CHAPTER II

LITERATURE REVIEW

2.1 Nile tilapia

Nile tilapia is an important aquatic economic animal in Thailand. Consumption and export of Nile tilapia has expanded recently with more than half of total fresh-water fish export consisting of tilapia, as shown in Table 2.1 (Customs Department, 2007). Farmers prefer to raise Nile tilapia because it is easy to grow, resistant to diseases, fast to harvest, and can tolerate a wide range of environmental conditions such as low dissolved oxygen, high salinity, high water temperature, and high ammonia concentrations (Macintosh and Little, 1995; Green et al., 1997; and Popma and Masser, 1999).

Table 2.1 Export value of Nile tilapia and total fresh water fish

Year	Total export Nile Tilapia		Total export fresh water fish	
	Quantity (kg)	Value (Baht)	Quantity (kg)	Value (Baht)
2002	3,245,499	181,274,507	6,225,866	352.039.808
2003	4,708,958	300,833,379	6,428,169	402,718,871
2004	7,798,012	482,916,939	10,014,614	618,126,201
2005	11,014,276	625,937,383	15,759,252	1,082,533,693
2006	14,948,984	784,142,594	20,904,125	1,352,711,402

Source Customs Department, 2007

2.1.1 Characteristics of Nile tilapia

Nile tilapia, is the generic name of a group of cichlids endemic to Africa. The cichlids group consisting of three aquaculturally important genera *Oreochromis. Sarotherodon* and *Tilapia* (see Figure 1), is mostly found in the Nile river of Africa. Nile tilapias have high tolerance and can adapt to any climate. It is able to live in both fresh water and brackish water with 20 g/L of salinity, pH 6.5-8.3, and can tolerate up to 40 degree Celsius. Nile tilapia are not tolerant of the temperature lower than 10 degree Celsius since its native land is located in the tropics.

2.1.2 Masculinization of Nile tilapia

Due to the high market demand for Nile tilapia, the yield can be increased by the inducing the sex of the fish. Male fish are bigger in size and grow faster than females (Penpan, 2004; Francesc, 2001). There are many techniques to induce the sex of the fish including an interspecific hybridization, breeding supermale tilapia (genetically-altered male tilapia, (GMT)), and using hormones such as methyltestosterone (MT). Among these techniques, the use of hormone-treated feed for the production of all-male populations is the most popular in tilapia aquaculture (Macintosh and Little, 1995).

