Impacts on Human Health and Safety of Naturally Occurring and Supplemental Hormones in Food Animals

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Introduction

Since the end of the Second World War, the United States has pursued a national policy of an abundant and inexpensive food supply. This policy has been hugely successful at meeting these goals. The average share of disposable personal income spent on total food by consumers in the U.S. from 1960 to 2018 fell from 16.8% to 9.7%, driven by a declining share of income spent on food at home (Figure 1). Concomitantly, increased animal productivity has improved efficiencies of animal production and reduced the carbon footprint for production of meat, milk, and eggs (Capper 2011; Capper and Cady 2020; Pelletier, Ibarburu, and Xin 2014; Putman et al. 2017; Thornton 2010).

Some components of the technologies employed by animal producers to improve efficiency of animal production include improved nutrition and reproduction; advances in genetics; and health and management practices; as well as feed additives, hormonal treatments, and growth enhancing technologies (GETs). These technologies have reduced cost of food production and reduced impacts of animal production on the environment (Avery and Avery 2007).
Originally, food was simply perceived as a means to obtain the necessary nutrients and energy for the body. As the evolution of nutrition science progressed, food began to be seen as a means of supporting adequate growth and development of the body. Currently, food is also perceived as a key factor influencing the prevention of some diet-related diseases (Pogorzelska-Nowicka et al. 2010). Thus, a substantial effort in the food production industry goes towards the improvement of food healthiness (Decker and Park 2010). In addition, there is the requirement to establish that residues of naturally occurring and synthetic compounds used to improve animal efficiency do not pose a human health risk.

The use of growth enhancing technologies (GETs) has improved the quality of meat products by repartitioning fat into muscle mass and reducing fat content of meat products.

All hormonal compounds have undergone rigorous testing for human and animal safety under guidance of the FDA Center for Veterinary Medicine.
In the United States, the oversight of food safety at the national level presently involves at least twelve agencies, of which four predominate: the U.S. Department of Agriculture (USDA), the FDA, the Environmental Protection Agency (EPA), and the National Marine Fisheries Service.

The FDA has jurisdiction over domestic and imported foods that are marketed in interstate commerce, except for meat and poultry products. The FDA's Center for Food Safety and Applied Nutrition (CFSAN) seeks to ensure that these foods are safe, sanitary, nutritious, wholesome, and honestly and adequately labeled. The CFSAN exercises jurisdiction over food processing plants and has responsibility for approval and surveillance of food-animal drugs, feed additives and of all food additives (including coloring agents, preservatives, food packaging, sanitizers, and boiler water additives) that can become part of food. The CFSAN enforces legal limits (tolerances) for pesticide residues that are set by the EPA and shares with USDA Food Safety and Inspection Service (USDA-FSIS) responsibilities for egg products. NOAA Fisheries is responsible for the stewardship of the nation's ocean resources and their habitat. They provide oversight for productive and sustainable fisheries, safe sources of seafood, the recovery and conservation of protected resources, and healthy ecosystems—all based on sound science and an ecosystem-based approach to management.

What are hormones?

Hormones are chemical messengers produced in one type of cell or tissue of an organism and transported in tissue fluids to regulate a specific set of cells or tissues in another part of the organism. Hormones are classified into different groups based on their chemical structures and resulting physiological functions. Hormones are able to exert their actions on specific cells types or tissues through interaction with a receptor that is specific to an individual hormone, similar to a lock and key system. The following section will describe the various hormone classification used in animal agriculture to improve production efficiency based on their chemical makeup.

Steroids

Steroid hormones as a group are lipids with a structure that contains four fused rings. Besides the commonly known reproductive steroids (testosterone, estrogens, progestogens), cholesterol, Vitamin D, and bile salts are also steroids. Because they are lipid soluble, steroid hormones can cross cell membranes which are composed of a phospho-lipid bilayer. Steroid hormones can easily cross cell membranes of the gut and are generally impervious to digestion so they are orally active. Steroid hormones exert their actions via receptors inside the cell (cytoplasmic, nuclear). Once bound to a specific receptor, a hormone is capable of inducing gene expression which results in activation of various pathways inside the cell resulting in specific activities such as growth, reproduction, and lactation.
Peptides/Proteins

There are 20 major amino acids and these comprise the more than 20,000 different proteins in the body, including the protein hormones. The amino acids are analogous to letters in the alphabet and each different protein has a specific sequence of amino acids that determine its three-dimensional structure and function. As stated previously, protein hormones are composed of varying numbers of amino acids and are usually water soluble and are not usually lipid soluble. Therefore, protein hormones cannot cross the cell membrane and require receptors on the cell surface which bind and then internalize the protein hormone. The cell surface receptor that a peptide/protein binds to is very specific to the hormone itself, and the hormone will bind to the receptor with very high affinity (Michael et al. 2006). The binding of the hormone to its receptor initiates an intracellular pathway that is responsible for stimulating the hormone’s action. These hormones regulate a large variety of physiological actions such as energy metabolism, ionic balance, reproduction, appetite control, growth and development, and secretion of other hormones (Michael et al. 2006). Insulin, for example is a protein hormone that regulates uptake of glucose in cells. Insulin is often needed by diabetics to help regulate their blood sugar but because it is a protein it cannot be given orally and must be injected. Examples of proteins or peptides which are used in animal agriculture include growth hormone (GH), also called somatotropin (ST), which is involved in regulation of growth and lactation, and GnRH, which is involved in regulation of reproduction.

