Synthetic Hormones in Animal Husbandry

Materials for the December 4-5, 2008 Meeting of the California Environmental Contaminant Biomonitoring Program (CECBP) Scientific Guidance Panel (SGP)

Agenda Item: "Consideration of Potential Designated Chemicals"

Introduction:

There are three synthetic hormones approved by the U.S. Food and Drug Administration (U.S. FDA) for growth promotion in cattle and sheep raised for meat production: the estrogenic compound zeranol, the anabolic steroid trenbolone acetate (TBA), and the progestin melengestrol acetate (MGA). The FDA approves and regulates the use of hormone implants, establishing tolerance levels (maximal allowable residue concentrations) and minimum withdrawal times for these hormones to prevent animal food products from exceeding tolerance concentrations. Hormonally active substances have been banned for use in food-producing animals in the European Community since 1988, and this ban applies to use in the production of meat imported to Europe.

In the United States synthetic hormones are often administered as implants and in combination with each other or with natural hormones. Volume-of-use data is not federally mandated to be reported and is therefore difficult to estimate. Hormone use in meat production appears to be quite prevalent. Almost 90 percent of steers and heifers weighing over 700 pounds and almost 86 percent weighing less than 700 pounds were implanted at least once in their lifetime with synthetic or natural hormones (17-β estradiol, progesterone, testosterone), and many cattle receive more than one implant (USDA 2000).

Humans are potentially exposed to the synthetic hormones by consumption of commercial meat products and from environmental exposures related to animal waste. Animal food products are monitored as part of the National Residue Program (NRP) conducted by the Food Safety Inspection Service (FSIS) of the USDA. This testing turns up very rare residue violations, but the extent of sampling is relatively small. For example, in NRP's September 2008 sampling plan for import and domestic products, it plans 270 samples of veal for zeranol, 180 samples of veal for trenbolone, and 300 samples of heifers for MGA (NRP, 2006).

Significant amounts of synthetic and natural hormones and their metabolites are excreted in animal waste (Kolok and Sellin 2008). Beef cattle wastes are strongly androgenic (Durhan et al. 2006). Synthetic hormones excreted by animals are present in manure applied as fertilizer and in feedlot retention ponds, and from there they may be retained in soil or transported to ground and surface water (Khan et al. 2008a and 2008b, Soto et al. 2004). Lange et al. (2002) calculated that if 63 percent of beef cattle (62 million head) are implanted with 20 milligrams (mg) estrogens and 200 mg androgens or progesterone, and if 8 percent of the applied hormone reaches the environment, an additional 100 kilograms (kg) of estrogens and 1000 kg of androgens and progesterone reach the environment via cattle excrement. These numbers represent an increase of approximately 0.2% (estrogens) and 20% (androgens or progesterone) over natural elimination rates (Lange et al. 2002). Livestock farming is thought to be the major source of

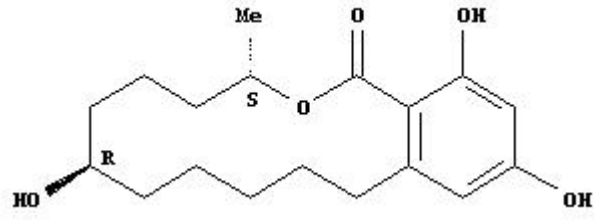
steroid hormones found in regional groundwater (Peterson et al. 2000) and external surface water (Kolodziej et al. 2004).

