Trenbolone and Other Cattle Growth Promoters: Need for a New Risk-Assessment Framework

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The "six-pack" of hormone growth promoters used in feedlot cattle deserves closer investigation from researchers and policy makers. The potential effects of exposure to such compounds are of particular interest in the field of endocrine disruption, which focuses on the effects these compounds can have during sensitive developmental periods, rather than using a carcinogenesis model. Steroid hormones participate in a delicate balance during fetal development. Interference with that balance can lead to disruptions that manifest as health problems at birth and later in life. Although we need more studies of the effects of feedlot compounds on human and wildlife development, some industry and government literature leads consumers to conclude that all the data are in. Governmental reviews of the literature from the European Union (where hormone use is banned) and Australia (where hormones are used) reveal different attitudes and conclusions about the same body of research, and these differences may relate to a different understanding of the value of the endocrine-disruption model. It is important to approach examinations of the effects of trenbolone and other growth promoters without bias and to acknowledge that cattle growth promoter research in the context of endocrine disruption has just begun. The scientific jury is still out regarding whether introducing these compounds into the human food supply and ecosystems—research suggests that hormonally active feedlot effluent may harm wildlife—can be deleterious.

Environmental Practice 8:58–65 (2006)

Each year in the United States, 36 million beef cattle are raised, and in two-thirds of these animals, bulk is increased using hormones. Of the six hormones used in bulk-inducing cocktails in the United States in various combinations of up to three hormones, three (estradiol, progesterone, and testosterone) are natural hormones, and three (zeranol, trenbolone acetate, and melengestrol acetate) are synthetic. All are intended as growth promoters to give cattle producers more meat for their investment on a per-animal basis. For example, according to a publication on cattle growth-promoting hormones produced by the Canadian Animal Health Institute, a trade association, hormones help improve nutrient retention, resulting in less need for feed for an animal to reach its "finish weight," or market weight (Canadian Animal Health Institute, undated). Also, according to the same publication, hormones improve meat quality by changing the deposition of fat and producing leaner meat. The publication states that without these economic benefits, the higher costs of raising beef cattle would be passed on to the consumer. As an example, at the time of this writing, the publication's authors cited the legislatively mandated hormone-free beef in the European Union (EU), where about a half pound of lean ground beef "will cost $4.60 in Germany while costing $3.19 in Canada" (Canadian Animal Health Institute, undated). Administration of these hormones to beef cattle is approved by the United States Food and Drug Administration (FDA).

On a global scale, the use of these compounds in cattle varies. The European Parliament has banned the use of the hormones as cattle growth promoters and issued a moratorium on imports of meats derived from animals dosed with such compounds. The World Health Organization and other global entities have attempted to adopt growth-promoting hormone standards, with a result that maximum residue limits for the synthetic hormones trenbolone and zeranol have been set (Joint FAO/WHO Expert Com-

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 DOI:10.1017/S1466046606060042

mittee on Food Additives, 2001a, 2001b, 2005). A total of 31 countries have approvals in place for the use of hormones in cattle; only the EU has banned them.

As a result of the EU ban on trade in beef containing growth-promoting hormones, the United States requested that the World Trade Organization (WTO) review the measure and rule on its compatibility with WTO agreements. The WTO tentatively ruled in favor of the US in 1997 and found definitively in favor of the US in 1999, when it ruled that the EU's ban on US beef had resulted in annual export losses of $116.8 million for the US. The Office of the US Trade Representative stated in its news release that "decades of scientific research—by both US food safety regulators and international bodies such as the World Health Organization—have proven the safety of the growth-promoting hormones used in US beef production" (United States Mission to the European Union, 1999). In spite of the ban, however, a black market in hormone-treated beef persisted in Europe (Raloff, 2002). The FDA's statement that scientific research demonstrates the safety of such compounds has been called into question by a new research context, which I will address in more detail below.

