CHAPTER II

LITERATURE REVIEW

2.1 Nile Tilapia

Nile tilapia is the generic name of a group of cichlids endemic to Africa (see Figure 2.1). The cichlids group consists of three aquaculturally important genera *Oreochromis, Sarotherodon* and *Tilapia*. Tilapia is more tolerant than most commonly farmed freshwater fish to low dissolved oxygen high salinity, high water temperature, and high ammonia concentrations. In many states in the U.S., tilapia is considered to be an exotic or non-indigenous species and their transport and culture restricted. Worldwide harvest of tilapia, has now surpassed 800,000 metric tons per year and second only to carps as the most widely farmed freshwater fish (Popma and Masser, 1999).
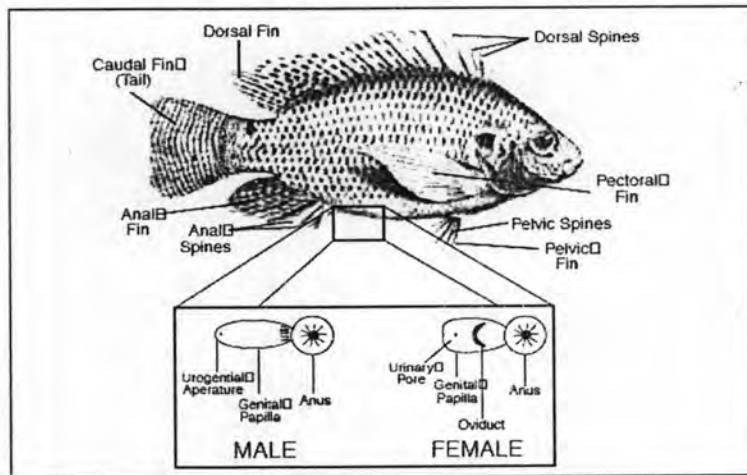

Figure 2.1 Components of Nile tilapia

To enhance growth potential and productivity, Nile tilapia are grown as all-male populations since no energy is shunted toward reproduction and there is no competition with younger fish (Green et al., 1997). Moreover, males grow to a larger final size than females (Macintosh et al., 1995). There are several techniques for masculinizing Nile tilapia. They include, physical sex characterization between male and female fish

(manual sexing); interspecific hybridization; genetically male tilapia (supermale tilapia), and; hormone-induced sex reversal by androgenic hormones.

2.2 Masculinization of Nile Tilapia Using MT

There are two routes for masculinization of Nile tilapia using MT. The first way is oral administration by feeding tilapia fry with MT-impregnated foods at a concentration of 60 mg MT/kg food approximately 5 times per day. The amount of food used for feeding fry is dependent on the age of the fry. In the first week, the amount of food fed is about 30 % of their body weight. It is reduced to about 20 % and 15 % during the second and their third week, respectively. By using this protocol, the percentage of male tilapia in a musculinization tank is about 86-100 % (คีรี, 2542; เพ็ญพรรณ, 2547). The second way is a single immersion treatment of fry after hatching for 14 days post hatch (DPH) with MT at a concentration of 1800 µg/l. This method gives a male tilapia population of 90 % (Gustavo et al., 2003)

2.3 Masculinization Ponds of Nile Tilapia Fry

There are 3 types of ponds for masculinizing Nile tilapia fry. The first is rectangular shaped clay pond with an area of about 50-1,600 m^2. The water retention height is about 1 m. This type of pond is the most effective in term of productivity because the condition in this pond is similar to that in a natural pond. The second is a rectangular or round cement pond, with an area more than 10 m^2. The water retention height is approximately 80 cm. For this type of pond, an aerator is needed to increase the production of tilapia. However, the cost of this type of pond may be more expensive because its size. The third type is using nylon netting as fish a pot inside a clay pond, natural pond, or natural receiving 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.

2.4 Masculinization of Other Fish Using MT

There are many species of fish and aquatic animals where their sex can be reversed to male by MT. For example, the effects of MT on sexual development and reproductive

performance in the fathead minnow (*Pimephales promelas*) were assessed and found to be 95% male when expose to more than 50 µg/l of MT (Pawlowski, 2004). There are many routes of exposure of MT for masculinization depending on species of fish, fish age, concentration of MT, and exposure time.