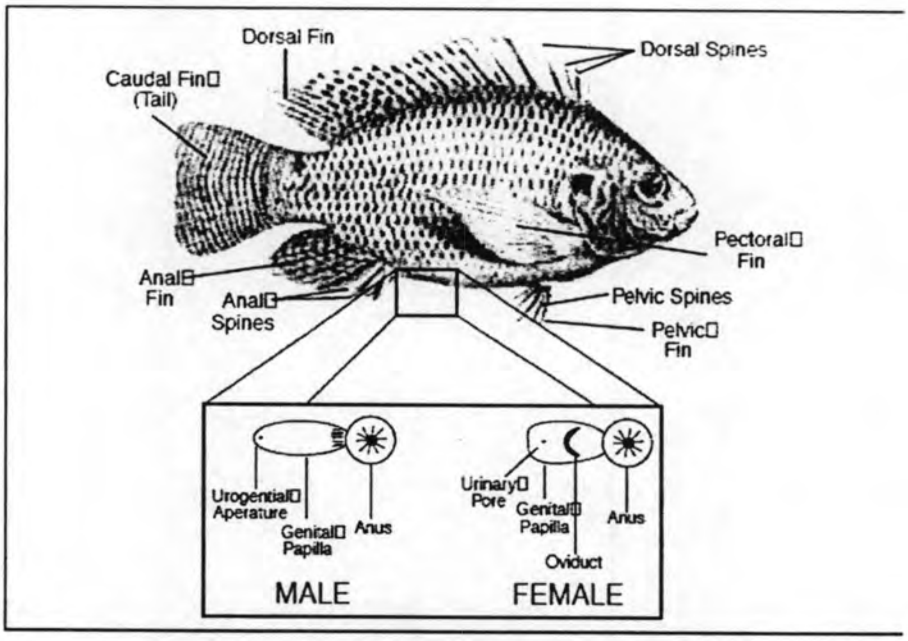

Figure 2.1 Components of Nile tilapia

There are several ways to administer the hormones to the fish. These include dipping the fish in a hormone solution, feeding the fish with hormone capsules or mixing hormone with the fish food. To change sex from female to male, several androgen hormones (male hormone) such as 19-norethyltesterone, fluoxymestirone, ethyltestosterone, and MT are used. Mixing with the feed is the most effective way for breeding fish in Cichlidae order. A common practice to induce the sex of tilapia is to use MT. MT is highly effective in changing the fish sex and the technique used is easy, and is low cost (Kuwaye et al., 1993; Penpan, 2004). More than 90% of fish can be changed to male after treatment (Kriangsak et al., 2006).

Fish farmers typically used about 0.06 mg of MT for every 1 kg of feed during the first 30 days of the fish breeding. The total amount of feed used for feeding the fry is dependent on the age of the fry. The frys are fed with the mixed food 5 times a day after the yolk sac start to disappear (3-4 days). In the first week, the amount of feed provided is approximately 30% of body weight of the fish. In the second week, the amount of feed provided is about 20% of body weight of the fish and the amounts used is decreased further to 15% of the body weight of the fish in the third week.. Using this procedure, the percentage of male proportion after 30-60 days is between 86 and 100% (Kere, 1999; Penpan, 2004).

2.1.3 Breeding ponds for masculinization of Nile Tilapia fry

The majority of the ponds for breeding Nile tilapia in Thailand are clay ponds, cement ponds, and nylon fish pot nets inside a pond or river (called Kra chang). Clay pond are rectangular in shape with an area of about 50-1,600 m^2. The depth of the water is about 1 m. Since this type of pond has similar conditions to a original pond, this type of pond is the most effective in term of productivity. The second type of ponds used is a rectangular or round cement pond with an area more than 10 m^2. The depth of the water is approximately 80 cm. For this type of pond, an aerator is needed to increase the production of tilapia. The third type of ponds used is fish pot nets made of inside a clay pond, original pond, or original body of water. The size of the nylon pot is around 5 m x 8 m x 2 m, with the height of water inside the net at about 1 m. Pillars are used to support the net at 4 corners.

2.2 Methyltestosterone

MT is an anabolic steroid that has a structure similar to testosterone (original androgen hormone). MT was developed as an oral form of testosterone without loss of bioavailability. The difference between the 2 hormones is the methyl function group in the 17 position. The testosterone itself is ineffective for oral use since it, is metabolized and destroyed by the liver during the "first pass" so that only 5-10% of testosterone enters the blood and becomes effective. MT is not broken down and can survive the first pass through the liver, and is effective as an oral agent, rather than being destroyed by the liver.

The action of MT is somewhat androgenic, with a moderate anabolic effect. As is typically seen with 17-alpha methylation, the resulting MT steroid has lower anabolic activity than its parent testosterone.

2.2.1 The chemical structure of Methyltestosterone

The chemical name of MT is 17β-Hydroxy-17α-methylandrost-4-en-3-one and synonyms are methyltestosteronum and methyltestosteronum, with the molecular formula of $C_{20}H_{30}O_2$. The chemical structure is presented in Figure 2.2, while the general physical-chemical properties of MT are shown in Table 2.2.