Prostaglandins

Prostaglandins are modified fatty acids produced in animals and humans that are formed chiefly by the action of the enzyme cyclooxygenase on arachidonic acid. Since they are fatty acid derivatives, they can also cross cell membranes and are orally active. Prostaglandins perform a variety of hormone-like actions such as controlling blood pressure and smooth muscle contraction. In animal agriculture prostaglandins are used to improve reproductive efficiency by regulating the reproductive cycles of domestic animals.

Amines

Amine hormones are derived from the amino acids tryptophan (serotonin and melatonin) or tyrosine (thyroid hormones, and the catecholamines epinephrine and norepinephrine) and are secreted from the pineal, thyroid, and adrenal gland. The primary class of amines used in animal agriculture are the catecholamines, a group of chemically related compounds which affect nerve transmission and smooth muscles compounds. Catecholamines are also involved in regulating energy metabolism which is the primary reason they are used in animal agriculture to improve growth efficiency.

Sources of Hormones in Foods

Produced by Animals

Hormones produced by animals that are involved in regulation of growth, reproduction, and other biological functions are present throughout the body and are found naturally in meat, milk, and eggs. The hormones in milk are
Milk contains numerous protein hormones, steroid hormones, thyroid hormones, peptide growth factors, and prostaglandins. Perhaps the most extensively studied and the presence of hormones in milk and their physiological significance was suggested during the early 1900s. Milk contains numerous protein hormones, steroid hormones, thyroid hormones, peptide growth factors, and prostaglandins (Grosvenor, Picciano, and Baunrucker 1993). The milk of all mammals, including humans, provides not only essential nutrients, but also numerous bioactive factors (e.g., cytokines, nucleotides, enzymes, vitamins, growth factors, and hormones) that modulate development and confer immune protection to the young. The first documented presence of steroid hormones in cows’ milk was in 1929 by Yaida. Since then, several naturally occurring bioactive peptides and hormones have been identified and detected in bovine milk and dairy products (Mills et al. 2011; Park and Nam 2015; Richardson and Mattarella 1977; Schams and Karg 1986; Vargas-Bello-Pérez, Márquez-Hernández, and Hernández-Castellano 2019).

Naturally occurring hormones either originate from maternal blood and are secreted into milk through an active transport or are synthesized by the mammary gland and excreted into milk. They serve messenger and signaling roles in the regulation of mammary function and enhancement of immune development and gastrointestinal tract maturation (Hamosh 2001). The various ranges of concentrations of hormones in bovine milk according to their origin are summarized by Jouan and colleagues (2006) in Table 1.

<table>
<thead>
<tr>
<th>Hormone Type</th>
<th>Example</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonadal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal</td>
<td>glucocorticoids</td>
<td>0–50 ng/ml</td>
</tr>
<tr>
<td>Pituitary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothalamic</td>
<td>somatostatin</td>
<td>10–30 ng/ml</td>
</tr>
<tr>
<td>Parathyroid hormone-related</td>
<td></td>
<td>40–100 ng/ml</td>
</tr>
<tr>
<td>protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td>5–40 ng/ml</td>
</tr>
<tr>
<td>Calcitonin</td>
<td></td>
<td>700 ng/ml</td>
</tr>
<tr>
<td>Melatonin</td>
<td></td>
<td>5–25 pg/ml</td>
</tr>
</tbody>
</table>

Table 1. Examples and concentrations of selected hormones present in bovine milk (Jouan et al. 2006).