MT is effective in inducing sex inversion by oral administration of different groupers such as *Epinephelus tauvina*, *Mycteroperca microlepis*, and *E. fario*, pellet implantation in *E. tauvina*, and *E. fario*, and by injection in juvenile *E. suillus* (*E.coioides*). (Gerald et al., 2001)

The effectiveness of 30 or 50 mg of MT/kg diet for sex-reversal of juvenile largemouth bass, *Micropterus salmoides*, was evaluated for periods of 4, 6, or 10 weeks starting when the fish were 25 mm total length. Chronic effects of MT on the reproductive status of medaka (*Oryzias latipes*) by observation showed that parental fish in the 27.75 ng/l treatment group exhibited male secondary sex characteristics in which no fish with ovary could be discerned. In addition, exposure of parental fish and F_1 generations treated with 9.98 mg/l showed male secondary sex characteristics. Swollen abdomens were observed in parental fish and F_1 female fish exposed to 9.98-ng/l. These swollen abdomens were induced by enlarged ovaries and were accompanied with declined fecundity and fertility in the F_0 generation. These results indicate that MT reduces the reproductive potential of medaka and that this species can be used to conducted a fish full life-cycle test (FFLC) to evaluate androgens (Masanori et al., 2004).

2.5 General information on steroids

Steroids are isoprenoic compounds that are of great importance in biology, medicine, and chemistry and are found in multiple forms in the environment (Figure 2.2). One class comprises of sterols with cholesterol (Figure 2.2-5) as the main compound in animals required to build and maintain the cell membranes. Plant sterols (phytosterols) such as β-sitosterol (not shown) also act as structural components in herbal cell membranes. Another class is steroid hormones derived biosynthetically from cholesterol, including estrogens (estradiol (Figure 2.2-1) and estrone (Figure 2.2-2)) and the androgens (testosterone (Figure 2.2-3)) and 4-androstene-3, 17-dione (Figure 2.2-4). These signal compounds regulate metabolism, growth and reproduction in vertebrates. Estradiol,

formed by the ovary and placenta, is the most potent naturally occurring steroid hormone in female organisms whereas testosterone is the most important one in male organisms, formed by the testis (Koolman et al., 1998).
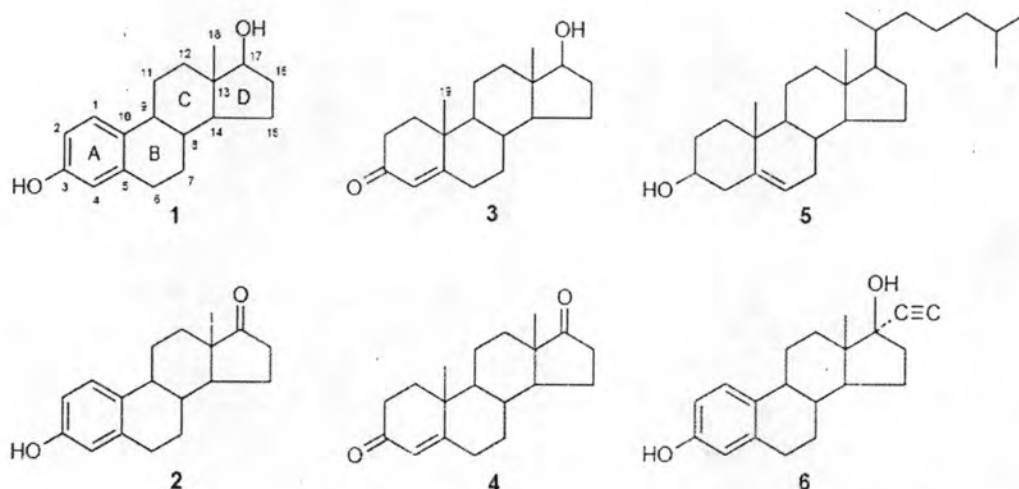

Figure. 2.2 Molecular structures of estradiol (1), estrone (2), testosterone (3), 4-androstene-3, 17-dione (4), cholesterol (5), and ethinyl estradiol (6).