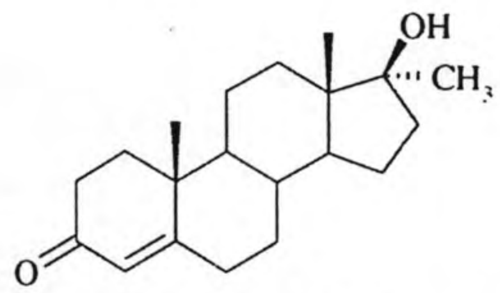

Figure 2.2 Chemical structure of 17 alpha-methyltestosterone

Besides masculinization of Nile Tilapia, MT is used to treat men with a testosterone deficiency and to treat woman with breast cancer, breast pain, swelling due to pregnancy, and with the addition of estrogen, it is used to treat symptoms of menopause. Anabolic steroids have been the subject of drug misuse and abuse, often producing adverse effects such as increased risk of cardiovascular disease, and antisocial behavior. Some of the masculinizing effects in women can be irreversible (Michael et al., 1998).

Table 2.2 Properties of methyltestosterone

Properties*	**Methyltestosterone**
Molecular Formula [a, f]	$C_{20}H_{30}O_2$
Cas Number. [f]	58-18-4
Molecular weight [f]	302.45
Color/Form [b, f]	White or creamy white crystals or crystalline powder
Odor [b, f]	Odorless
Melting point [a, f]	162-168 °C
Vapor Pressure [e]	1.85×10^{-8} mmHg at 25°C
Solubilities [a, b, d, f]	- Soluble in methanol, ethanol, ether and other organic solvents - Sparingly soluble in vegetable oil - In water: 3.4 mg/L at 25 °C
Octanol/Water Partition Coefficient (log Kow) [c]	3.36
Henry's Law constant [e]	4.7 x 10-9 atm-m^3/mole at 25 °C
Hydroxy radical reaction rate constante [e]	1.0 x 10-10 cm^3/mole-sec at 25 °C
Ozone reaction rate constante [e]	1.1 x 10-17 cm^3/mole-sec at 25 °C

[a] O'Neil, (2001) [b] Lewis et al. (1997) [c] Hansch et al. (1995) [d] Yalkowsky et al. (2003) [e] US EPA (2003)
[f] Sigma-Aldrich material safety data sheet

2.2.2 Adverse effect of Methyltestosterone

Anabolic steroids may be carcinogenic. They can stimulate growth of sex-hormone dependent tissue, primarily the prostate gland in men. Precocious prostatic cancer has been described after long-term anabolic steroid abuse (Roberts & Essenhigh., 1986). Cases of hepatic cancers associated with anabolic steroid abuse have been reported (Overly et al., 1984).

Chronic ingestion of high doses of anabolic steroids can cause elevations in blood pressure, left ventricular hypertrophy and premature coronary artery disease (McKillop et al., 1986; Bowman., 1990; McNutt et al., 1988). Orally active anabolic steroids can cause abnormalities of hepatic function, manifest as abnormally elevated hepatic enzyme activity in biochemical tests of liver function, and sometimes as overt jaundice. The histological abnormality of peliosis hepatics has been associated with anabolic steroid use (Soe et al., 1992), Angiosarcoma (Falk et al., 1979) and a case of hepatocellular carcinoma in an anabolic steroid user has been reported (Overly et al., 1984). The effects of MT are also shown in the reduction of Insulin secretion and on the hepatic function in woman (Michael et al., 1998).

2.3 Pathway of Methyltestosterone to environment

Drugs are commonly administered as medicated feed, injection or in the case of local applications as a bath formulation. Hormone treatment is also administered in a similar manner. Since feed mixed with MT are not completely consumed in the breeding ponds, it is possible that MT can be released from these ponds and contaminate the environment and impact other aquatic organisms. When infected by MT, cultured fish show reduced appetite and thus feed intake. Therefore, a proportion of the medicated feed that is not eaten by the fish passes through the cages and may be distributed or disperse to the surrounding areas of the fish farm. It is also probable that uptake of the hormone by the fish may be low resulting in the hormone entering the environment via feces and urine (Rinchard et al., 1999). In recent years improved husbandry practices have reduced the amount of waste feed generated. Nevertheless, deposition of drugs from uneaten feed or faeces in under-cage sediments can be a major route of environmental contamination for pharmaceuticals and hormones used in aquaculture (Lunestad, 1992; Jacobsen and Berglind, 1988). A study by Fitzpatrick et al., (1999) on soils exposed to MT-impregnated food showed that the MT concentration in soil were 2.8-2.9 ng/g even after more than 2 months of exposure. In addition, the sludge recovered from the fish ponds may be applied directly to land which may become a potential pathway for the hormones to enter environment and the aquatic environment (Boxall et al., 2004).