The United States Department of Agriculture (USDA) states in its 1999 *Primer on Beef Hormones* (United States Department of Agriculture, 1999) that the EU markets use uncastrated bulls as a beef source rather than the castrated steers used in the US, and that bulls have more than ten times the levels of testosterone as steers. They conclude that this translates into US beef's having lower levels of hormones than most European beef, although they do not address the issue of the five other hormones potentially being given to US beef cattle, or the fact that heifers also are slaughtered for beef.

In the same primer, the USDA argues that estradiol equivalent levels in beef are less than those found in eggs, milk, and foods containing phytoestrogens, and additionally, the USDA fact sheet argues that "the amounts of estradiol, progesterone, and testosterone in animals raised using hormones as growth promoters are extremely low compared with their production in humans" (United States Department of Agriculture, 1999). It cites as an example young boys, who, according to the fact sheet, would have to consume daily 16 pounds of beef raised using estradiol in order to produce a 1% increase in their production of this hormone. The comparison also makes no mention of the other five hormones variously used in beef cattle. The fact sheet also points out that pregnant women produce nine million times more estradiol than is found in a half-pound portion of beef from an animal raised using estradiol.

While the primer raises questions, some of which I will address below, it also provides an interesting implication. It implies that these hormones do persist in the beef, or at least, that testosterone and estradiol do, at very low concentrations. There is some evidence, discussed below, that hormones persist in parts per billion or parts per trillion concentrations in beef from cattle exposed to single hormones or combinations of two or three of the "six-pack" of growth-promoting chemicals, although slaughter practices call for cessation of hormone administration a few days before slaughter to allow the animals to process out the hormones. Of course, during the hormone-administration period and after cessation, the animals still excrete hormone products. It is known that metabolites of at least some of these compounds pass into feces, and at least one metabolite of trenbolone is excreted in urine (Lange et al., 2002). Issues of persistence have importance not only for effects on humans, but for effects on wildlife, as well.

It should be noted that cattle do not receive the six-pack all at once or even receive all of the hormones, and the androgen combination of implants is usually used in the final 60 to 90 days of feeding. In the United States, rules stipulate that no more than three of the six hormones will be used in a combination at any one time and all combinations used require FDA approval.

Problems in Literature Interpretation

In examining the current literature on feedlot hormones in the context of endocrine disruption, I encountered a surprising and interesting interpretive conflict. The EU, in its 2002 *Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health* [SCVPH], describes a group of 17 studies it relied on in making its most recent decision to continue prohibition of hormone-treated beef from European countries (Scientific Committee on Veterinary Measures Relating to Public Health, 2002). After a review of these 17 studies, the panel concluded, "after re-appraisal of the data from the 17 studies and recent scientific literature, the SCVPH confirms the validity of its previous *Opinions* (in 1999 and 2000) *on the Assessment of Potential Risks to Human Health from Hormone Residues in Bovine Meat and Meat Products*, and that no amendments to those opinions are justified" (Scientific Committee on Veterinary Measures Relating to Public Health, 2002).

The Australian government, which allows use of hormones in beef cattle (other countries where they are approved include the United States, South Africa, Japan, Mexico, New Zealand, and Chile), has produced a similar document listing the same 17 studies and including the comments of an advisory panel on the conclusions and merits of each study (Australian Advisory Committee on Pesticides and Health, 2003). A draft of this document was approved in 2003 by Australia's Advisory Committee on Pesticides and Health. In an interesting counterpoint to the EU's conclusions, this document contains many negative comments on these studies, including, "This report [Metzler and Pfeiffer, 2001] lacked detail and transparency in both the experimental methods and results," and faults several reports in a similar manner (Australian Advisory Committee on Pesticides and Health, 2003).

The authors of the Australian document conclude that "(1) a review of the new data does not indicate any grounds for amending Australia's current regulatory position with respect to HGPs [hormone growth promotants]—i.e., that there is unlikely to be any appreciable health risk to consumers from eating meat from cattle that have been treated with HGPs according to GVP [good veterinary practice]; (2) there is no new scientific evidence to indicate a need for the reconsideration by the Australian Pesticides and Veterinary Medicines Authority of the present use of HGPs under GVP conditions; and (3) in view of the complexity of assessments of total dietary hormone intakes and the contentious views expressed in various international forums, the use of HGPs in meat producing animals should be kept under ongoing consideration by the Advisory Committee on Pesticides and Health" (Australian Advisory Committee on Pesticides and Health, 2003).The Australian report also contains many references to concerns about carcinogenicity in adults, but it contains only two (out of 175) pages that focus on endocrine-disruption concerns.