Like their biosynthetic precursor, cholesterol, the four rings of the steroid skeleton of estrogens and androgens are in trans-trans-trans conformation (Breitmaier and Jung, 1995). One main difference between cholesterol and steroid hormones is the absence of the aliphatic side chain (Figure 2.2). The aromatic ring A in estradiol shows phenolic properties. Oxygen-dependent aromatization of testosterone to estradiol results in the removal of the methyl group 19 between ring A and B (Stryer, 1996). Steroid hormones of lower estrogenic activity are estrone and 4-androstene-3, 17-dione. Ethinyl estradiol (Figure 2.2-6) is a synthetic derivative of estradiol and is the key component of oral contraceptives. The acetylene residue at position 17 circumvents an oxidation at this carbon atom and makes this compound more recalcitrant in the environment. Basically, steroids have similar physical chemical characteristics (Table 2.1), as they display low water solubility and comparatively high melting points. All these molecules possess one or two quaternary carbon atoms, C-10 and C-13.

Table 2.1 Selected physicochemical properties of steroid hormones

Steroid homone	Molecular weight (g/mol)	Water solubility (mg/l)	Log K_{ow}	Melting point (°C)	References
17β-Estradiol	272.4	3.9-13.3	3.1-4.0	171	Hanselman et al., 2003
Estrone	270.4	0.8-12.4	3.1-3.4	259	Hanselman et al., 2003
17α-Ethinyl estradiol	296.4	4.8	3.6-4.1	183	Lee et al., 2003
Testosterone	288.4	18.0-25.0	3.2	155	Lee et al., 2003
4-Androstene-3,17-dione	286.4	37.0-41.0	NA*	173	Lee et al., 2003
Cholesterol	386.6	2.0	NA*	149	Windholz et al., 1983

* No data available.

2.6 Steroid hormones in the environment

2.6.1 Natural and anthropogenic sources and deposits

Estradiol and estrone, detected in the aquatic environment, mainly originate from municipal effluent discharges (Ternes et al., 1999b), runoffs from agricultural production, and farmyard manure applied as organic fertilizer (Hanselman et al., 2003). The importance of estrogens from animal sources has to date been of less concern than their discharges from wastewater treatment plants, although runoff from manure largely contributes to estrogens entry in the environments (Hanselman et al., 2003; Raman et al., 2004). Cattle and poultry manure have been reported as a source of environmental loadings of testosterone (Lee et al., 2003). It is clear that intensive animal breeding could generate large quantities of both the steroidal estrogens and androgens from urinary and fecal deposition and, indeed, high concentrations (up to 2 µg/l) of estradiol and testosterone have been found in runoff from poultry manure (Finlay et al., 2000). Other significant sources of androgens appear to include pulp and paper mill effluents and

sewage treatment effluents (Jacobsen et al., 2005). Another source could be the microbial conversion of phytosteroidal compounds (e.g.,β-sitosterol) to steroid hormones including 4-androstene-3,17-dione in anoxic aquatic sediments (Jenkins et al., 2003).

Steroids can be found as fossil fuel markers in coals, petroleum and sedimentary rocks. These markers represent modified molecules of biochemical precursors such as cholesterol and other steroids, formed by microbial degradation, pressure, temperature and mineral catalysis, while being buried for millions of years in deep sediments. These steroids are thermodynamically more stable than the steroids found in the organisms but possess the basic structure of their predecessors. They can be used to determine the constitution of a community of organisms in a specific time of earth history, or to trace contamination of soils, plants and groundwater by petrol and petroleum-derived products (Mackenzie et al., 1982, Payet et al., 1999).

2.6.2 Potential impact on the environments

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. Recent investigations have shown that many of the same synthetic and natural chemicals that disrupt endocrine signaling in vertebrates also disrupt the binding of phytoestrogens to the bacterial nodulation D protein (NodD) receptor. As a result the potential binding of estrogens to the bacterial NodD receptor could have a destabilizing influence on the establishment of this vital symbiotic relationship.