2.4 Effect of Methyltestosterone to environment

Steroid hormones are frequently detected in the environments and are likely to have endocrine disrupting effects on aquatic wildlife at concentrations in the nanogram per liter range (Hanselman et al., 2003, Sumpter and Johnson, 2005). Endocrine disrupting chemicals (EDCs) have been defined as "exogenous agents that interfere with the production, release, transport, metabolism, binding, action, or elimination of the substance in the body of an organism responsible for the maintenance of homeostasis and the regulation of developmental processes" (Kavlock, 1991). The potential endocrine effects of estrogens, such as vitellogenin production and feminization of male fish, have been well documented (Jobling et al., 1998, Panter et al., 1998). Although the study of estrogens has received considerable attention, much less effort has been directed at studying the potential endocrine-disrupting effects of androgens such as testosterone. Information of androgens in the environment is limited, but aquatic organisms downstream of pulp and paper mills have demonstrated biological responses consistent with exposure to these substances, including masculinization of female fish (Howell and Denton, 1989; Thomas et al., 1989).

Besides their potential impact on the internal physiological signal pathways, steroid hormones also do seemed to have a disrupting effect on the chemical signaling pathways between different organisms. These external signaling pathways include fundamental processes such as the nodulation in leguminous roots mediated by phytoestrogens (Fox, 2004). Phytoestrogens like the flavonoid luteolin act as recruiting signals to attract soil bacteria of the genus *Sinorhizobium*, responsible for the symbiotic nitrogen fixation.

2.5 Adsorption and Absorption

Adsorption is the accumulation of molecules at the interface of two phases. Adsorption plays a critical role in the transport, bioavailability, and fate of contaminants in both original and engineered aquatic systems (Benjamin, 2002). For instance, adsorption can cause both nutrients and contaminants to accumulate in soils or sediments, rather than remaining in the aqueous phase that passes through such systems. These reactions determine whether the absorbable species are available to organisms and for chemical reactions they might undergo. Absorption is the uptake of the contaminant into the physical structure of the solid or the incorporation of the contaminant within the structure of the sorbent. When both absorption and adsorption are occurring or it is not possible to distinguish between the two processes, the term sorption is the commonly used.

The sorption behavior of substances in soils can be described through experimentally-determined parameters. An important parameter is the adsorption coefficient which is defined as the ratio between the concentration of the substance in the soil/sludge and the concentration of the substance in the aqueous phase at adsorption equilibrium.

2.5.1 Factors affecting for sorption

2.5.1.1 Solubility

Adsorption of a solute is inversely proportional to its solubility in the solvent. The greater the solubility, the stronger the solute-solvent bond and the smaller is the extent of adsorption. MT has a solubility of 3.39 mg/l indicating that it has a tendency to be highly adsorbed in soil and sediment.

2.5.1.2 Temperature

Adsorption reaction are normally exothermic, thus the extent of adsorption generally increases with decreasing temperature.

2.5.1.3 Salinity

An increase in salinity can significantly lower the adsorption of polar or slightly polar compounds. Normally, neutral molecules are generally less affected by salinity, but often show an increased adsorption with increasing salt concentration. The salt in the solution increases the ionic strength of the solution and, if the chemical is non-polar, the salt in the solution can cause the nonpolar compounds to be preferentially transfer from the solution to nonpolar surfaces on the soils or sediments or may result in a decrease in the aqueous solubility of the nonpolar compound which favors sorption.

