosmetics additives low cost) ( US FDA, 2009). Among several kinds of photocatalytic oxidation, the TiO<sub>2</sub>/UV process is the most respective treatment ( Magara.*et.al.*,1994). Lazar *et al.* (2012) found that the efficiency of TiO<sub>2</sub> photocatalysis depends upon three main factors i.e. 1) different TiO<sub>2</sub> morphologies or surface treatment on TiO<sub>2</sub> can increase photocatalytic efficiency in degradation reactions ( ISK web,2013) . 2) the designed photocatalytic reactors can decrease energy consumption. 3) Coupling the TiO<sub>2</sub> photocatalysis with other water-treatment technologies can be more beneficial, especially in large-scale treatments.

TiO<sub>2</sub> photocatalysis presents a lot of advantages such as 1) high oxidizing ability, as oxidative OH radicals produced by TiO<sub>2</sub> photocatalysis have high oxidation potential shown in Table 2.10, TiO<sub>2</sub> photocatalyst exhibits high oxidizing ability. 2) chemical stability, TiO<sub>2</sub> is chemically stable and not dissolved in water, although some other semiconductor photocatalytic compounds are dissolved when irradiated in water

(Vidal *et al.*,2012;Vilela *et al.*,2012). TiO<sub>2</sub> is so chemically stable that it is not dissolved in almost all acidic, basic and organic solvent. 3) safety, TiO<sub>2</sub> is regarded as the most interesting attributes, since it shows the good performances i.e. safe (Cinkarna, 2012), inert material in general, high stability as eco-friendly alternative technology for keeping the global clean by destruction of pollutants (Lazar *et al.*, 2012). The overall rates of degradation depend upon many factors, especially surface area and number of active sites of catalyst for adsorption of contaminant.

**Table 2.10** Comparison of oxidation potential of various oxidizing agents

Oxidizing agent	Reaction	Oxidation potential (V)
<b>Fluorine</b>	$F_2 + 2e = 2F^-$	2.87
<b>OH radical</b>	$OH\cdot + H^+ + e = H_2O$	2.80
<b>Ozone</b>	$O_3 + 2H^+ + 2e + H_2O = O_2$	2.07
<b>Hydrogen peroxide</b>	$H_2O_2 + 2H^+ + 2e = 2H_2O$	1.77
<b>Manganese peroxide</b>	$MnO_4^- + 8H^+ + 5e = Mn^{2+} + 4H_2O$	1.51
<b>Hypochlorous acid</b>	$HClO + H^+ + 2e + Cl^- = H_2O$	1.50
<b>Chlorine</b>	$Cl_2 + 2e + 2Cl^-$	1.36
<b>Oxygen</b>	$O_2 + 4H^+ + 2e = 2H_2O$	1.23

However, before TiO<sub>2</sub> photocatalytic treatment can be proposed as a general and trouble free method, it is required that the chemistry of various classes of pollutants under these conditions are known in detail.

Since the chemistry of such processes is complex, careful analytical monitoring using different techniques is essential in order to control all transformation steps, to identify harmful intermediates and to understand and interpret the reaction mechanism. The assessment of pollutant disappearance in the early steps is not sufficient to ensure the absence of residual products (Kostedt IV and Magyck, 2006) because the heterogeneous photocatalytic treatment may give rise to a variety of organic intermediates which can themselves be toxic, and in some cases, more persistent than the original substrate

(Konstantinou and Albanis, 2002). TiO<sub>2</sub> photocatalysis is also used for water treatment as a promising technique for pollutant destruction Sabin *et al.*( 1992). Because of the special property of TiO<sub>2</sub> and can mineralize a wide spectrum of pollutants, TiO<sub>2</sub> is still important for utility (Devi *et al.*, 2009). Furthermore, the price is not expensive and simple infestation compare with other system. TiO<sub>2</sub> photocatalysis could degrade many compounds ( Kim *et al.*,2006) that scientists considered on spread out of practical use and business. (Lazar *et al.*, 2012). Different TiO<sub>2</sub> morphologies have been synthesized and found to be effective for the photocatalytic degradation of various compounds. Surface treatment of TiO<sub>2</sub> is another option for increasing catalytic activity (Konstantinou and Albanis, 2002). Ideally, the end products of these processes are carbon dioxide, water and inorganic mineral salts.

