

Risks Associated with Using Methyl Testosterone in Tilapia Farming

Donald J. Macintosh

Despite widespread use of the androgen 17 α -Methyl Testosterone (MT) in tilapia farming, the implications of tilapia hormone treatment in relation to human health and the environment have not been well articulated to the fish trade, or the general public. The purpose of this white paper is (a) to explain clearly why MT is widely used by the producers of farmed tilapia; and (b) to demonstrate why there are no risks to consumers, and no known risks to producers or the environment, provided the recommended best practices for MT use in aquaculture are followed. These best practices are described, so that tilapia dealers can ensure that their suppliers are taking the necessary steps to protect consumers, fish farm workers and the environment.

General Conclusions

- MT treatment of tilapia fry is the most simple and reliable way to produce all-male tilapia stocks, which consistently grow to a larger/more uniform size than mixed sex or all-female tilapias. It is highly effective on the Nile tilapia, *Oreochromis niloticus*, the main species farmed commercially worldwide, thus MT treatment has become the standard technique to produce all-male tilapias.
- The quantities of MT used worldwide in aquaculture are not reported, but it can be concluded that the great majority of tilapia products traded internationally (including virtually all tilapia fillets) are derived from MT treated fish.
- MT is a synthetic male hormone which closely mimics the naturally-produced hormone testosterone and, consequently, it is used widely in human medicine as a hormone supplement and in agriculture to promote weight gain in livestock.
- While the legal status of hormone use in aquaculture may vary from country to country, the main conclusions reached from the available scientific evidence is that MT treatment of tilapia carries no human health risks, provided it is applied only during the early fry stage, at the recommended dosage.
- The quantities of MT consumed by tilapia fry during all-male treatment are insignificant compared to the levels of testosterone produced by both men and women, and consumed in other foodstuffs, especially meat and dairy products.

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- Tilapias rapidly excrete ingested hormone, with MT levels falling to less than 1%

within 100 hours of withdrawing MT. Thus MT is not detectable in adult tilapias, which require a grow out period of at least five months to reach international marketable size.

- There are no reported health effects among workers at tilapia farms where MT is used, but standard procedures for dealing with all chemicals and pharmaceuticals should be applied to MT as a routine precaution.
- Little is known about the environmental impacts of releasing wastewater from tilapia production facilities that utilize MT treatment, because research to date has focused on the much greater concerns surrounding environmental estrogens and their anti-androgen effects on wild fish and other animals. However the quantities of MT that may be entering the environment are orders of magnitude lower than the hormones being released from agricultural wastes and sewage.
- Steroid hormones like MT are absorbed rapidly onto sediments and similar substrata. It has been shown that gravel and sand filters, or biofilters including wetlands, can rapidly remove hormones from water (within 24 hours).

Recommendations

MT treatment in tilapia farming is considered to be entirely safe provided the following recommended best practices are adopted by producers:

1. Restrict tilapia MT treatment to the early fry stages, specifically to the first month from the time the fry are free-swimming/first-feeding.
2. Limit the dosage of MT used to a maximum of 50 mg MT/kg fry feed.
3. Rear MT treated tilapia fry to adult size for at least five months after hormone treatment ends to ensure zero hormone residue remains in the fish.
4. As a precautionary measure, adopt safe handling protocols when preparing and administering MT treated tilapia feed; use latex gloves and a protective face mask to avoid dermal contact or inhalation of MT.
5. Keep a careful inventory of the amounts of MT supplied to and used in each tilapia hatchery, and ensure that access to the hormone supply and record-keeping are controlled by the farm manager or hatchery supervisor.
6. Avoid direct release of hatchery water used for MT treatment of tilapia fry into the environment. As a precautionary measure, tilapia hatcheries should utilize a gravel and sand filter, plus a shallow vegetated pond or an enclosed wetland, to receive and hold the hatchery wastewater for several days before discharge into the general environment.