2.5.1.4 pH

Only chemicals that tend to ionize are much affected by pH. One of the influences of pH is the change in the character of the surfaces. For example, at low pH, humic materials are nearly neutral and more hydrophobic. Changes in pH will affect the organic acids and bases of the humic material. As hydrogen and hydroxide ions are adsorbed quite strongly, the adsorption of other ions will be influenced by the pH of the solution. When the pH is changed, surface charges also change and the sorption of charged species will be affected.

2.5.1.5 Soil Texture

Soils with mostly clay and organic matter tend to hold water and preferentially adsorbed dissolved chemicals. Clay especially intermixed with organic materials, adsorb most out of the three main soil textures (clay, silt, and sand) because of its small particle size, high surface area, and high surface charge.

2.5.1.6 Organic Matter Content

Soil organic matter influences how much water a soil can hold and how well it will be able to adsorb chemicals. Increasing the soil's organic content increases the soil's ability to hold organic chemicals. A study by Lee *et al.*, 2003 using batch experiments to examine the sorption and dissipation of three hormones (testosterone, 17 β-estradiol, and 17α-ethynyl estradiol) in five types of soils showed that the sorption coefficient K_d of each hormone were directly proportional to the organic content of the soil.

2.5.1.7 Soil Permeability

Soil permeability is a measure of how fast water can move downward through a particular soil. Water moves quickly through soils with high permeability and therefore transport dissolved chemicals faster with the percolating water.

2.5.1.8 Surface Area

Adsorption is a surface phenomenon and the extent of adsorption is directly related to surface area of the sorbent. An increase in surface area typically would increase the adsorption of molecules especially polar molecules.

2.5.2 Sorption Coefficient (K_{oc})

Since the sorption of nonpolar organic compounds are affected by the amount of organic material present, a common generalized expression of sorption coefficient is the organic carbon sorption coefficient, K_{oc}. The K_{oc} is given by:

$$K_{oc} = \frac{K_d \times 100}{\%\ \text{organic carbon}} \qquad \text{Equation (1)}$$

where

K_{oc} = Sorption coefficient normalized with organic carbon content (L/kg)

K_d = Sorption Distribution coefficient (L/kg)

The adsorption coefficient normalized to the organic carbon content of the soil, is a useful indicator of the binding capacity of a chemical onto organic matter of soil and sewage sludge. The K_{oc} allows for comparisons to be made between different chemicals or different soil/sludge. This parameter can be estimated using various correlations relating the water solubilities and the n-octanol/water partition coefficients of organic compounds (Kümmerer, K., 2004).

2.6 Adsorption isotherms

Adsorption is usually described using isotherms which relates the amount of adsorbate sorbed on the adsorbent and the aqueous equilibrium concentrations.

2.6.1 Linear isotherm

When sorption of a compound is directly related to the aqueous concentrations, the sorption can be modeled using a linear isotherm. The distribution can be described using the linear distribution coefficient (K_d). K_d is defined as the ratio of mass sorbed per unit mass of the sorbent and the aqueous equilibrium concentration and is a dimensionless value when concentrations in both phases are expressed on a weight/weight base. When the concentration in the aqueous phase is given on a weight/volume base then the units are $ml \cdot g^{-1}$. The equation is given below (Hemond and Fechner-Lery, 2000):

$$K_d = \frac{C_{soil}}{C_{aq}} \qquad \text{Equation (2)}$$

where

C_{soil} = Concentration of substance in soil at equilibrium, $[g_{substance}/g_{soil}]$

C_{aq} = Concentration of substance in aqueous phase,

$[g_{substance}/g_{aqueous}\ or\ ml_{aqueous}]$

2.6.2 Freundlich isotherm

When the mass sorbed per unit mass of sorbent with respect to the aqueous equilibrium concentrations is nonlinear, an empirical Freundlich model can be used to describe the adsorption. The Freundlich model is given by:

$$C_s = K_f\ C_w^{\ n} \qquad \text{Equation (3)}$$

Where:

n	= Freundlich exponent
K_f	= Freundlich adsorption constant
C_s	= Concentration of substance in soil at equilibrium, $[g_{substance}/g_{soil}]$
C_w	= Concentration of substance in aqueous phase, $[g_{substance}/\ mL_{aqueous}]$

The parameters in the Freundlich model can be determined by taking the logarithm and rearranging equation (3):

$$\log q = \log K_f + n \log C_w \qquad \text{Equation (4)}$$

The coefficients K_f and n can be estimated from slope of the line fitted to a graph of log C_s versus log C_w (Bohn, H.L. et al., 2001).