Steroid hormones, including estrogens, progesterone, and testosterone, are present in milk, but most attention by consumers is given to estrogens because of concerns about estrogen-dependent cancers. Estrone (E1) and estradiol (E2) are detectable in milk and a major factor that affects their concentration in milk is the amount of milk fat (Pape-Zambito, Magliaro, and Kensinger 2008; Pape-Zambito, Roberts, and Kensinger 2010). For instance, for skim milk and whole milk, respectively, E1 concentrations were 2.9 and 7.9 pg/ml and E2 concentrations were 0.4 and 0.9 pg/ml. Concentrations in high-fat ice cream, which tend to be premium brands were even greater. In a survey of retail milk across the United States (Vicini et al. 2008), whole organic milk had significantly more E2 than whole conventional milk. Likewise, progesterone was also greater in organic milk, and this is not because of the fat content of these samples, which were not affected by the production method claim on the label. Pape-Zambito and colleagues (2010) also examined organic compared to con-
Certain protein hormones are naturally in milk and of these, somatotropin and insulin-like growth factor 1 (IGF-1) have received the most attention in the last 25 years because of commercialization of recombinant bovine somatotropin (rbST). In general, the concentrations of bST in unpasteurized milk are at or near the limits of detection of highly sensitive analytical assays. Somatotropin does not bind to the human receptor for growth hormone and rbST is denatured by pasteurization (JECFA 2014). Conversely, bovine IGF-1 is detectable in milk, is not affected by pasteurization, and does have biological activity in humans. Amounts of IGF-1 in samples of milk from individual cows vary considerably from 1 to 13 ng/ml (JECFA 2014) and retail milk contains approximately 3 ng/ml, with organic retail milk having slightly less (Vicini et al. 2008). In general, circulating concentrations of IGF-1 are greater for cows with positive nutrient balances (energy and protein) and are reduced with negative nutrient balances (McGuire et al. 1992; Vicini et al. 1991); however, the concentrations of IGF-1 are greater in milk from cows in early lactation, when cows tend to be in lower nutrient balances.

In general, the amounts of naturally occurring hormones in milk and dairy products are significantly lower than production of the same hormones by humans (JECFA 2000a, b). In some cases, the biological actions of the hormones are species-specific, and most times they do not reach systemic circulation because they are naturally degraded in the small intestine. The FDA guidelines state that no physiologic effects could be expected when consumption is ≤1% of the endogenous quantities produced by the segment of the population with the lowest daily production (FDA 2018). An illustrative example was performed by Macrina and colleagues (2012), who tested the potential ingestion of estrogens by consumption of retail milk and dairy products. Production rates of E1 plus E2 in humans range from 54,000 to 630,000 ng/day. These authors estimated total E1 intake from three servings of whole milk was 68 ng/day, which represents 0.01% to 0.1% of the daily production rate in humans. These levels are far below the current guidelines for safe consumption and should rule out concerns regarding disorders or hormone imbalances based on the consumption of dairy products.

Other animal products, such as eggs, beef, poultry, pork and fish also contain hormones, of which steroid hormones have been characterized. Consumption of steroids in the diet are considerably lower than steroids produced in humans including children (Hartmann et al. 1998).
No hormonal products are or have been approved or used for poultry production, therefore no exogenous hormonal residues exist in eggs. Eggs contain the natural hormones E2 (less than 0.03 to 0.22 µg/kg), progesterone (12.5 to 43.6 µg/kg), and testosterone (0.04 to 0.49 µg/kg) (Doyle 2000).

For endogenous sex steroid hormones, also known as gonadal steroids, the FDA Center for Veterinary Medicine (FDA-CVM) establishes incremental increases beyond exposure because of endogenous exposures rather than an acceptable daily intake (ADI). Therefore, because of the existence of these naturally occurring hormones in humans and food-producing animals, the human consumer is exposed throughout his/her lifetime to rather large quantities of these steroid hormones through his/her own daily production and, to much lesser quantities, from untreated food-producing animals. Daily steroid production values in pre-pubertal boys and girls, the most sensitive segment of the population, are presented in Table 2.

The FDA-CVM concluded that no physiological effect could be expected in consumers eating animal products containing additional amount of the hormone that is less than or equal to 1% of the amount produced by the human daily.

<table>
<thead>
<tr>
<th>Sex status</th>
<th>Estradiol</th>
<th>Progesterone</th>
<th>Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female-menstrual cycle</td>
<td>270 to 445</td>
<td>418 to 19,580</td>
<td>240</td>
</tr>
<tr>
<td>Female-gestation</td>
<td>2,000 to 37,800</td>
<td>94,000</td>
<td>320</td>
</tr>
<tr>
<td>Female-postmenopausal</td>
<td>8</td>
<td>326</td>
<td>140</td>
</tr>
<tr>
<td>Female-ovariectomized</td>
<td>n/a</td>
<td>239</td>
<td>n/a</td>
</tr>
<tr>
<td>Female-prepubertal</td>
<td>31</td>
<td>253</td>
<td>32</td>
</tr>
<tr>
<td>Male-adult</td>
<td>48</td>
<td>416</td>
<td>6,480</td>
</tr>
<tr>
<td>Male-prepubertal</td>
<td>6</td>
<td>150</td>
<td>65</td>
</tr>
</tbody>
</table>

Table 2. Steroid production in humans during 24 hours (µg/24 hours) (Farber and Arcos 1983).

The FDA-CVM concluded that no physiological effect could be expected in consumers eating animal products containing additional amount of the hormone that is less than or equal to 1% of the amount produced by the human daily. An additional safety factor is present in that only about 10% of the ingested steroid would be absorbed. The FDA-CVM calculated a permitted increased exposure above the amount naturally present in untreated target animals (1% of the daily production values; Table 3).