INTRODUCTION

Use of the hormone 17 α -Methyl Testosterone (MT) to induce sex reversal in farmed tilapias has become a common practice in many parts of the world. MT is a simple and reliable way to produce all-male tilapia stocks, which consistently grow to a larger/more uniform size than mixed sex or all-female stocks. Thus, MT usage in tilapia farming is expected to continue to increase rapidly as the global demand for large whole tilapia and tilapia fillets grows. Currently, tilapia is farmed in at least 85 countries, making it the most widely farmed finfish worldwide and second in volume only to carps (FAO, 2006a). Tilapia production has expanded dramatically in recent years, from about 1 million metric tons in 1998, to nearly 2.4 million tons in 2006, of which almost 2 million tons were contributed by a single species, the Nile tilapia (*Oreochromis niloticus*).

Although the extent of MT treatment is unknown, it can be assumed that a high proportion of the tilapias imported into western consumer markets as whole fish, and virtually all fillets, have been MT-treated. The global production of farmed tilapia is projected to rise to 3 million tons by 2010, with the increase in demand being greatest for tilapia fillets (Fitzsimmons, 2008); hence use of MT is expected to increase in line with this growth in tilapia production. Much of the increased production is expected to come from Nile tilapia farming (FAO, 2006b).

BACKGROUND

Description and Uses of 17 α -Methyl Testosterone

17 α -Methyl Testosterone (MT) is a synthetically produced anabolic and androgenic steroid hormone; i.e. it promotes both muscle growth and the development of male sexual characters. MT closely mimics the naturally-produced hormone testosterone and, consequently, this and other synthetic forms of testosterone have been used widely in human medicine as a hormone supplement to treat men with testosterone deficiency (e.g. Bhasin *et al.*, 1998). MT has also been used to treat women with breast cancer or breast pain and, together with estrogen, to treat symptoms of the female menopause.

Endogenous testosterone is produced by the testicles in men and in much lower quantities by the ovaries in women. The adrenal glands also produce small amounts of testosterone in both sexes. High dosages of exogenous male hormones, including MT, are known to cause side effects, especially liver damage, but lower levels actually produce various health benefits, including reduced risks from cardio-vascular disease and cancer (Valcour, 2001). Overall, it has been shown that the side effects of testosterone supplementation in humans are minimal when plasma testosterone levels are kept within the normal physiological range (Bhasin *et al.*, 1998).

Recently, a more potent form of testosterone, 17 α -1-testosterone, has become widely available. It is advertised to body-builders and others who want to achieve muscle growth and/or fat loss, and to men with deficient testosterone levels. The typical dose of

MT or 17 α -1-testosterone in human hormone therapy, or as a supplement, is 20 to 40 mg daily for up to four weeks, taken orally. MT is a white crystalline compound sold in powder or tablet forms; it is also known as 17 beta-Hydroxy-17 alpha-methyl-4-androsten-3-one or 17-Methyl Testosterone. Although MT has now been replaced with other forms of testosterone for clinical use in hormone replacement therapy, at least in Europe, it is still widely available - including on the internet - under various trade names, e.g. *Android*, *Testred* (e.g. www.drugs.com/pro/testred.html).

Anabolic hormones (both androgens and estrogens) are also widely used in agriculture to promote weight gain in cattle and sheep. Commercially sold hormone implants for livestock typically contain a mixture of both natural and exogenous male and female hormones, including 200mg testosterone. These implants (placed in the ear) contain relatively large quantities of hormone which are released continuously over several months. No withdrawal time for the implant is required before the animals are slaughtered. Another hormone, melengestrol acetate (MGA), a synthetic female hormone, is added to livestock feed at a rate equivalent to 0.25-0.50mg/animal/day. Consequently, in many countries worldwide the meat and milk products reaching consumers still contain detectable levels of both exogenous and endogenous hormones (Velle, 1982). On average, the adult person's consumption of hormones in food is about 10 μ g progesterone/day and 0.05 μ g testosterone/day, principally from meat and dairy products (Shore and Shemesh, 2003).