2.6.3 Langmuir isotherm

Langmuir derived a relationship to describe an adsorption isotherm by making several reasonable assumptions. These assumptions include a uniform surface, a single layer of adsorbed material, and no interactions between sorbed molecules. The Langmuir isotherm is given by:

$$C_s = \frac{C_{s.m}KC_w}{1 + KC_w} \qquad \text{Equation (6)}$$

where

C_s	= Concentration of substance in soil at equilibrium, $[g_{substance}/g_{soil}]$
$C_{s.m}$	= Concentration of substance in soil at a complete of monolayer, $[g_{substance}/g_{soil}]$
C_w	= Concentration of substance in aqueous phase, $[g_{substance}/\ mL_{aqueous}]$
K	= Langmuir's sorption affinity [L/mg]

The Langmuir parameters can be determined by taking the reciprocal of the Langmuir equation and plotting $1/C_s$ versus $1/C_w$ as shown below;

$$\frac{1}{C_s} = \frac{1}{C_{s.m}} + \frac{1}{KC_{s.m}C_w} \qquad \text{Equation (7)}$$

The slope of the plot is equal to $1/K_aq_m$ and the intercept equal to $1/q_m$ (Bohn, H.L. et al., 2001).

2.7 Sorption of hormones

From the previous study, several studies indicated that sorption of the four hormones under consideration is correlated with the presence of organic carbon and clay content. Lee *et al.* (2003) used batch experiments to examine the sorption and dissipation of three hormones (testosterone, 17 *β*-estradiol, and 17α-ethynyl estradiol) in four types of soils and freshwater sediment and found that the Kd of each hormone increased with increasing organic content of the soils or sediments. Casey et al., (2003) used a series of batch sorption and miscible-displacement model experiments to study the sorption and transport of radiolabeled [^{14}C]17*á*-estradiol in field soils. Their results indicated that sorption was correlated to the mineral particle size and organic matter content. The higher the organic carbon and clay present in the soil or sediment, the higher the quantity of steroid hormones sorbed to the soil or sediment (Casey *et al.*, 2005; and Mansell *et al.*, 2004.).

It should also be noted that not only the presence of organic carbon is a condition for sorption of the steroid hormones but also the presence of salinity may increase sorption of these compounds. For example, in study of Lai *et al.*, (2000), the present of saline water was found to increase the sorption of estrogen in the water phase. The effect of salinity can be implied from a study of Rios et al., (2004) where sorption of residual oils to soils were determined. The K_d values was found to increase with increasing age of spill (age of the oil residual) and soil salinity and decrease when the salinity of the initial aqueous concentration was greater than the soil salinity. And also the result from research of Bowman et al. (2002), the Kd of oestrone is slight increase from 74 mL/g to 114 mL/g at the salinity 0.1 to 33.2.