2.6.3 Fate of steroid hormones

Concerns over the potential negative ecological effects of steroid hormones from human- and animal-derived wastes have resulted in an increased interest on the mobility and persistence of these compounds in the environment. Removal of steroid hormones from water, sediments, and soils is expected to be largely the result of a combination of sorption and biodegradation. Being predominantly hydrophobic organic compounds of low volatility, sorption to the solid phase is likely to be a significant process. Studies by Holthaus et al. (2002) on the sorption of estradiol to river sediments revealed that less than 1 % of the present steroids were predicted to be removed from the aqueous phase by suspended sediments. Andersen et al. (2003) reported that sorption of estradiol and estrone to activated sludge and anoxic sewage sludge occurred to a minor degree and the sorbed steroids appeared to decrease slightly along the treatment train. The strong decrease of dissolved estrogens along the treatment train despite the almost equal amounts of sorbed estradiol and estrone in the activated sludge of the same sample indicates that sorption kinetics was slow with no equilibrium between the sorbed and dissolved estrogens. Soils were also found to bind estrogens (Hanselman et al., 2003; Lee et al., 2003). In addition, androgens have been shown to be sorbed to soils or to be accumulated in sediments (Jenkins et al., 2003, Lee et al., 2003).

Steroid hormones are mainly excreted from humans and livestock as soluble conjugates (e.g., sulfate- and glucuronic acid-esters), which are cleaved during wastewater treatment (Ternes et al., 1999a). Recent studies demonstrated that

unconjugated, active estrogens are degraded under oxic conditions during normal activated sludge process and lab scale experiments (Layton et al., 2000; Ternes et al., 1999a). However, other investigations indicated only partially elimination of estrogens, depending on the facility and location of the respective wastewater treatment plant (Ternes et al., 1999b; Desbrow et al., 1998; Belfroid et al., 1999). Layton et al. (2000) have performed a series of biodegradation studies with radio-labeled estradiol and testosterone in laboratory assays inoculated with activated sludge obtained from different wastewater treatment plants. Differences in mineralization of estradiol by sludge from a municipal wastewater treatment plant compared to that from an industrial plant were observed. In contrast to estradiol, testosterone was mineralized to carbon dioxide in all investigated plants.

In recent years, co-metabolic transformations of estrogens in activated sludge and by the nitrifying bacterium *Nitrosomonas europaea* were described (Vader et al., 2000; Shi et al., 2004). It was proposed that the enzyme ammonium monooxygenase unspecifically hydroxylates steroid hormones such as estradiol or ethinyl estradiol to derivatives with lower estrogenic activity However, co-metabolism is mostly used to describe a microbial process for which no exact explanation exists (Wackett, 1996). Furthermore, the relevance of *Nitrosomonas europaea* for wastewater treatment is disputable, since it is known that other nitrifying bacteria (e.g., *Nitrosomonas mobilis* and *Nitrosomonas marina*) are mostly abundant and functionally important during sewage sludge treatment (Wagner et al., 2002).

2.7 17Alpha-Methyltestosterone

17alpha-methyltestosterone (MT) is a naturally occurring androgen ("male" sex hormone) that is produced in the testes of men and, in small amounts, by the ovaries and brains in women. It is a synthetic derivative of testosterone. It is a hormone used to treat men with a testosterone deficiency and also used in women to treat breast cancer, breast pain, swelling due to pregnancy, and, with the addition of estrogen to treat symptoms of menopause.