<table>
<thead>
<tr>
<th>Hormone/Tissue</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>0.12 µg/kg</td>
<td>0.24 µg/kg</td>
<td>0.36 µg/kg</td>
<td>0.48 µg/kg</td>
</tr>
<tr>
<td>Progesterone</td>
<td>5 µg/kg</td>
<td>15 µg/kg</td>
<td>30 µg/kg</td>
<td>30 µg/kg</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.64 µg/kg</td>
<td>1.3 µg/kg</td>
<td>1.9 µg/kg</td>
<td>2.6 µg/kg</td>
</tr>
</tbody>
</table>

Table 3. Incremental increases allowed for the natural steroid hormones (Code of Federal Regulations 2019).

Products containing these natural steroids are approved (label) for use in beef cattle only, therefore the tissues for human consumption are muscle, liver, kidney, and fat. No residues resulting from the use of these steroids is permitted.
in excess of the increments above the concentrations of the steroid naturally present in untreated animals.

**Produced in plants or by molds**

Although not the scope of this paper, it should also be noted that animal products are not the only source of hormones in the human diet. There are plant-derived estrogens, known as phytoestrogens and some can be present at levels in feeds to cause a negative effect on reproductive performance, milk yield, or health of animals (Adams 1995). The most common phytoestrogens in the human diet are isoflavones that are found in soy products and are consumed in large amounts in some populations. Mycotoxins, such as zearalenone, can also have strong estrogenic activity and be found in foods. Because they have weak estrogenic activity, it is possible that they can block estrogen receptors and decrease estrogen-induced maladies, such as breast cancer, or they could reduce the effectiveness of hormone replacement therapy (Cornwell, Cohick, and Raskin 2004; Ehling and Reddy 2015). However, this effect might be dependent on the estrogen background, which could account for the equivocal results from health studies (Lee et al. 2009).

**Hormones used in animal production-reproductive management**

**Beef, and dairy cows and heifers**

During the 1950s frozen bovine semen was developed and artificial insemination (AI) with progeny-tested bulls became recognized as an effective way to make rapid genetic progress for milk yield and beef production. A major deterrent to AI in cattle is the requirement for daily estrus detection throughout the year for dairy cattle and daily for 60 to 90 days or more for beef cattle. Therefore, research over the past 60 plus years led to identification of effective estrus and breeding management protocols (estrus synchronization) to allow for AI during one pre-determined day or over a few day interval (Lauderdale 2009). Products were identified and granted FDA-CVM approval for use in cattle for estrus and breeding management (Code of Federal Regulations 2019) according to label directions. The protocols can use steroids, GnRH, and Prostaglandin F2-Alpha (PGF2α) to achieve single day timed breeding (use of GnRH plus PGF2α or GnRH plus PGF2α plus steroid) or several day pre-determined breeding (PGF2α or steroid plus PGF2α). Each product has regulatory authority approval for their labeled indicated use (Freedom of Information for each New Animal Drug Application [NADA] or generic [ANADA] as cited for each product below).

1. Steroids: Steroid products are a progestogen (an analog of progesterone), melengestrol acetate (NADA 034-254; 039-402), and the natural steroid, progesterone, delivered with an intravaginal device called a continuous intravaginal drug releasing device (CIDR) (NADA141-200). Progesterone and progestogens block estrus; if delivered for sufficient time, removal of the progestogen block will allow cattle to return to estrus in a pre-determined synchronized interval.
2. GnRH: GnRH products that are salts of the natural GnRH, gonadorelin, are Cystorelin® (NADA 098-379), Factrel® (NADA 139-237), Fertagyl® (ANADA of Cystorelin, 200-134, OvaCyst® (ANADA of Cystorelin 200-069), and GONABreed® (ANADA of Cystorelin, 200-069). GnRH induces the release of an ovulatory surge of luteinizing hormone, resulting in ovulation of a dominant follicle or initiation of a new follicular wave.

3. Gonadotropins, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH): are natural products but not used since their releasing hormones (GnRH, LHRH, FSH-RH) are available through regulatory authority approval and are less expensive.

4. Prostaglandins: PGF products are salts of either the natural PGF—dinoprost—and are Lutalyse®, Lutalyse®HighCon (NADA 108-901), ProstaMate® (ANADA of Lutalyse 200-253, and In-Synch® (ANADA of Lutalyse 108-901) or a PGF analog—cloprostanol—and or Estrumate® (NADA 113-645, estroPLAN® (ANADA 200-310), and SYNCHSURE™ (ANADA 200-310). PGF,α and PGF,α analogs regress the corpus luteum (CL) when administered during days 6 through 18 of the estrus cycle, resulting in cattle returning to estrus on about days 2 through 6 post-injection.

Sows

1. Steroid: The steroid product is the progestogen altrenogest (Matrix®; NADA 131-310). This progestogen is labeled and used for estrus synchronization in gilts and sows.