Health effects of concern for human exposure to the three synthetic hormones are cancer, reproductive effects, and other endocrine disruption outcomes. The natural hormones that these compounds model are carcinogenic; for example, estrogen at current background levels in the population is thought to be carcinogenic. Furthermore, anabolic steroids are reproductive toxicants and listed as such under Proposition 65. While trenbolone is not an approved human pharmaceutical, it appears to have extensive illicit use as an anabolic steroid in body building and various case reports of effects from acute use can be found. TBA, zeranol, and MGA cross the placenta and are detectable in fetal tissues in rabbits (Lange et al. 2002a). There is also some evidence that these xenobiotic growth promoters and their metabolites may be genotoxic (Metzler and Pfeiffer 2001). Determining health effects in humans is complicated because hormones levels are regulated by feedback systems and baseline levels vary among people and impacts differ depending on lifestage, and an individual's hormonal status. An expert committee in the United Kingdom, reviewing the health effects from veterinary use of hormones, noted that natural feedback systems may be less effective in the fetus, postmenopausal women, and pre-pubertal children, leaving them more vulnerable to the effects of exogenous hormone exposure (UK VPC 2006). In addition, the additive effects of exposure to multiple exogenous hormones in addition to pre-existing levels of natural hormones are difficult to quantify. Information on the toxicity of the individual compounds is presented in the summaries that follow.

Synthetic hormone metabolites have biological effects on aquatic organisms. Feedlot discharges have been shown to be biologically active in laboratory testing and to cause endocrine and reproductive effects in fish (Durhan et al. 2006, Gray et al. 2006, Hewitt et al. 2003, Jensen et al. 2001, Orlando et al. 2004, Parks et al. 2001).

Although there have been few attempts made to measure these synthetic hormones in humans, sensitive methods for detecting hormone use in animals have been developed because their use is banned in the European Union (Aman et al. 2006, Buiarelli et al. 2003, Launay et al. 2004, van Bennekom et al. 2002), and their use in the U.S. is under regulatory surveillance. Furthermore, methods to detect trenbolone in human and equine urine have been developed because of abuse of these hormones in sports and horse racing.

Zeranol [CASRN 26538-44-3]



Exposure or potential exposure to the public or specific subgroups:

Zeranol (α-zearalanol) is a non-steroidal, synthetic estrogen that is administered to livestock by implantation of a continuously releasing hormone pellet (12-36 mg) in the ear. Zeranol is metabolized to β-zearalanol (taleranol) and to a lesser extent, to zearalanone (Kleinova et al. 2002). Zeranol and its metabolites are resorcylic acid lactones. Zearalenone, also a resorcylic acid lactone, is produced by the *Fusarium* genus, fungi that are common contaminants of corn. Therefore, human exposure to zeranol metabolites may occur via zeranol use in animal husbandry or fungal infestation of corn crops (Kleinova et al. 2002). Zeranol is not generally used at finishing feedlots in large quantities because estrogen, which is a higher potency growth promoter, is used instead; therefore, zeranol is not thought to be prominent in waste streams from feedlots (Kolok and Sellin 2008). Zeranol has been found in low concentrations (nanograms [ng]/liter) in sewage discharges (Laganá et al. 2001, 2004). In a recent NRP report (2006), out of 323 samples analyzed, there were no residue violations for zeranol.[1]

Known or suspected health effects:

Zeranol and its metabolites act as estrogen receptor agonists and exert typical estrogenic effects on animals (Lamming 1987, Le Guevel and Pakdel 2001, Leffers et al. 2001, Nagel et al. 1998, Nikaido et al. 2005, Galbraith 2002). The presence of mycotoxins and serum levels of zearalanone were associated with early thelarche and mastopathy in Hungarian girls (Szuets et al. 1997). Furthermore, the presence of mycotoxins was strongly correlated with precocious puberty, and exposure to the mycoestrogenic zearalenone is thought to trigger central precocious puberty in young girls (Massart et al. 2008). Natural estrogen is a known cause of human breast and uterine cancer and increased exposure to zeranol may similarly increase risk from the existing burden of the natural compound. Most, but not all, of the short-term assays to assess mutagenicity of zeranol and some metabolites (zearalanone and taleranol) were negative (Metzler and Pfeiffer 2001).