It is interesting that two bodies of scientists could review the same articles and the same data and reach such directly opposing opinions. Most of the articles discussed are published in peer-reviewed journals, implying that reviewers thought the findings warranted publication. How can scientific groups in two countries be so polarized in their conclusions about the same group of studies? I argue that the EU is erring on the side of caution in this situation, not wanting to wait until more data are in, and basing the decision on a small but growing body of evidence that the use of hormones in cattle can disrupt on many levels. It appears that the Australian panel may also have thought that some of the findings were valid, but that most were not conclusive enough to warrant an expensive and extensive policy change. In the delicate balance of deciding where to err, each side appears to have decided in favor of its existing policy.

Problems in the Context of Endocrine Disruption

The information provided by government agencies and industry groups in countries that use hormones in many cases does not address the concerns of researchers in the field of endocrine disruptors. These concerns include mixture effects, effects of exposure at sensitive developmental periods, and the differences in physiological response to pharmaceutical versus natural steroid hormones (Crews, Willingham, and Skipper, 2000). In addition, one tenet of the endocrine-disruption model is that compounds can have effects at doses previously thought to be ineffectual or that they exhibit hormesis, with similar effects at low and high doses (Crews, Willingham, and Skipper, 2000). To my knowledge, no studies of the effects of growth-promoting hormones address dose–response curves during development in the context of hormesis.

Often, the information disseminated to allay the public's concerns about hormones in beef is not meaningful in the context of endocrine disruption. For example, in the Canadian Animal Health Institute's fact sheet (undated), the authors make a comparison between estrogen levels produced daily in an adult male (136,000 ng) and those found in a 6-oz serving of beef from a treated animal (3.8 ng). The implication is that adult males produce an amount of estrogen five orders of magnitude greater than that found in a bit of beef; the implied conclusion is that 3.8 ng is negligible.

In a further comparison, the fact sheet states that a 6-oz serving of beef from an animal not treated with hormones contains about 2.6 ng of estrogen, implying that this difference of a miniscule 1.2 ng between the two is negligible; however, this is really an increase of 7 ng/kg in the amount of estrogen in beef from a treated animal. In the delicate world of steroid hormone activity, an increase of that nature can be meaningful, especially if it translates into an increase at a developmentally sensitive period (such as fetal development) or an increase in combination with the introduction of other hormonally active compounds (Crews, Willingham, and Skipper, 2000). This increase can be more significant if the hormone is a synthetic that binds the native receptor with a greater affinity than does the natural

ligand. The fact sheet does not provide information about levels of hormone in the fetal environment, how the levels in the beef compare, or whether the hormone can enter the fetal environment. Although answers to some of these questions are available, more research is needed on the effects the ingestion of low doses of hormones (especially the synthetics) can have *in utero*. The information also does not address potential differences in effects between ingested and endogenously produced estrogens or the differences in potency between natural and synthetic hormones (Bauer et al., 2000).