### Mechanism and Kinetics

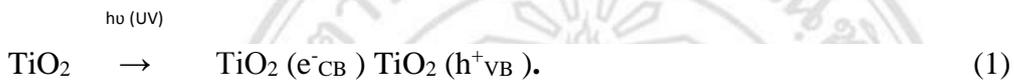
The principles of the photocatalysis process are well established throughout the scientific community ( Tanaka *et al.*,2000) . Two basic types of photocatalysis are heterogeneous and homogeneous types. Heterogeneous photocatalysis can occur by photocatalytic (UV light) and photo assisted degradation (VIS light)(Chun et al,2000). Their briefly principles are given in Figure 2.4, in which the chemical residue is used as the model pollutant (Catellote and Bengtsson.2011). Among these AOPs given in Table 2.10 only TiO<sub>2</sub> photocatalysis will be further presented.

**Table 2.11** Two types of advanced oxidation processes (Lomoră *et al.*, 2011)

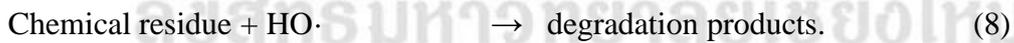
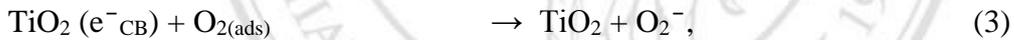
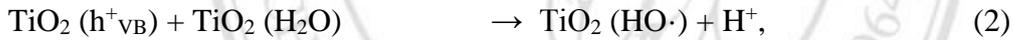
<b>Heterogeneous processes</b>	→ O <sub>2</sub> /TiO <sub>2</sub> /UV
	→ O <sub>3</sub> /TiO <sub>2</sub> /UV
	→ Fe <sup>3+</sup> /TiO <sub>2</sub> /UV
<b>Homogeneous processes</b>	→ Fe <sup>3+</sup> /UV
	→ H <sub>2</sub> O <sub>2</sub> /Fe <sup>2+</sup> /UV
	→ H <sub>2</sub> O <sub>2</sub> /UV
	→ O <sub>3</sub> /UV
	→ Cl <sub>2</sub> /UV

## UV Heterogeneous Photocatalysis

Under UV light at wave length at  $\lambda \leq 400$  nm), photo-excitation initiate in the semiconductor/catalyst substrate ( Goswami *et al.*,2004) . This due to the photons absorption of the TiO<sub>2</sub> semiconductor particles. The radiation of UV-light, containing energy photons which higher (or equal) than the semiconductor band gap energy ( $E_g \approx 3.2$  eV for the anatase TiO<sub>2</sub> polymorphic form), will influence the promotion of an electron (e<sup>-</sup>), from the valence band (VB) to the conduction band (CB). Photo-electrons (photo-e<sup>-</sup>) and photo-holes (photo-h<sup>+</sup>) pairs are therefore generated ( Hachem *et al.*, 2001), according to Eq. (1):



Some of the photo-generated e<sup>-</sup> and h<sup>+</sup> will participate in charge/energy transfer reactions with the adsorbed reactants (dye molecules, oxidants) on the surface of TiO<sub>2</sub> catalyst, giving to the initiation of highly reactive radicals (HO·) and to various intermediary products (Eq. 2-8):