In comparison to the hormone dosages applied in human medicine, or livestock production, the use of MT in tilapia farming involves exposing only the early fry stages to minute amounts, typically less than 0.02mg per tilapia in total. Moreover, sex reversed tilapias are reared for at least five months after hormone treatment ceases, until they reach marketable size, by which time no hormone residue remains.

The use of MT in tilapia farming dates from the late 1960's, when various hormones and treatment methods were experimented with in order to produce single sex tilapia stocks to overcome the widespread problem that tilapias reproduce excessively in most grow-out systems (Clemens and Inslee, 1968; Guerrero, 1975; Nakamura, 1975; Owusu-Frimpong and Nijjhar, 1981; Phelps *et al.*, 1992). By feeding small amounts of male hormone to tilapia fry before and during sexual differentiation, virtually all the treated fish develop as males morphologically and the potential of the stock to reproduce is thereby eliminated. This form of sex control has the added benefit that male tilapias generally grow faster than females, with a result that all-male fish are larger and more uniform in size than mixed sex tilapias (e.g. Smith and Phelps, 1997; Hussain, *et al.*, 2005). The larger size and greater uniformity of MT treated tilapias make them highly suited for export, especially to supply the fast-growing demand for fresh and frozen fillets.

These desirable growth characteristics are shown particularly by MT treated Nile tilapia (*Oreochromis niloticus*), which is the major tilapia species farmed commercially worldwide (FAO, 2006b). For European markets, the target body weight per fish exceeds 600g, which takes a production period of at least 10 months. Body weight differences between male and female *O niloticus* are significant even after 100 days and the male to

female size superiority continues to increase with age (Rutten *et al.*, 2005).

Monosex tilapia stocks can also be produced by other methods, such as hand-sexing, or genetic manipulation to produce hybrids or genetically enhanced males known as “supermales” (Mires, 1982; Hulata, 1997, Mair, *et.al.*, 1997). However hormonal sex reversal is widely recognized by producers to be much simpler, more effective and consistent than these other techniques. Rutten *et al.*, 2005 noted that, at around 10 months, Nile tilapia males can reach almost double the body weight of females; and thus “product uniformity is especially compromised when mixed sex populations are used for production”. Similarly, genetic selection is not considered favourable as a mechanism to reduce the great difference in body size between male and female *O niloticus* (Falconer and Mackay, 1996). Consequently, treatment of tilapia fry with MT has emerged as the standard technique to produce all-male fish for commercial tilapia farming.

Although the aquaculture statistics do not separate MT treated fish and filets from untreated tilapias, it can be assumed that the great majority of tilapia products (including virtually all tilapia fillets) traded internationally are derived from MT hormone-treated fish.

MT treatment in tilapia farming

The standard hormone treatment procedure for tilapias involves adding MT to commercial fry feed, which is then administered to batches of fry of similar age during the short period of their early development when they are most susceptible to the masculinisation effect of this hormone.

The Nile tilapia and the other *Oreochromis* species that dominate commercial tilapia farming are mouth brooders. After their eggs are released and fertilized, the female broodfish carry the eggs orally until they develop into fry (described by Macintosh and Little, 1995). Treatment with MT should begin from the second or third day after the fry are released from maternal care. The most common sex-reversal treatment involves giving the first-feeding (and still sexually undifferentiated) tilapia fry powdered fish feed containing 30-60 mg MT/kg of diet. The MT hormone-treated feed is provided for 28-31 days. At the time of cessation of hormone treatment, the average size of the fry is still only 0.2-0.4 g approximately (FAO, 2006b). Based on a modest FCR (food conversion rate) of 1:1 (i.e. 1g feed produces 1g fish), then the consumption of hormone per fish is not more than $60\text{mg} \times 0.4\text{g}/1000\text{g} = 0.024\text{mg}$. In practice, tilapia producers try to minimize hormone use and maximize FCR, in order to reduce costs and increase production efficiency. The largest tilapia producer in Thailand, for example, uses about 10g of 17-alpha-MT per one million fry, equivalent to 0.01mg hormone per fish.