2.7.1 Modification of MT

MT was developed synthetically in the search for an androgen that could be given orally without loss of bioavailability. Testosterone itself is ineffective when taken orally since the majority of the compound is metabolized and destroyed by the liver during the "first pass" so that at most 5-10% of the compound enters the blood and becomes effective. MT is a 17-alpha steroid molecule, with a methyl group added to the C-17-alpha position of the molecule. Thus, MT is not broken down and deactivated as fast as oral testosterone by the liver. Still, it reaches the blood quickly and has only a low half-life time. Since MT, in part, is reabsorbed through the mucous membrane in the mouth, this substance is also available for sublingual intake. Synthetic anabolic steroids are based on the principal male hormone testosterone, modified in one of three ways (Murad et al., 1985):

- alkylation of the 17-carbon
- esterification of the 17-OH group
- modification of the steroid nucleus

2.7.2 Structure and Properties of MT

MT has a chemical name of 17beta-Hydroxy-17alpha-methylandrost-4-en-3-one. And synonyms are Methyltestosteronum and Methyltestosteronum. In addition MT has a commercial namesuch as Android® Virilon® etc. The chemical structure is presented in Figure 2.3 while its physical chemical properties are presented in Table 2.2.
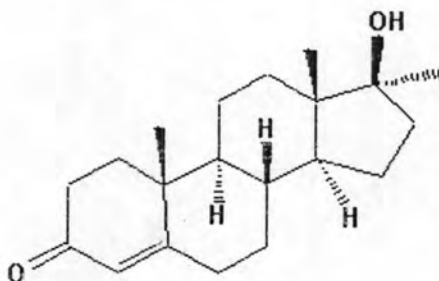

Figure 2.3 Chemical structure of MT.

Table 2.2 Physical-chemical properties of MT

Property	Value
Molecular formula [a]	C_{20}-H_{30}-O_2
CAS number	58-18-4
Molecular weight	302.45
Color/Form [b]	White or creamy white crystals or crystalline powder
Odor [b]	Odorless
Melting Point [a]	161-166 °C
Octanol/Water Partition Coefficient (log K_{ow}) [c]	3.36
Solubilities [a, b, d]	- Soluble in methanol, ethanol, ether and other organic solvents - sparingly soluble in vegetable oil - In water: 3.4 mg/L at 25 °C
Vapor Pressure [e]	1.8×10^{-8} mm Hg at 25° C
Henry's Law constant [e]	4.7×10^{-9} atm-m^3/mole at 25 °C
Hydroxy radical reaction rate constant [e]	1.0×10^{-10} cm^3/mole-sec at 25 °C
Ozone reaction rate constant [e]	1.1×10^{-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)

2.8 Effects on Human

2.8.1 Adverse Effect

The adverse effects of anabolic steroids include weight gain, fluid retention, and abnormal liver function as measured by biochemical tests (Soe et al., 1992). Administration to children can cause premature closure of the epiphyses. Men can develop impotency and azoospermia. Women are at risk of virilization (Malarkey et al., 1991).

2.8.2 Main Risk and Target Organ

There is no serious risk from acute poisoning, but chronic use can cause harm. Main risks of exposure to excessive androgens include: menstrual irregularities and virilization in women and impotence, premature cardiovascular disease and prostatic hypertrophy in men. Both men and women can suffer liver damage with oral anabolic steroids containing a substituted 17-alpha-carbon. Psychiatric changes can occur during use or after cessation of these agents (McKillop et al., 1986; McNutt et al., 1988 and Bowman, 1990).

2.8.3 Carcinogenicity

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 and Essenhigh, 1986). Cases of hepatic cancers associated with anabolic steroid abuse have been reported.

2.9 Environmental Fate of MT

So far not much is known about the fate of MT in the fish farms and in the receiving waters. However, it is highly probable that the remaining and unmetabolized MT-impregnated foods are accumulated in the sediments of the masculinization ponds and released into the receiving waters. A recent study showed that MT concentration in soils were between 2.8 and 2.9 ng/g for nearly three months after cessation of treatment demonstrating the persistency of MT in soils (Mcelwee et al., 2000). MT residues in receiving water may impact humans who consume contaminated water, affecting their endocrine and reproductive systems.

2.9.1 Terrestrial Fate

Based on a classification scheme by Swann et al., 1983, MT with an estimated K_{oc} value of 1,600, (determined from a log K_{ow} of 3.36 and a regression-derived equation (Lyman et al., 1990) is expected to have low mobility in soil. Volatilization of MT from moist soil surfaces is not expected to be an important fate process since the estimated Henry's Law constant is 4.7×10^{-9} atm-cu m/mole, using a fragment constant estimation method (Meylan et al., 1991). MT is not expected to volatilize from dry soil surfaces

based on the estimated vapor pressure of 1.8×10^{-8} mm Hg, determined from a fragment constant method. Biodegradation data were not available.