Indeed, the information disseminated often neglects these primary concerns of researchers in the field of endocrine disruption. An information sheet from Health Canada (2004) produced for consumers states that the concerns about the use of hormones relates to worries about the impact on human health, "in particular, whether these compounds may be cancer causing . . ." (Health Canada, 2004). The link between embryonic hormonal events and cancer interests endocrine-disruptor researchers; the origins of such research trace back to the discovery of cancers and reproductive disorders in children of women who used diethylstilbestrol (DES) during pregnancy (McLachlan, 1977; Newbold et al., 1998; Newbold et al., 2000). But the implied—and probable—meaning of this reference to cancer in the Health Canada literature is induced carcinogenesis in adults. In the field of endocrine disruption, a compound's carcinogenic potential in adults is not the primary concern (Crews, Willingham, and Skipper, 2000); cancer enters the picture as another potential latent effect of *in utero* exposure. The main concern of endocrine-disruption investigations—and research into the effects of feedlot hormones falls squarely into that arena—is the effect that pharmaceutical hormones and other disruptors can have during embryonic development and other sensitive developmental periods, such as puberty (Landrigan, Garg, and Droller, 2003). Some researchers focus on human health; others, on wildlife health; but the bottom line is that sensitive developmental periods are the current focus. Nowhere in any of the government or industry consumer literature on this topic did I find reference to this concern specifically as it relates to endocrine disruption and the latent effects these developmental exposures to minute (parts per billion or parts per thousand) concentrations can have (Crews, Willingham, and Skipper, 2000).

There are several questions relevant to the use of hormones in beef cattle in the context of endocrine disruption:

1. *Risk:* In humans, do residues occur in ingested food and at what concentrations, and which of the compounds—if any—cross the placenta and could affect a developing fetus? In animals, what is the persistence of the compounds in the environment, and what are the various potential routes of exposure?
2. *General developmental effects:* What, more specifically, are potential developmental effects of exposure to environmentally relevant levels via probable routes?
3. *Mixtures:* What mixtures occur in beef that people consume or in environments adjacent to or downstream in feedlots, and what are the effects of these mixtures in people and wildlife?

As a part of this last question, studies should address dietary intake of other hormonally active compounds as a component of the mixture equation.

Risk and Exposure: How Much Risk is Associated with Exposure to These Compounds?

Given that *in utero* effects are of paramount interest, the question arises as to whether compounds like trenbolone can even reach the fetus. The USDA publication (1999) mentions that pregnant women produce nine million times more estradiol than is found in a half-pound serving of beef from an animal raised on estradiol. What it does not mention is that much of this huge amount of estrogen a pregnant woman produces is sequestered from the fetus by sex hormone binding globulins. Sex hormone binding proteins increase in pregnant women to levels six to ten times higher than those of nonpregnant women (Diagnostic Products Corporation, 1997), and the fetus itself produces an alpha-feto binding protein to modulate hormonal activity. Why? Because the fetus is producing its own carefully controlled hormonal milieu in response to fetal events; an imbalance in that milieu could lead to an imbalance and disruption of fetal endocrine-governed development. The average USDA reader would infer from the publication that pregnant women make so much estrogen that a tad more won't make a difference. It may not, but there are no studies to examine the effect of this apparently minor increase via exogenous exposure in humans. Additionally, the USDA information sheet fails to mention exposure to one or more of the other five compounds used in beef cattle, including testosterone and trenbolone, which do cross the placental barrier (Lange et al., 2002).

If binding proteins do not recognize ingested hormones and synthetics such as trenbolone and these compounds can cross the placental barrier (see below), then they may be capable of disrupting the delicate fetal hormonal milieu. Some research suggests that this is, in fact, what happens. We know, for example, that DES crosses that barrier (McLachlan, 1977; Newbold et al., 1998; Newbold et al., 2000), and other compounds that mimic hormones—but aren't sequestered—can cross as well. In a specific example, Bauer et al. (2000) examined the ability of anabolics and their metabolites to compete with natural sex hormones for the sex hormone binding globulin and found that trenbolone does not bind to these proteins in the blood with high affinity.

The major focus of issues of low concentrations of residues is placed here in an endocrine-disruption context. This model of risk differs from that for carcinogenicity in its basic assumptions (Crews, Willingham, and Skipper, 2000). One assumption is that there may be a lack of a threshold dose for effects of compounds that are hormonally active during sensitive developmental periods, such as embryogenesis. The basis of this assumption is that for each individual, endogenous hormone levels are those that are appropriate for normal development. Anything exceeding this dose exceeds the threshold and tips the hormonal balance inappropriately. Because some synthetics are not recognized by binding proteins, they may, in fact, exert greater hormonal influence during development than their natural counterparts.