In the most of the hormone sorption studies, high initial concentrations were applied to ensure a measureable concentration range above the detection limit of the analytical technique. However, the initial concentration affects the sorption rate. At high concentrations rather than at the environmentally found concentrations, sorption equilibrium can be reached within a day. However, studies were conducted at concentration 20 times lower than their solubility limits (S), equilibrium was reached within two weeks (Yu *et al.*, 2004). A study by Kim et al., (2007) on the sorption of two male hormones, testosterone and androstenedione, by four soil and sediment samples showed that sorption equilibrium was achieved within one-two weeks for an initial hormone concentration of 10,000 µg/L or about 30% of their solubility limits and two to three weeks when the initial concentration was 300 µg/L or less than 1% of their solubility limits. Their study showed that the sorption of testosterone and androstenedine was nonlinear. The Freundlich distribution coefficient, log K_f, values were 2.3 to 3.7 and the parameter n ranged from 0.698 to 0.899 for all soil–solute systems. This study suggests that the sorption rates of male hormones may continue over 14 d or longer and when concentrations fall into the ng/L range those soils and sediments may have larger sorption distribution coefficients than at higher concentrations (Kim et al, 2007). Isotherm nonlinearity leads to an inverse correlation between single-point organic carbon–normalized sorption distribution coefficients (K_{OC}) and equilibrium androgen concentration (C_e). When $C_e/S_w = 0.012$, the log K_{OC} values for testosterone and androstenedione on the various sorbents ranged from 6.18 to 6.75 and 6.83 to 6.04, respectively, compared to 6.30 to 6.80 and 6.16 to 6.92 when $C_e/S_w = 0.004$.

2.8 Summary

In the presently, the consumption and export of Nile tilapia are increasing from a lot of its advantages. As the raising the tilapia, many ways to produce an all male population of Nile tilapia are presented. The common and popular ways is using of male-inducing hormones by dipping them into a solution containing the hormone or by mixing the hormones with fish feed. MT is the most common hormone used and is effective, easy to use, and of low cost. More than 90% of fish can be changed to male after hormone treatment (Kriangsak et al., 2006; Kere, 1999; Penpan, 2004).

It has chance that MT is sorbed onto the sediments of the fish pond and may contaminate the environment and impact other aquatic organisms, if the sediments are released. However, deposition of MT from uneaten feed or faeces in under-cage sediments can be a major route of environmental contamination for MT used in aquaculture (Lunestad, 1992; Jacobsen and Berglind, 1988). From the study of Fitzpatrick et al:, (1999), MT remained in soils at concentrations between 2.8 and 2.9 ng/g, eight weeks after ending treatment with methyltestosterone-impregnated food.

MT is also the steroid hormones are present as Endocrine disrupting chemicals (EDCs). A numerous of potential endocrine effects on the study of estrogens, nevertheless much less effort has been directed at studying the potential endocrine-disrupting effects of androgens such as testosterone. Information of androgens in the environment is limited, but aquatic organisms downstream of pulp and paper mills have demonstrated biological responses consistent with exposure to these substances, including masculinization of female fish (Howell and Denton, 1989; Thomas et al., 1989).

Furthermore, MT is anabolic steroids, the effect of hormone to human are reported as the carcinogenic. The abuse of anabolic steroid can stimulate precocious prostatic cancer (Roberts & Essenhigh., 1986) and also the hepatic cancers (Overly et al., 1984). Several study of the anabolic steroids effect was presented in study of McKillop et al., 1986; Bowman., 1990; McNutt et al., 1988; Soe et al., 1992; Falk et al., 1979. In the addition, the anabolic steroids can cause elevations in blood pressure, left ventricular hypertrophy and premature coronary artery disease and also shown in the reduction of Insulin secretion and on the hepatic function in woman (Michael et al., 1998).

Since feed mixed with MT are not completely consumed in the breeding ponds, it is possible that MT can be released from these ponds and contaminate the environment and impact other aquatic organisms. Nevertheless, deposition of drugs from uneaten feed or faeces in under-cage sediments can be a major route of environmental contamination for pharmaceuticals and hormones used in aquaculture (Lunestad, 1992; Jacobsen and Berglind, 1988). In addition, the sludge recovered from the fish ponds may be applied directly to land which may become a potential pathway for the hormones to enter environment and the aquatic environment (Boxall et al., 2004).

From the study of Fitzpatrick et al., (1999) and a several study in the fate and transport of hormone, it shows the possibility of MT to sorbed on to the soil and the sediment. However, there are not much information on the sorption of MT onto sediments and soils. The study on the sorption of MT are necessary. In the addition, Many factor that have effect to the soption of MT, so the effect of many factor to the sorption of MT also needed to study.