2.9.2 Aquatic Fate

The estimated K_{oc} value for MT in Section 2.9.1, indicates that MT is expected to be adsorbed to suspended solids and sediment. Volatilization from water surfaces is not expected based on the estimated Henry's Law constant of 4.7×10^{-9} atm-cu m/mole. With an estimated BCF of 77 (based on its log K_{ow} (Hansch, 1995) and a regression-derived equation (Meylan et al., 1999)), the potential for bioconcentration in aquatic organisms is expected to be moderate (Franke et al., 1994). Data on biodegradation of MT in aqueous system are not available.

2.9.3 Atmospheric Fate

According to a model on gas/particle partitioning of semivolatile organic compounds in the atmosphere MT with an estimated vapor pressure of 1.8×10^{-8} mm Hg at 25 °C (Lyman, 1985), is expected to exist in the particulate phase in the atmosphere. Particulate-phase MT may be removed from the air by wet and dry deposition. MT does not absorb light at wavelengths >290 nm (Lide, 1994) and therefore is not expected to be susceptible to direct photolysis.

2.10 Aerobic degradation and metabolite of steroid hormones and sterols

Steroid hormones of human and animal origins have been delivered into the environment over thousands of years, and to an increasing extent in recent years due to growing population and more intensive farming. Several bacteria have acquired pathways to make use of these compounds as growth substrates. Certain bacteria are able to grow on steroid hormones or sterols as the sole source of carbon and energy by the expression of a set of steroid catabolizing enzymes.

2.10.1 Aerobic degradation and metabolites of Testosterone

Under aerobic conditions, steroids can be degraded by certain bacterial species (Fujii et al., 2002; Fujii et al., 2003; Yoshimoto et al., 2004; Kieslich, 1985). Although bacteria

are generally capable of growing on estradiol or estrone as sole source of carbon and energy, only a few degradation mechanisms have been proposed (Coombe, 1966). On the other hand, the degradation pathway of testosterone, 4-androstene-3,17-dione, in *Comamonas testosteroni* (Talalay et al., 1952; Tamaoka et al., 1987) and cholesterol in various genera (Kieslich, 1985), known as 9,10-seco pathway, was studied in great detail since the 1960s (Shi and Whitlock, 1968; Horinouchi et al., 2004).

This oxygen-dependent pathway with its mode of cleavage of the steroid nucleus appears to be a general degradation pathway of C-19 steroids and cholesterol in aerobic bacteria (Figure 2.4). Cholesterol and testosterone both enter this pathway, the former via side chain cleavage, and the latter by oxidation. After Δ1-dehydrogenation to 1,4-androstadiene-3,17-dione (step 1), 9α-hydroxylation of the C-9 atom is followed by the nonenzymatic transformation of 9α-hydroxy-1,4-androstadiene-3,17-dione into 3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene- 9,17-dione (step 2). Furthermore, the phenolic ring A is opened by *meta*-cleavage (steps 3 and 4) yielding 4,5,9,10-diseco-3-hydroxy-5,9,17-trioxoandrosta-1(10),2-dien-4-oic acid. This compound is hydrolyzed (step 6) to 2-oxo-4-hexenoic acid and 9,17-dioxo-1,2,3,4,10,19- hexanorandrostan-5-oic acid. Only the ketoform (2-oxo-4-hexenoic acid) of the two tautomeres 7 is further metabolized to 4-hydroxy-2-oxo-hexanoic acid, which is finally cleaved to propionaldehyde and pyruvic acid (steps 8 and 9). 9,17-dioxo-1,2,3,4,10,19-hexanorandrostan-5-oic acid is thought to be further degraded (steps 10, 11, 12, 13) to succinic acid (Schubert et al., 1969). Still these last steps have to be further investigated (Horinouchi et al., 2004), especially the quaternary carbon atom in the product of step 11 poses a problem for further degradation, as well as the following tertiary alcohol (Hylemon and Harder, 1999).