From a wildlife-effects perspective, none of these compounds will have any effect if they are not present in the environment and thus unavailable to wildlife. Taking trenbolone again as a specific example, research has found that it persists in manure for 260 days and that 10% of the amount given to an animal passes into its feces (Schiffer et al., 2001). These studies demonstrate the presence of trenbolone residues in manure, the concentrations in liquid manure representing 12% of the administered dose and, in solid dung, 20%. The behavior of the excreted anabolic steroids in soil can be compared to well-documented agricultural or industrial pollutants. In fact, the behaviors of these residues in soil are similar to those of chlorinated organic compounds and aromatic hydrocarbons (Schiffer et al., 2001; Weissenfels, Klewer, and Langhoff, 1992). When spread on fields, trenbolone degrades within a week, and it degrades in 2 months in dried dung fertilizer, although there is some question as to whether this is true degradation or dissipation as a result of runoff. Some wildlife work points to runoff as the reason for this decrease (Orlando et al., 2004).

Trenbolone: A Specific Case

Trenbolone acetate is quickly hydrolyzed to trenbolone (TbOH)-17β, and metabolites of TbOH include TbOH-17α and triendione (Pottier et al., 1981). Both forms of TbOH have an affinity for human androgen receptor (Bauer et al., 2000), and the β-form has a stronger affinity for this receptor than does one of its natural ligands, 5α-dihydrotestosterone (DHT) (Bauer et al., 2000). Additionally, TbOH-β can be more effective than DHT *in vitro* (Wilson et al., 2002), competing for the human androgen receptor more efficiently than DHT at concentrations of 10 and 100 parts per million.

Although TbOH-β is the metabolite most suspected of exerting a disruptive influence, it is possible that TbOH-α may also exert DHT-like effects. Some DHT-like effects with TbOH-β have been recorded; for example, subcutaneous administration of high doses of TbOH-β to pregnant rats resulted in their female offspring's having a greater anogenital distance length and regressed nipples, both DHT-dependent phenomena (Wilson et al., 2002). It must be pointed out that Wilson et al. (2002) found that giving trenbolone subcutaneously results in an increase in its potency of about 100-fold compared to oral ingestion; the latter, of course, would be the route of concern for human consumption. In addition, the dosages used in the Wilson et al. study, by one reviewer's calculations, would be equivalent to a daily intake of over 600 kg of trenbolone-acetate–contaminated liver (based on the value reported by Marchand et al., 2000) or 6,000 kg of contaminated muscle (based on the values identified by Sadek et al., 1998). These would be, of course, huge intakes for a person.

Some research indicates that in maternal transfer, TbOH-α crosses the placental barrier more efficiently and is the primary metabolite found in maternal tissues and in the liver and kidney of fetuses (Lange et al., 2002). The research, which employed rabbits as models, showed that there were higher proportions of TbOH-α in fetal fat and muscle than in maternal fat and muscle, implying that perhaps further metabolization could occur (Lange et al., 2002). Thus, it is possible that even the DHT-like effects seen with TbOH-β administration by Wilson et al. (2002) could actually be the result of DHT activity of the α-form after more efficient placental transfer or transformation.

The fact that either form can partition into liver or fat is important for species at risk; in the case of an aquatic species, for example, it is possible for the mother to take up runoff contaminants through the environment and then pass them to offspring via transfer in yolk. Studies have shown that developing turtle embryos take up steroids from the yolk through the course of development (Elf, Lang, and Fivizzani, 2002); it is possible that they may take up maternally transferred steroid-like compounds—such as trenbolone—from yolk, as well.