Need to assess efficacy of public health actions:

Because of the potential toxicity of these compounds, whether from natural or synthetic sources, it is important to prevent human exposure. Identifying zeranol in biospecimens is not sufficient to prove exposure to the synthetic hormone, as ingestion of *Fusarium* contaminated corn can produce similar results. However, laboratory methods exist to distinguish between metabolites

[1] U.S. FDA states in regulation: "The safe concentration for total zeranol residues in uncooked edible tissues of cattle is 150 parts per billion (ppb) in muscle, 300 ppb in liver, 450 ppb in kidney, and 600 ppb in fat….A safe concentration refers to the total residue concentration considered safe in edible tissues….No residues of zeranol may be found in the uncooked edible tissues of sheep …." (CFR Title 21, Part 556). OEHHA was unable to find a recent analysis using current knowledge about cumulative effects of hormones and has not conducted a quantitative analysis of the health effects of zeranol.

resulting from exposure to zeranol and those resulting from exposure to the *Fusarium* mycotoxin. Elevated levels of zeranol or its metabolites could indicate that measures aimed at keeping the synthetic hormone out of the food supply are not adequate or that efforts to prevent *Fusarium* contamination of the food supply are not adequate.

Potential to biomonitor:

Physical and chemical properties:
Vapor pressure: 1.01×10^{-14} torr
Water solubility: < 0.1 g/100 ml (at 20 °C).
Bioaccumulation: Bioconcentration factor (BCF) 504 (@ pH 1-6, T 25 °C.

Past biomonitoring studies: Zearalanone is stored in adipose tissue (Pillay et al. 2002). In one study (Nagel et al. 1998), an oral dose of zearalanone had a half-life of 22 hours in human blood. Both the presence of mycotoxins and serum levels of zearalanone in the range of 18.9-103µg/L were associated with early thelarche and mastopathy in Hungarian girls (Szuets et al. 1997).

Availability of analytical methods:

Since zeranol use has been banned in the European Union, the interest in detecting illegal zeranol use has resulted in the development of GC-MS (Blokland et al. 2006) and immunoassay (Tuomola et al. 2002) techniques that can be used to distinguish between exposure to zeranol and exposure to *Fusarium* toxins in biological specimens. LC/MS methods exist to detect zeranol in sub-ppb quantities in animal urine (Launay et al. 2004, Schmidt et al. 2008, Rúbies et al. 2007).

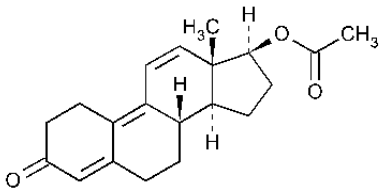
Availability of adequate biospecimens:

Urinary metabolites include conjugates of zeranol and zearalanone with glucuronic acid and sulfuric acid. Almost ¼ of the dose in humans is not accounted for and assumed to represent unknown metabolites, including a hydroxylated zearalanol or ring-opened zearalanone (Metzler and Pfeiffer 2001).

Incremental analytical cost:

GC/MS and LC/MS equipment are available in the laboratory, but development work is necessary to establish and validate methods.

Trenbolone Acetate [CASRN 10161-34-9]



Exposure or potential exposure to the public or specific subgroups:
Trenbolone acetate (TBA), a synthetic androgen, is administered by implantation of a continuously releasing hormone pellet (300mg) in the ear. TBA is hydrolyzed to 17β-trenbolone, a potent androgen receptor agonist that is oxidized to trendione and then reduced to 17-α trenbolone (Schiffer et al. 2001). Approximately 95 percent of TBA is excreted as 17α–trenbolone (Ankley et al. 2003, Schiffer et al. 2001). Feedlot effluents contain detectable concentrations of both 17α-trenbolone and 17β-trenobalone (Durhan et al. 2006). TBA metabolites have been detected at levels of 1700 picograms (pg)/kg effluent and 5-75 ng/kg solid manure (Schiffer et al. 2001). In the most recent NRP report, out of 497 samples analyzed, there were two residue violations (0.01%) for TBA (NRP 2006).[2]