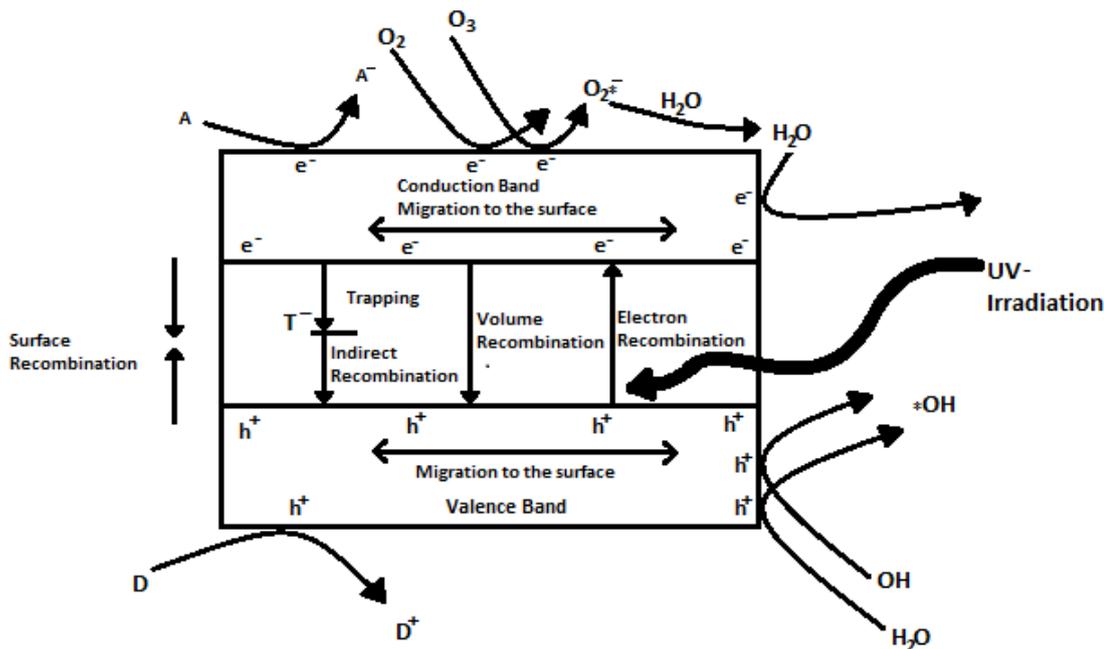
Pollutant photocatalytic degradation in aqueous solutions by TiO<sub>2</sub> powder is simplified mostly by a hydroxylation reactions series induced by hydroxyl radicals (•OH). Illumination of UV initiates TiO<sub>2</sub> photocatalyst electron-hole pairs. Positive charges holes produce •OH and H<sup>+</sup> ions by contacting with water molecules. Superoxide ions (O<sub>2</sub><sup>•-</sup>), forming by reaction between electron and dissolved oxygen, react with molecules of water to produce hydroxide ions (OH<sup>-</sup>) and peroxide radicals (•OOH), which react with H<sup>+</sup> ions to produce •OH and OH<sup>-</sup>, and holes oxidize OH<sup>-</sup> to •OH Reyes *et al.*(2006) . Hence, all of these finally simplify the production of •OH which attack the pollutants in aqueous solution (Lazar *et al.*, 2012).

The photocatalytic reactors design is basis area when earnestly research is in process (Reyes *et al.*, 2006). An ideal photocatalytic reactor should be easy, energy efficient, low-priced to build and operate, and able to manage high volumes of wastewater. Reactors operating with solar radiation will hold great promise. Coupling TiO<sub>2</sub> photocatalysis with other technologies has great potential in large-scale water treatment, and further research is necessary (Konstantinou and Albanis, 2002). It is proved that conduction band electrons (e<sup>-</sup>) and valence band holes (h<sup>+</sup>) are generated when aqueous TiO<sub>2</sub> suspension is irradiated with light energy greater than its band gap energy. The photogenerated electrons could reduce the organic substrate or react with electron acceptors such as O<sub>2</sub> adsorbed on the Ti surface or dissolved in water, reducing it to superoxide radical anion O<sub>2</sub><sup>•-</sup>. The photogenerated holes can also oxidize either the organic molecule directly, or the OH<sup>-</sup> ions and the H<sub>2</sub>O molecules adsorbed at the TiO<sub>2</sub> surface, to OH<sup>•</sup> radicals. Together with other highly oxidant species (peroxide radicals) they are reported to be responsible for the heterogeneous TiO<sub>2</sub> photodecomposition of organic substrates. According to this, the relevant reactions at the semiconductor surface causing the degradation of pesticides can be expressed as follows:



It is well established that conduction band electron (e<sup>-</sup>) and valence band holes (h<sup>+</sup>) are generated when aqueous TiO<sub>2</sub> suspension is irradiated with light energy greater than its band gap energy (Eg. 3.2 eV) (Konstantinou and Albanis, 2004). The photogenerated hole/electrons pairs participate to the reactions. The resulting ·OH radical, being a very strong oxidizing agent (standard redox potential +2.8 V) can oxidize most of pesticides to the mineral end products. Substrates not reactive toward ·OH radicals are degraded employing TiO<sub>2</sub> photocatalysis with rates of decay highly influenced by the valence band edge position. Several intermediates of the photocatalytic degradation have been identified corresponding to oxidation, dechlorination if chlorine substitute is present in aromatic ring, and hydrolysis of the ester functional group. Oxidant attack of the ·OH on the P=S bond occurred firstly in the case of phosphorothioates, resulting the formaion of oxon derivatives. The continuous attack of ·OH followed by the rupture of

P-O bond, resulted in the formation of the corresponding phenols and different trialkyl and dialkyl phosphorothioate or phosphate esters.



**Figure 2.4** Schematic representation of the mechanism of  $\text{TiO}_2$  photocatalysis ( Kumar, 2013 )

## 2.7 Bioassay systems and techniques (Bheem, 2012)

This technique found on the biological method by using laboratory animals (mouse, rat, guinea pig and rabbits etc.), plant inoculation (using plant component to treat a sample such as haemolytic activity) microbiological or cell based assay (microorganism; bacterias, fungi, actinomyces or cultured cells for antibiotic screening etc.). For these techniques they can be derived into three parts as follow;

### 2.7.1 *In vivo* techniques

The objective of theses techniques to study on response of treatment in living laboratory animals directly. Rodent (mouse and rat) and rabbit etc. are used as inoculated animals.

### **2.7.2 *In vitro* techniques**

*In vitro* operates on cell culture of considerable biological system to examine the result of treatment under the condition that different from normal environment. These testing units (stem cells, cell culture and microbes) will be incubated in cultured media along the experiment.

### **2.7.3 *Ex vivo* techniques**

The experiment launch on cells or tissues to examine the result of treatment under suitable condition within the considered time of survived organ 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 treatment. Then, organ is isolate to equipment to study the result of treatment within survival time of the organ outside body with arrangement only necessary factors for maintain cell integrity e.g. oxygen, glucose and isotonic salts.

## **2.8 Brine shrimp bioassay for chemical toxicity test**

Brine shrimp lethality bioassay is a simple, high throughput cytotoxicity test of bioactive chemicals. It is based on the killing ability of test compounds on a simple zoological organism-brine shrimp (*Artemia salina*) ( Nunes *et al.*,2006) . The brine shrimp lethality bioassay is widely used in the evaluation of toxicity of heavy metals, pesticides, medicines especially natural plant extracts and etc (Wu, 2014).

*A. 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 (Sorgeloos, 1978). 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. It feeds on

microorganisms such as algae and bacteria and helps rid the lake water of contaminants 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 month (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<sub>50</sub> values for bisexual populations were 0.95 mg L<sup>-1</sup> significantly different from other strains. In addition, Sánchez-Fortún and Barahona (2009) studied on toxicity and characterization of cholinesterase-inhibition induced by diisopropyl fluorophosphate in *A. 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.