2. Gonadotropins, LH, and FSH, the natural gonadotropins, rather than GnRH products, have a label for use in sows and gilts. PG600 (NADA 140-856) is a combination of FSH and LH and is used to stimulate estrus in non-estrous cycling gilts and sows to facilitate breeding.


4. Prostaglandins (PGF): PGF products are Estrumate®, Lutalyse®, and ProstaMate®. These products are used to induce farrowing or terminate unwanted pregnancy at an early stage.

5. Oxytocin (NADAs 046-788; 109-305; ANADA 200-328), a peptide is used to stimulate uterine activity to assist with farrowing and to stimulate milk ejection to assist with piglet nursing.

Ewes

Ewes are seasonal breeders, therefore, some producers desire to produce lambs out-of-season to meet market demands. Progesterone delivered with a CIDR, along with ram introduction, is the program of choice. For in-season breeding, usually in small flocks, a CIDR and a PGF are used for estrus synchronization, similar to cattle.
Impact of hormones used for reproductive management on residues in meat and milk

Hormones used for reproductive management are administered during the time of breeding or at the end of gestation. Since the objective of the animal production enterprise is to produce animals safely, humanely, and profitably, there is an inherent interval of days to months between use of the hormonal product and harvest for human food. For milk, there is no inherent interval between use of the products and milk consumption. Each product identified has been granted FDA-CVM approval for its label use. In order to receive FDA-CVM approval, adequate studies were completed to satisfy FDA-CVM requirements:

(1) For products using natural hormones, humans are exposed to their own production of these hormones (Tables 2 and 3). In addition, the same hormones are present in animal products consumed by humans as a result of internal production of these hormones by the animal. These products are approved by regulatory authorities based on studies documenting that the amount of additional natural hormone following product use is below a level deemed to be consistent with human food safety, therefore, humans are not at risk from eating food from animals supplemented with these hormones.

(2) For analogs of the natural hormones, the FDA-CVM required information and laboratory animal toxicological testing to determine safe levels in the animal products that we eat. Furthermore, the FDA required that the manufacturers demonstrate that the amount of hormone left in each edible tissue after treatment is below the appropriate safe level. A safe level is a level which would be expected to have no harmful effect in humans as codified by FDA-CVM.

Hormones used in animal production-growth management

In the United States, livestock producers have used various types of GETs (Table 4) such as steroidal implants and β-AA since the early 2000s to improve carcass leanness, increase average daily gain (ADG), alter dry matter intake (DMI), and produce heavier weight and leaner animals when harvested at equal duration of days on feed. Steroidal implants can be used multiple times throughout the life of a beef animal; it is not uncommon for a beef animal to be administered a suckling-, growing-, and finishing-phase growth promoting implant.

Steroidal implants increase frame growth (i.e., long-bone growth) resulting in a heavier market weight when the beef animal reaches an industry acceptable level for fatness. Steroidal implants can be used multiple times throughout the life of a beef animal; it is not uncommon for a beef animal to be administered a suckling-, growing-, and finishing-phase growth promoting implant. The USDA-APHIS (2011) reported that greater than 90% of all feedlot cattle in the United States receive a steroidal implant during the feedlot phase of production. Steers administered a single finishing phase implant 143 days before harvest have increased carcass weight by 30 kg over non-implanted steers (Smith et al. 2018). β-AA are typically only fed during the last 21 to 42 days on feed in pigs and...
The most common and widely used type of GET are steroidal implants with anabolic activity that are used for beef cattle. More than 30 commercially available implants are marketed in the United States to beef cattle producers.

Steroidal implants with anabolic activity have been proven safe over multiple years of study and have a zero-day withdrawal prior to harvest, because research shows that by harvest time, no residue remains that would be concerning to human health.

Beta-adrenergic agonists are a GET approved by the FDA that is delivered through the animals’ feed. These compounds are approved as growth regulators in meat animals including cattle, swine, and turkey, and are fed during the last 7 to 42 days prior to harvest depending upon the species.

cattle and 7 to 14 days on feed in turkeys. Pigs and cattle fed ractopamine hydrochloride (HCl) have increased daily gains and decreased carcass fatness. Samuelson and colleagues (2016) conducted a survey of 22 consulting nutritionists that managed approximately 14 million head of cattle annually (representing more than half of all feedlot cattle in the United States) and reported that approximately 85% of their clients used ractopamine HCl for approximately 31 days prior to harvest.

**Steroidal implants with anabolic activity**

The most common and widely used type of GET are steroidal implants with anabolic activity that are used for beef cattle. More than 30 commercially available implants are marketed in the United States to beef cattle producers. These are classified from low to high potency (Johnson and Beckett 2014). Implants are classified into these groups based on the differing amounts and ratios of anabolic compounds contained in the implant pellets. The active ingredients contained in steroidal implants belong to one of three major categories of hormones: androgens (e.g., trenbolone acetate or testosterone propionate), estrogens (e.g., estradiol-17β, estradiol benzoate, or zeranol), and progestins (e.g., progesterone). Steroidal implants are administered primarily using small pellets that are placed under the skin on the back of the animal’s ear; this location ensures that no pellets will enter the food chain since ears are removed from the animal early in the harvest process and are not consumed by humans. Steroidal implants with anabolic activity have been proven safe over multiple years of study and have a zero-day withdrawal prior to harvest, because research shows that by harvest time, no residue remains that would be concerning to human health (Table 3).