This level of hormone consumption per tilapia fry (0.1-0.2mg MT/fish) is not only minute, but the MT ingested is rapidly eliminated and declines within days to undetectable levels in the viscera, carcass, blood and muscle of treated fish. For example, within 100 hrs of terminating MT treatment, this hormone was present at less than 1% of

its initial level in the Mozambique tilapia (Johnstone *et al.*, 1983). Hormone elimination is rapid in fish because it is believed to occur mainly via excretion in the faeces and via the gills (Cravedi *et al.*, 1993).

POTENTIAL HAZARDS ASSOCIATED WITH MT USE IN TILAPIA FARMING

The potential hazards associated with the use of 17 α -Methyl Testosterone (MT) in tilapia farming can be divided into three main aspects: a) risks to tilapia consumers; b) risks to fish farm workers; c) risks to the environment. The nature of the potential hazards involved, and the known levels of risk in each case, are elaborated in the following sections, together with guidance on best practices to reduce the identified risks to a minimum.

a) Tilapia consumers

Over the past 25 years, scientific studies have repeatedly shown that MT does not accumulate in the meat of tilapias and other fish species. Johnstone *et al.* (1983) reported on the elimination rate of MT in both trout and tilapia. They found that whole fish body levels of MT were not detectable only 100 hours after withdrawal of the hormone-treated diet. In the fish carcass, MT was undetectable after only 50 hours of hormone withdrawal. This rapid rate of clearance of MT was similar in both species. Comparable results showing rapid loss of MT in rainbow trout were also reported by Fagerlund and Dye (1979). A number of subsequent studies have corroborated these earlier findings, namely that dietary administered MT does not find its way into the fish meat. For example, Gulla *et al.*, 2007 detected no hormone residue in the meat of rainbow trout fed 3mg 17 alpha-MT per kg of fish feed for 35 days, while Guerrero (2008) quotes studies showing that hormone levels in tilapia fall to normal level five days after hormone feeding is stopped.

In experimental studies on tilapia fillets Green and Teichert (2001) estimated the concentration of MT in one portion of edible tissue (57 to 143g skinless fillet) to be in the low parts *per trillion* range, or 1.2- 3.4 ng MT. Again, these figures were based on a 21 day withdrawal period and do not take into consideration the metabolism and excretion associated with a longer withdrawal period. Based on the scientific evidence that MT is rapidly eliminated from fish, there is no possibility that MT will persist in adult fish after the several months required for farmed tilapias to reach marketable size.

The most important conclusion to draw is that the quantities of MT consumed by tilapias during the fry treatment period (equivalent to 0.1-0.2 mg MT/fish on average) represent less than 0.001% of the typical daily dosage of MT prescribed in human medicine (20-40mg), and that even this minute quantity declines to less than 0.00001% of the daily human dosage within one week of terminating tilapia MT treatment. It should also be noted that dairy and meat products contribute vastly more hormones to the human diet than MT treated tilapia could ever do, yet even these hormone-rich foodstuffs represent

less than 0.1% of the endogenous sex hormone produced by humans (Velle, 1982). The testes of an adult man release about 15 mg of endogenous testosterone per day, while about 10mg of androgens are excreted daily (Shore and Shemesh, 2003)..

In conclusion, provided MT is administered to farmed tilapias only during the early fry stage, there are absolutely no human health risks to consumers when MT treated tilapias enter the market as whole adult fish or fillets.