Interestingly, bacteria that are using the 9,10-seco pathway are unable to metabolize estradiol or estrone, since the existence of the C-19 carbon atom is a prerequisite for the cleavage of ring B. For example, when 19-nor-androst-4-ene-3,17-dione is incubated with *Comamonas testosteroni*, estrone accumulates in the medium (Levy and Talalay, 1959). Another important feature is that the genes for steroid degradation in *Comamonas testosteroni* are not constitutively expressed, but are induced by their respective steroid substrates (Marcus and Talalay, 1956; Möbus et al., 1997; Skowasch et al., 2002). In

addition, studies of the mechanisms regulating the steroid-inducible gene expression revealed, that regulator proteins, binding proteins or intermediate compounds produced in the course of testosterone degradation are likely to be involved in microbial steroid metabolism (Xiong and Maser, 2001; Xiong et al., 2003; Pruneda et al., 2004; Thomas et al., 1989).
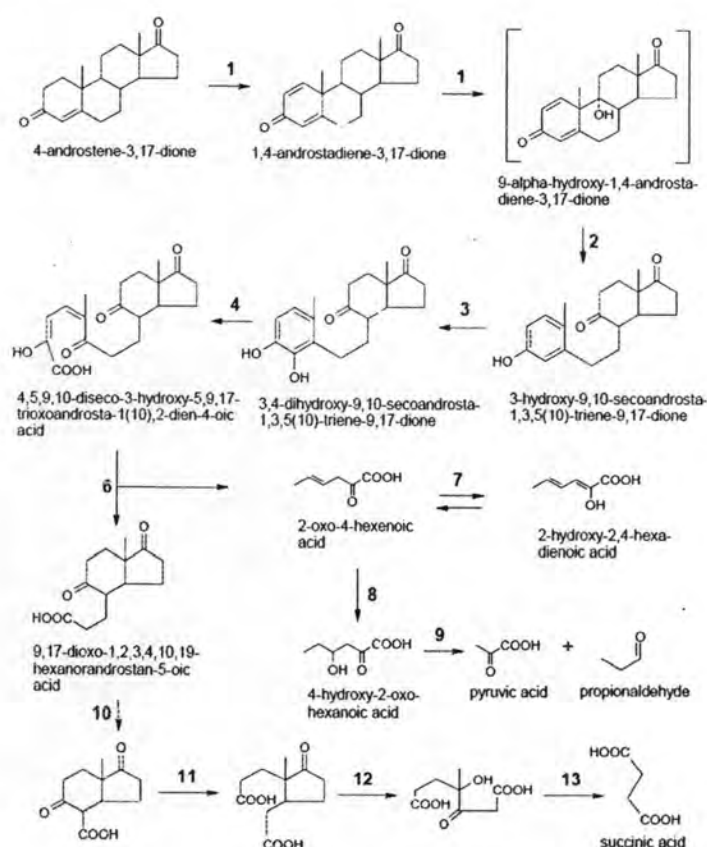

Figure 2.4. Proposed aerobic cholesterol and testosterone degradation pathway (9,10-seco-pathway). The side-chain cleavage of cholesterol and the dehydrogenation of the 17β-hydroxyl group on testosterone are not shown. Steps 1 to 6 have been taken from Horinouchi et al. (2004), while the final steps from 7 to 13 have been taken from Kieslich (1985). The degradation steps 10 to 13 have yet to be clarified. Numbers displayed refer to the description of the reaction steps in the text.

2.10.2 Aerobic degradation and metabolites of MT

2.10.2.1 Metabolites in human

Shinohara et al., (2000) studied four healthy adult male volunteers who refrained from all medications for 7 days prior to the study day and 3 days following drug

administration. After an overnight fast, each subject was orally administered 500 mg of 17α-methyltestosterone dissolved in 50 ml of 5% ethanol. Urine volumes were measured, and the samples were stored frozen in polyethylene bottles at −20°C until analysis. The main metabolites of 17α-methyltestosterone were 17α-methyl-5α-androstan-3α, 17β-diol and 17α-methyl-5β-androstan-3α, 17β-diol (see Figure 2.5), 17α-Methyl-[²H₃]-5α-androstan-3α,17β-diol and 17α-methyl-[²H₃]-5β-androstan-3α,17β-diol were used as internal standards. Results of this study showed that the method provides a specific, sensitive and reliable technique to determine the urine levels of 17α-methyl-5α-androstan-3α,17β-diol and 17α-methyl-5β-androstan-3α,17β-diol, and can be applied to pharmacokinetic studies of 17α -methyltestosterone.