Human health concerns related to residues associated with GETs were largely linked to the use of Diethelstilbesterol (DES). The use of orally administered DES for cattle was approved by the U.S. Food and Drug Administration in 1954, and the use in of implants in growing-finishing cattle rations was approved in 1956. These products were rapidly adopted by the beef cattle industry (Raun and Preston 2002). The discovery of a low incidence of DES residues in the livers of cattle associated with misuse was later discovered and reported. These residues, along with the report of cervical cancer in daughters of mothers treated with prescription DES during pregnancy, led the Food and Drug Administration to remove oral DES for cattle from the market in 1972 and implants the following year (Table 4). The removal of DES from the market led to the development of a number of other growth stimulation products for cattle (Raun and Preston 2002).

**Beta adrenergic agonist**

Beta-adrenergic agonists are GETs approved by the FDA that is delivered through the animals’ feed. These compounds are approved as growth regulators in meat animals including cattle, swine, and turkey, and are fed during the last 7 to 42 days prior to harvest depending upon the species. These growth regulators are absorbed from the digestive tract then enter the bloodstream where they bind with receptors on both fat and muscle tissue (Mersmann 1998). When bound to fat, it triggers increased fat breakdown and impedes lipogenesis (formation of new fat) (Hosford et al. 2015; Parr et al. 2014). When bound to muscle, it results in an increase in muscle accumulation and a de-
<table>
<thead>
<tr>
<th>Growth Regulator</th>
<th>Year of FDA Approval</th>
<th>Applicable species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral diethylstilbestrol (DES)</td>
<td>1954</td>
<td>Cattle</td>
</tr>
<tr>
<td>DES implant</td>
<td>1956</td>
<td>Cattle</td>
</tr>
<tr>
<td>Estradiol benzoate / progesterone implants</td>
<td>1956</td>
<td>Castrated male cattle</td>
</tr>
<tr>
<td>Estradiol benzoate / testosterone propionate implants</td>
<td>1958</td>
<td>Intact and ovariectomized female cattle</td>
</tr>
<tr>
<td>Oral melengestrol acetate</td>
<td>1968</td>
<td>Intact female cattle</td>
</tr>
<tr>
<td>Zeranol (36 mg) implants</td>
<td>1969</td>
<td>Cattle</td>
</tr>
<tr>
<td>Oral DES removed from market</td>
<td>1972</td>
<td>-</td>
</tr>
<tr>
<td>DES implants removed from market</td>
<td>1973</td>
<td>-</td>
</tr>
<tr>
<td>Silastic estradiol implant</td>
<td>1982</td>
<td>Cattle</td>
</tr>
<tr>
<td>Estradiol benzoate / progesterone</td>
<td>1984</td>
<td>Cattle</td>
</tr>
<tr>
<td>Trenbolone acetate (TBA) implants</td>
<td>1987</td>
<td>Cattle</td>
</tr>
<tr>
<td>Estradiol (17-β) / TBA implants</td>
<td>1991</td>
<td>Castrated male cattle</td>
</tr>
<tr>
<td>Bovine somatotropin</td>
<td>1993</td>
<td>Lactating dairy cows</td>
</tr>
<tr>
<td>Estradiol (17-β) / TBA implants</td>
<td>1994</td>
<td>Intact and ovariectomized female cattle</td>
</tr>
<tr>
<td>Zeranol (72 mg) implants</td>
<td>1995</td>
<td>Cattle</td>
</tr>
<tr>
<td>Estradiol (17-β) / TBA implants</td>
<td>1996</td>
<td>Grazing cattle not fed in confinement</td>
</tr>
<tr>
<td>Ractopamine hydrochloride</td>
<td>2000</td>
<td>Pigs</td>
</tr>
<tr>
<td>Ractopamine hydrochloride</td>
<td>2003</td>
<td>Cattle</td>
</tr>
<tr>
<td>Zilpaterol hydrochloride</td>
<td>2006</td>
<td>Cattle</td>
</tr>
<tr>
<td>Initial and delayed release: Estradiol (17-β) / TBA implants ²</td>
<td>2007</td>
<td>Castrated male cattle</td>
</tr>
<tr>
<td>Ractopamine hydrochloride ²</td>
<td>2008</td>
<td>Turkeys</td>
</tr>
<tr>
<td>Gonadotropin Releasing Factor – Diphtheria Toxoid conjugate ²</td>
<td>2011</td>
<td>Pigs</td>
</tr>
<tr>
<td>Extended release: Estradiol benzoate / TBA implants²</td>
<td>2014</td>
<td>Cattle</td>
</tr>
<tr>
<td>Initial and delayed release: Estradiol (17-β) / TBA implants ²</td>
<td>2017</td>
<td>Intact and ovariectomized female cattle</td>
</tr>
<tr>
<td>Delayed release: Estradiol (17-β) / TBA implants ²</td>
<td>2017</td>
<td>Cattle</td>
</tr>
</tbody>
</table>

Table 4. Chronological sequence of FDA approval of growth regulators used in the U.S. animal production industry.