MT treatment could still be perceived as hazardous to tilapia consumers if MT is applied at higher than the recommended dosages, or is used for longer periods, i.e. beyond the fry treatment stage and into the grow out phase. While this represents an identifiable risk, there are in fact very strong incentives for producers to minimize use of MT, mainly for cost reasons. Moreover, there is no evidence that higher dosages or longer treatment periods improve the sex reversal effectiveness of MT. Similarly, there is no growth benefit associated with longer term MT treatment (Phelps *et al.*, 1992). In fact research findings indicate that MT is actually less effective if the recommended dosage and duration of treatment are exceeded (e.g. Yoshikawa and Oguri, 1978). But as a safeguard, tilapia dealers should ensure that the producers they buy from apply this hormone only during the fry rearing period.

The recommended best practice is to (a) restrict tilapia MT treatment to the early fry stages, specifically to the first month from the time the fry are free-swimming/first-feeding; (b) limit the dosage of MT used to a maximum of 50 mg MT/kg fry feed.

b) Tilapia farm workers

Tilapia producers purchase commercially available MT in powder or tablet form. As described above, the hormone is usually added to powdered tilapia feed which is then offered to tilapia fry several times per day during the hormone treatment period (FAO 2006b; Guerrero, 2008). Farm workers may come into contact with MT in two ways; (a) when it is being added to tilapia fry feed; (b) when MT treated feed is being administered to tilapia fry in hatchery tanks or hapas. Although the chemicals and pharmaceuticals used in aquaculture are not particularly hazardous, the risks to farm workers of exposure to MT can be minimized by following standard procedures for the handling of such substances (e.g. OSHA, 2008; Syndel, 2008).

MT is highly insoluble in water, so it is normally dissolved in an ethanol solution before it is mixed, or sprayed, into tilapia feed; the MT-feed mixture is then allowed to dry. Mixing and drying of the MT-feed material should be done in a large, well ventilated room, or open area, to disperse the ethanol as it evaporates, thereby reducing the risk of MT inhalation. Use of protective surgical gloves and a face mask are also strongly recommended to further minimize the risk of uptake of the hormone by inhalation, or via skin contact.

The MT feed preparation stage requires careful dose calculation and weighing of the

hormone in relation to the quantity of MT treated feed being prepared. This should normally be done by the same person, or by a very small team of well-trained staff, a practice that also has the advantage that most general farm workers need not be exposed to the pure hormone, or hormone-ethanol solution. Similarly, access to these substances should be carefully controlled, by keeping them in a secure and locked cabinet or refrigerator to which only designated staff have access. A precise record of the quantities of MT used should also be maintained.

Farm workers who administer the MT-treated feed to tilapia fry should take basic precautions to avoid dermal contact with the feed by wearing gloves, and by always using a plastic scoop or similar implement, to dispense the feed. A protective face mask is also advisable if there is any risk of inhaling feed particles. Although the use of MT in commercial tilapia hatcheries is becoming more and more commonplace around the world, to date there have been no reports of adverse health effects to farm workers. OSHA considers that exposure from inhalation is “not relevant”, but does not have data on the effects of dermal exposure to MT.

While OSHA states that inhalation is irrelevant and that there is no existing data to be found on the handling risk associated with MT, it is common sense that safe handling protocols should be applied to MT use, as when dealing with any other chemical substance. In addition to wearing gloves to minimize dermal exposure to MT, farm workers who remove sediment from ponds used for MT fry treatment should wear protective boots and clothing.