Known or suspected health effects:
Trenbolone is an illicit drug of abuse in sports because of its effectiveness as an anabolic steroid, and it is considered to cause reproductive toxicity for the purposes of Proposition 65. TBA metabolites, including 17α-trenbolone and 17β-trenbolone, found in feedlot effluent samples have demonstrated androgenic activity *in vitro* (Jegou et al. 2001) and in fish living downstream from animal feedlots (Jegou et al. 2001, Orlando et al. 2004). 17β-trenbolone has anabolic activity several times above that of testosterone (Khan et al. 2008b), is a potent androgen and reproductive toxicant (Ankley et al. 2003, Gray et al. 2006, Hotchkiss et al. 2007, Wilson et al., 2002), and has a higher affinity for the testosterone receptor than does endogenous testosterone (Ankley et al. 2003). The affinity of both and 17β- and 17α-trenbolone for the human androgen receptor (AR) has also been reported (Bauer et al., 2000, Wilson et al. 2002). Thus, human exposure is a significant concern. 17α-trenbolone is an unexpectedly potent reproductive toxicant in fish, which may be due to conversion of 17α-trenbolone to 17β-trenbolone by the fish (Jensen et al., 2006). Trenbolone can cross the placenta in late pregnancy (Lange et al. 2001). Trenbolone has also been shown to cause suppression of delayed type hypersensitivity response (Hotchkiss and Nelson, 2007). Studies of genotoxic potential of TBA and its metabolites have mostly been negative (Metzler and Pfeiffer 2001).

[2] According to the U.S. FDA, "[t[he safe concentration for total trenbolone residues in uncooked edible tissues of cattle is 50 parts per billion (ppb) in muscle, 100 ppb in liver, 150 ppb in kidney, and 200 ppb in fat….A safe concentration refers to the total residue concentration considered safe in edible tissues" (CFR Title 21, Part 556). OEHHA was unable to find a recent analysis using current knowledge about cumulative effects of hormones and has not conducted a quantitative analysis of the health effects of trenbolone.

Need to assess efficacy of public health actions:
Finding trenbolone or its metabolites by biomonitoring could signal its use as an illicit drug or a break down in the control of its use in food-animal production, although blood tests alone may not be sufficient to distinguish between these two exposures.

Potential to biomonitor:
Physical and chemical properties:
Vapor pressure: 1.26×10^{-9} torr
Water solubility: 0.012 g/L
Bioaccumulation: Bioconcentration factor (BCF) 162
Persistence: TBA metabolites are stable in animal waste and the environment. Levels decreased from 1700 pg/kg to 1100 pg/kg (liquid manure) after 5 months of storage, which translates to a half-life of 267 days in liquid manure (Schiffer et al. 2001). TBA metabolites were detectable in soil 58 days after fertilization with solid dung (Schiffer et al. 2001).

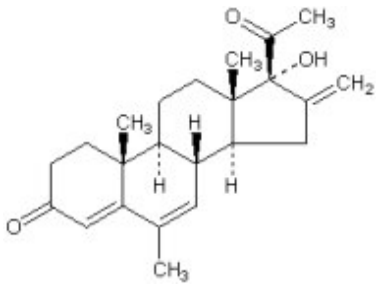
Past biomonitoring studies: Oral uptake in rats fed meat from cows treated with trenbolone was in the range of 3-7 percent. Most of what the rats consumed was excreted unabsorbed in feces (JECFA/WHO 1988).

Availability of analytical methods:
Because of the illicit use of trenbolone in body building and in horse racing, and its use in food-animal production, methods to detect trenbolone metabolites through biomonitoring have been well developed. According to Thevis et al. (2008) "liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been the method of choice for the detection of these analytes in sports drug testing. Recent findings of trenbolone and methyltrenbolone in doping control urine samples of elite athletes demonstrated the importance of a sensitive and robust analytical method, which was based on an enzymatic hydrolysis of target compounds, liquid-liquid extraction and subsequent LC-MS/MS measurement." In humans, 17β-trenbolone is mainly excreted in urine as 17α-trenbolone; 17β-trenbolone and trendione are also present (Spranger and Metzler 1991).