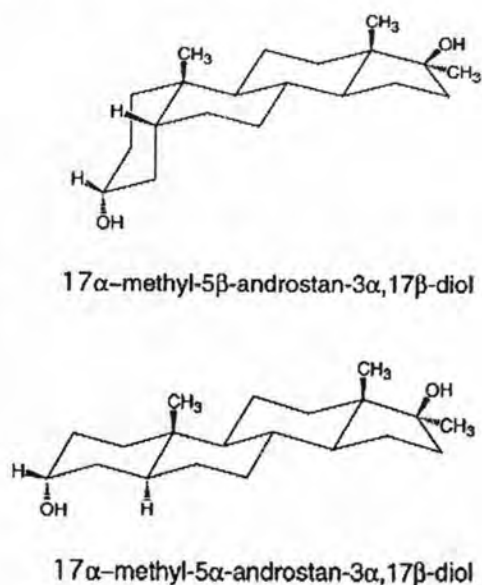17α–methyl-5β-androstan-3α,17β-diol

17α–methyl-5α-androstan-3α,17β-diol

Figure 2.5 Structure of 17α-methyl-5β-androstan-3α, 17β-diol and 17 and 17α-methyl-5α-androstan-3α, 17β-diol

2.10.2.2 Metabolites in Cow

Blokland et al, (2004) studied the metabolism and excretion of 17α-methyltestosterone, norethandrolone, methylboldenone (see Figure 2.6) and metabolites in a heifer treated with these steroids by intra-muscular injection. Samples of urine were collected and analyzed using gas-chromatography coupled to a mass-spectrometer. It was concluded that degradation of 17α-methyltestosterone, norethandrolone and

methylboldenone showed similar metabolic pathways. The main route was hydroxylation of the parent compound. The formation of oxygenated products was observed, but this was a minor pathway.
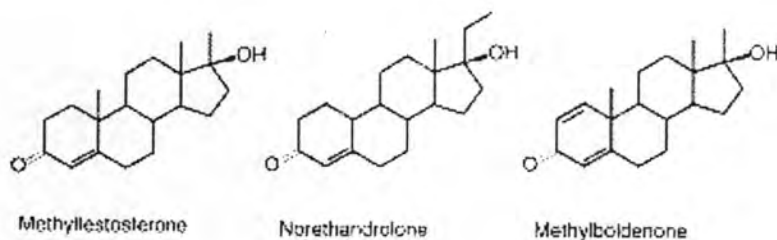

Figure 2.6 Structure of methyltestosterone, norethandrolone and methylboldenone.

The most dominant metabolite formed after administration of methyltestosterone is 17α-methyl-5β-androstane-3α, 17β-diol, 17α-methyl-5α-androstane-3β, 17β-diol and 17α-methyl-5β-androstane-3β, 17ß-diol. A minor oxygenated metabolite formed is, 17α-methyl-5x-androstane-3x, 16x, 17β-triol (x = α or β).

From the literature review and revealed document above, it is highly probable that the remaining and unmetabolized MT-impregnated foods accumulates in the sediments of the masculinization ponds and are released into the receiving waters. Moreover, using MT for masculinization in Nile tilapia can affect on aquatic life and ecological seystems. When the residue of MT release in to natural water body, it can induce sex reversal of other species fish that live in that area in term of chronic effects. There are some review found that MT at low concentration can effect on ecological system. In addition, so far there is no standard for of effluent from fish farm and not much known about the fate of MT. Consequently biodegradation is the one of interesting fate to study on the fate of MT. Because biodegradation is exactly disappear or remove of MT in environment.