A more likely hazard would arise from the intentional consumption of MT by farm workers because of its well-known anabolic (muscle-building) and perceived libido-enhancing properties. This area of risk can be eliminated by keeping a strict inventory of the amount of hormone supplied to and used in the hatchery; and by keeping all supplies securely locked and under the individual control of the farm manager or hatchery supervisor. For cost reasons, there are strong disincentives for tilapia producers to be lax regarding the availability and possible misuse of this hormone.

c) MT release into the environment

There is growing attention being given to the impact of pharmaceutically active compounds, including hormones, released into the environment via wastewater discharge (e.g. Heberer, 2002). Animal manure, sewage outflows and municipal and agricultural wastewater are the prime sources of such compounds. Put into perspective, if all the projected tilapia production by 2010 of 3 million tons was contributed by MT treated fish, the hormone use would be not more than 100 kg. In comparison, 33 tons of estrogens and 7.1 tons of androgens are excreted annually by farm animals in the European Union, and 49 and 4.4 tons respectively in the USA ((Lange *et al.*, 2002). Wastewater discharge from tilapia hatchery facilities represents only a tiny fraction of the total waste discharge into the environment but, nonetheless, a precautionary approach should be taken.

Unfortunately, little is known about the environmental impacts of releasing wastewater from tilapia production facilities that utilize MT treatment. One reason is that, to date, nearly all the research on sex hormones in the environment has focused on estrogens, especially estrone and estradiol-17 β , rather than androgens. These female hormones are released in much greater quantities and are already present in environmental concentrations above their lowest observable effects on wild fish, other animals and plants (Shore and Shemesh, 2003).

Use of the standard MT treatment for all-male tilapia production will result in the release of this androgen into the water system for up to one month. The hormone will be released from uneaten feed, via faecal and urinary excretion, and via the gills. Since nearly all (>99%) of the MT taken up by tilapias is voided within 100 hours after hormone treatment is stopped (Johnstone *et al.*, 1983), it follows that virtually all the administered MT, or its derivatives, will enter the water culture system. This means that hatcheries operating with recycled or semi-recycled water supplies will release less hormone residue into the environment than those operating flow-through water systems.

While releasing water containing MT residues remains a potential hazard that merits further research, the very limited number of studies conducted to date indicate that no detectable levels of hormone will enter the general environment, provided the hatchery wastewater (or water from ponds used to hold fry treatment facilities such as hapas) is recycled and filtered, or is retained for several days before being released into the natural environment.

It is recommended that tilapia producers who practice MT treatment should utilize a trickle filter of gravel and sand, plus a shallow vegetated pond (e.g. containing water plants), or an enclosed wetland, to receive and hold the hatchery wastewater before discharge into the general environment. In one study, testosterone in water that was passed through a gravel and sand filter was reduced substantially, from 166 to 7 ng/l and estrogen from 73 to 2 ng/l (cited by Shore and Semesh, 2003). Contreras-Sanchez (2001) reported lower concentrations of MT in the water of hatchery systems that included soil or gravel media; while use of active charcoal to filter water from systems without such substrata reduced the MT present in the water to almost background levels.

Lagoons and natural or constructed wetlands are known to be highly effective in reducing the concentration of many pharmaceutical compounds present in municipal wastewater, via a combination of photolysis, plant uptake, microbial degradation and soil sorption, or sequestration (White *et al.*, 2006). Steroid hormones in water are rapidly absorbed into sediments, or are reduced to inorganic compounds via mineralization (Shore and Semesh, 2003). For example, biosolids from a municipal sewage treatment plant were found to rapidly mineralize added testosterone, equivalent to its more than 90% removal from the aqueous phase within 24 h (Layton *et al.*, 2000).

By implication, the use of biofilters in tilapia hatcheries, followed by release of the wastewater into a holding pond or an enclosed wetland area, would substantially

eliminate MT and its derivatives before they could enter the general environment.

Finally, one positive aspect of MT treatment and the environment concerns tilapia escapees. Tilapia fry that escape from hatchery facilities where MT is used will be morphologically male and, consequently, they will have much lower potential to reproduce, including interbreeding with wild tilapia stocks, compared to normal fry (an environmental problem identified in the white paper on “The Potential Risks from Farm Escaped Tilapias”). It is well established that the reproductive potential of tilapia populations is determined by the number of reproductively active females, rather than by the number of males. MT treatment eliminates the reproductive capacity of virtually all the genetically female fish.

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