<table>
<thead>
<tr>
<th>Item</th>
<th>ADI, µg/kg of BW</th>
<th>Liver, µg/kg</th>
<th>Muscle, µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clenbuterol</td>
<td>0-0.004</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Ractopamine HCl</td>
<td>0.1</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>Zilpaterol HCl</td>
<td>0-0.04</td>
<td>3.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table 5. Maximum residue levels of beta-adrenergic agonists. From Joint FAO and WHO Expert Committee on Food Additives 2014.
There are two β-AA that have been approved by the FDA for use in meat animal production in the United States. One compound is identified as β1-AA and is approved for use in cattle, swine and turkey—ractopamine HCl and the other is identified as β2-AA which is approved for use in cattle—zilpaterol HCl (Table 5). Ractopamine HCl is fed with a zero-day withdrawal prior to harvest in cattle, swine, and turkey; while cattle fed zilpaterol HCl are subjected to a 72-hour withdrawal period prior to harvest.

**Melengestrol acetate**

Melengestrol acetate (MGA) (e.g., Heifermax® or MGA) is a synthetic progestin which is orally active. It is used in finishing heifers as a means to combat estrous cyclicity and is also used in female breeding synchronization programs (Perry et al. 2005; Sides et al. 2009). Although progesterone is not generally thought of as anabolic in nature, this compound increases endogenous estradiol secretions which have been shown to increase gains by 6 to 9% in treated heifers compared to non-treated heifers (Bloss et al. 1966). When MGA is fed to breeding animals the risk for environmental exposure through meat is even less because it is likely that these females will be bred, become pregnant, deliver a calf, and raise that calf to weaning prior to the cow entering the beef supply chain.

**Gonadotropin releasing hormone antagonist**

Gonadotropin releasing hormone antagonist (e.g., Improvest®) is used in beef and pork production around the world as an alternative to castration. Animals might receive injections of the antagonist throughout the course of production and this results in reduced endogenous testosterone production which is a major causative agent of off flavors and odors associated with pork harvested from intact males (Bilskis et al. 2012).

**Produced in animals by transgenes.**

There is one FDA-approved transgenic animal for food use, AquAdvantage™ salmon. All salmon produce GH, and this Atlantic salmon has a transgene with a regulatory element derived from ocean pout and a DNA sequence for growth hormone from Coho salmon. By using this genetic modification, the salmon can grow year-round as compared to seasonal growth of conventional salmon. Regulatory studies were conducted to determine residues of GH and other hormones related to the GH axis in salmon tissues (FDA-CVM 2010). The study examined tissues from farmed salmon from Canada (n=10), farmed salmon from the study sponsor (n=33), and transgenic salmon from the sponsor (n=30). Tissue GH was below the assay’s limit of quantitation (LOQ) for all 73 samples and there were no significant differences between groups for concentrations of estradiol, testosterone, 17-ketotestosterone, T3, or T4. IGF-1 was not detectable for 100%, 67% and 73% of the farm control, sponsor control and transgenic salmon, respectively. FDA concluded that the difference in IGF-1 was insignificant compared to normal levels of exposure to salmon in the diet.
Hormones used in animals-lactation management

**Bovine Somatotropin**

Perhaps the most recognized hormone used in dairy management is bST, which is naturally produced by the pituitary gland to regulate growth and lactation. Its physiological function is exerted upon binding to receptors in responsive tissues, like any other protein hormone. It has been known since the 1930s that this hormone has the ability to increase milk production in cows. The mechanism of action of endogenous bST to promote galactopoiesis is well understood. Briefly, it involves changes in the metabolism and mobilization of nutrients from tissues (e.g., muscle, liver, and adipose) to support increased milk production. In other words, it makes nutrients available for the synthesis of milk by the mammary gland (Etherton and Bauman 1998). Advances in biotechnology and engineering allowed the production of bST by recombinant DNA technology in the 1970s. Recombinant bST (rbST) is a synthetic version of the natural hormone that is commercially produced by the same process used to make human insulin for diabetics: purified from recombinant bacteria.

Differences in the ability of somatotropin from one species to elicit biological effects in other species have been extensively reported. This term was defined as “species limited” (Bauman 1992). Clinical studies demonstrated that bST elicited no biological actions of somatotropin in humans even if injected or orally administered to humans (Collier and Bauman 2014). The sequence of bST differs by about 35% from human somatotropin, which makes bST unable to bind to the human somatotropin receptors in body tissues (Juskevich and Guyer 1990). The digestive tract secretes enzymes that break all ingested proteins down to amino acids that are absorbed. Therefore, both bST and rbST are not hormonally active in humans and, if ingested, they are rapidly digested to small peptides and amino acids because they are protein hormones.