Trenbolone has also been detected in beef intended for human consumption, and, as the government literature implies, so have estrogens. Henricks et al. (2001) report trenbolone α and β residues in liver after solo treatment with trenbolone in amounts up to 4.5 μg/kg and 1.1 μg/kg, respectively; Marchand et al. (2000) identified 9 μg/kg and 1.5 μg/kg for trenbolone α and β residues in liver, respectively. Sadek et al. (1998) report having found trenbolone levels in samples of beef from Egyptian markets with levels up to 1.2 μg/kg. Based on the table provided by Sadek et al. (Table 1 in their article), they present 12 samples of beef from various districts. For each sample, they assayed levels of trenbolone acetate, zeranol, DES, and estradiol. They found trenbolone in μg/kg levels in every sample, estradiol in some but at extremely low levels (parts per trillion), and zeranol in none; DES was also present in very low levels. The Australian government's National Residue Survey Annual Report, 2002–2003, reports having found trenbolone in six of 340 beef liver samples tested, although exact levels were not reported because the amounts fell below the Australian Standard cutoff of 0.01 mg/kg (0.01 parts per million) (National Residue Survey, 2003). An unspecified number of European meat and liver samples were found to contain residues of anabolic compounds (Marchand et al., 2000). Residues of melengestrol acetate (1.5 μg/kg) were found in some meat samples. Two trenbolone metabolites—TbOH-α (9.0 μg/kg) and TbOH-β (1.5 μg/kg)—were detected in one liver sample, and zeranol (1.2 μg/kg) was detected in another (Marchand et al., 2000).

There is no conclusive evidence either way, but the findings so far point to a possibility that wildlife is exposed to these compounds and may be adversely affected, and there is some indication humans may consume some residues via meat consumption. If people do consume these residues in low levels, can these compounds or their metabolites even gain access to a developing fetus? As mentioned, one study indicates that trenbolone, zeranol, and melengestrol acetate can all cross the placental barrier in rabbits (Lange et al., 2002) and do accumulate in fetal tissues, as well as in maternal tissues.

Mixtures

Evidence in government literature was cited to indicate that the dietary intake of estradiol in an egg is greater than that in meat from both treated and untreated cattle; the median level in untreated cattle, treated cattle, and in a egg is less than 2.5 ng/250 g steak, 5 ng/250 g steak, and 6.5 ng/50 g egg, respectively. Because many people consume both meat and eggs and other sources of dietary estrogens, effects of mixtures are also of paramount interest in the field of endocrine disruption. The use of combinations of up to three hormones from the six-pack of compounds promoting growth in cattle provides an excellent example of hormonally active compounds often occurring in mixtures, a key focus of research in the endocrine-disruption context (Crews, Willingham, and Skipper, 2000). Because investigations into the effects of this group of cattle growth promoters in an endocrine-disruption context has just begun, few data on the effects of mixtures of these compounds in that context exist. Here, again, the industry and government literature intended for public consumption are lacking, citing only examples of single hormones, rather than stating how much of each hormone might occur in a half pound of beef from a hormonally treated animal receiving combination treatments of two or three of the hormones.

It is necessary to establish how much, if any, of these compounds end up in meat products that people consume and in what concentrations and mixtures. The FDA may require this information, but in an international context, the data on what residues are found, at what levels, and in what concentrations, are conflicting and variable, as the data cited in this commentary indicate. These data must be followed by investigations into the effects these mixtures can have on endocrine-governed processes during vertebrate embryonic development in an endocrine-disruption context, looking at both human and wildlife exposures. That context includes assumption of the possibility of a no-threshold-dose effect (Crews, Willingham, and Skipper, 2000).

More Research is Needed

More research on three fronts in this area is needed. We need to investigate carefully, with good controls and clearly

reported data, whether residues of these compounds end up in food that we consume and in what levels and tissues. In addition, we need further investigation into the effects that exposure to these compounds may have on wildlife, an area with a clearer suggestion of a problem at this time; for example, when one research group investigating contamination searched for a reference site in a study of residue runoff from feedlots, they could not locate a feedlot that did not employ trenbolone in their cattle (Orlando et al., 2004).

If residues are found, we need careful investigation into the potential effects residues at this level may have on developmental processes in a mammalian system, using the endocrine-disruption model and not the carcinogenic model. Mixtures—from dietary and contaminant sources—should be taken into account. Such research should lead to clearer government guidelines and a better-informed public. It may also possibly lead to greater agreement among governments on interpretation of the same data sets.

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Submitted March 4, 2005; revised January 31, 2006; accepted February 16, 2006.