Availability of adequate biospecimens: Urine

Incremental analytical cost: The laboratory has LC/MS equipment, but development work is necessary to establish and validate the methods.

Melengestrol Acetate [CASRN 2919-66-6]



Exposure or potential exposure to the public or specific subgroups:
Melengestrol acetate (MGA) is a synthetic progestin and is administered in cattle feed at a dose of 0.25 to 0.50 mg/day (Velle 1981) for 9-150 days for the purpose of estrus synchronization and suppression in feedlot heifers and to promote growth by improving feed efficiency and causing weight gain (Schiffer et al. 2001). Twelve percent of MGA is unabsorbed in the gastrointestinal tract and excreted in feces (Schiffer et al. 2001). Levels of MGA in solid manure were in the range of 0.3-8 ng/kg (Schiffer et al. 2001). MGA has been found in waterways downstream from cattle feeding operations (Kolok et al. 2007). Lange et al. (2002) calculated that if 30 percent of beef heifers (1.65 million head) are fed 0.5 mg of MGA each day for 120 days, an additional 10 kg of MGA will reach the environment via beef heifer excrement. In the most recent U.S. Department of Agriculture (USDA) National Residue Program (NRP) report, out of 342 samples analyzed, there were no violations of MGA residue levels set for cattle (NRP 2006).[3] However, when cows were fed three times the recommended dose of MGA, the mean MGA residue in fat reached 29 ppb which is higher than the violation level of 25 ppb (Daxenberger et al. 1999).

Known or suspected health effects:
MGA has progestional and glucocorticoid activity (Lauderdale et al. 1977). MGA suppresses estrus in female cattle by maintaining luteinizing hormone (LH) secretion and acting with follicle-stimulating hormone (FSH) to stimulate estrogen production and growth-promotion (Galbraith 2002). MGA has greater affinity for the bovine progestin receptor than does progesterone (Bauer et al. 2000). MGA delayed menses in normal ovulating women and induced withdrawal bleeding in amenorrheic women (Lauderdale et al. 1977). Progesterone is listed as known to cause cancer under Proposition 65. MGA has not shown genotoxic potential, but one commercial MGA formulation was contaminated with an apoptosis-inducing compound (Metzler and Pfeiffer 2001).

[3] According to U.S. FDA regulation: "A tolerance of 25 parts per billion is established for residues of the parent compound, melengestrol acetate, in fat of cattle." (CFR Title 21, Part 556). We were unable to find a reanalysis using current knowledge about cumulative effects. Independent analysis by OEHHA was not conducted.

Need to assess efficacy of public health actions:
Finding MGA by biomonitoring would indicate that the control of its use in veterinary medicine has not been successful.

Potential to biomonitor:
Physical and chemical properties:
Vapor pressure: 9.29×10^{-11} torr
Water solubility: 3.1×10^{-3} g/L
Bioaccumulation: Bioconcentration factor (BCF) 926
Persistence: MGA is stable in soil; levels in solid manure ranged from 160-7,760 pg/g initially to 420-6,030 pg/g after 4.5 months of storage (Schiffer et al. 2001). MGA was present in soil 195 days after fertilization with solid dung and after cultivation of a maize crop (Schiffer et al. 2001).

Past biomonitoring studies: Radioactive MGA given to women orally had a half-life of 3.5 days; urine metabolites included hydroxyl, glucuronide, and sulfated compounds (Cooper et al. 1967).

Availability of analytical methods:
MGA residues accumulate preferentially in fatty tissues. ELISA, LC-MS, and HPLC-UV are options for detection. Rapid screening methods have been developed for detection in feces (heifer) by liquid extraction/ELISA (Lange et al. 2003), and methods have been developed for bovine and porcine plasma (Mortensen and Pedersen 2007). In 1977, levels of detection were below 10 ppb in fat, liver, muscle, and kidney (Lauderdale et al. 1977).

Availability of adequate biospecimens: Methods exist for plasma, feces, and various body tissues.

Incremental analytical cost: Development work is necessary to establish and validate the methods.

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