Composition of milk (fat, protein, lactose, cholesterol, minerals, and vitamins) and manufacturing characteristics are not substantially altered with bST use (Bauman 1992; Lynch et al. 1992). For example, bST treatments do not impact milk minerals and enzymes, freezing point, pH value, milk flavor, distribution of whey casein and protein, cheese coagulation time, standard curd firmness, or cheese yields (Laurent et al. 1992; Van Den Berg 1991). A recent meta-analysis by Saint Pierre and colleagues (2014) summarizing 26 studies published in peer-reviewed journals or reviewed by a regulatory agency concluded that despite increased milk and component yields in cows treated with rbST, body condition score was not altered. The FDA reported that there is no legal basis requiring the labeling of milk from cows that were supplemented with rbST since the milk is indistinguishable from milk from cows not supplemented with bST. “Indeed, milk labeled as rbST-free and organic or unlabeled conventional milk do not differ in composition (O’Donnell et al. 2010; Vicini et al. 2008), and there is no validated test that can distinguish among these milk sources.”

Concentration of IGF-1 in milk from cows treated with rbST is within the range of IGF-1 concentrations in milk from untreated cows (Collier et al. 1991; Prosser, Fleet, and Corps 1989). It is known that some biological actions of bST are in part mediated by IGF-1. Unlike other milk proteins, IGF-1 is not denatured (inactivated) by pasteurization and therefore is present in retail cows’ milk.

**Clinical studies demonstrated that bST elicited no biological actions of somatotropin in humans even if injected or orally administered to humans.**

**The FDA reported that there is no legal basis requiring the labeling of milk from cows that were supplemented with rbST since the milk is indistinguishable from milk from cows not supplemented with bST.**

**IGF-1 is not inactivated by pasteurization and therefore is present in retail cows’ milk.**

**0.09% of the normal daily production of IGF-1 in adults.**
milk. However, IGF-1 makes up only 0.00003% of total milk proteins, and the additional amount of IGF-1 that might be absorbed by humans drinking milk from rbST cows (assuming no degradation and complete absorption) represents 0.09% of the normal daily production of IGF-1 in adults. Many body fluids including gastrointestinal secretions of humans contain IGF-1. The amount of IGF-I in 1.5 liters of milk from rbST-treated cows as compared with milk from untreated cows is only about 0.8% of gastrointestinal secretion of IGF-1 (WHO 1998). The levels of IGF-1 found in the milk of rbST-treated cows are still within the physiological range typically observed in early lactation of untreated cows and less than that found in human milk or saliva (Collier et al. 1991; Collier and Bauman 2014). Furthermore, similar to bST results, studies with laboratory animal models have demonstrated that IGF-1 has no biological activity even if administered orally at high doses (Juskevich and Guyer 1990). Given that proteins are digested if consumed orally, the possible absorption of orally consumed IGF-1 has been directly examined in humans, specifically premature neonates and young adults; results are convincing and provide no evidence that orally consumed IGF-1 is absorbed in humans (Corpeleijn et al. 2008; Mero et al. 2002).

Some studies have reported a potential relationship between blood levels of IGF-1 and the risk of prostate (Harrison et al. 2017) and breast cancer (Hankinson 1998). However, recent studies have failed to confirm that milk consumption is associated with increased cancer risk (López-Plaza et al. 2019; Preble et al. 2019).

The FDA, WHO, and National Institutes of Health have independently stated that dairy products from rbST-treated cows are safe for human consumption. Although the use of rbST is still approved in the United States, the demand for the product has decreased in recent years. Many large grocery store chains no longer carry milk from cows treated with this recombinant hormone.

A recent assessment on the use of rbST by Collier and Bauman (2014) summarized that (1) consuming milk from dairy cows treated with bST poses no risk for human health and (2) the welfare does not differ between bST treated cows and untreated high-producing dairy cows. Rigorous sequential safety assessments of rbST have made it the most highly tested and exhaustively studied recombinant product for use in ruminants. Although the use of rbST is still approved in the United States, the demand for the product has decreased in recent years. Many large grocery store chains no longer carry milk from cows treated with this recombinant hormone.

Oxytocin

Oxytocin is a small peptide hormone made up of a sequence of nine amino acids. The main physiological function of oxytocin is to cause contraction of smooth muscle cells. In females, oxytocin is best known for its ability to stimulate labor, by promoting uterine contractions, and milk ejection, by contracting the myoepithelial cells surrounding the milk alveoli in the mammary gland (Crowley and Armstrong 1992). This hormone has also been linked to emotional and behavioral processes, including maternal behavior and mother-offspring bonding (Caldwell et al. 2017; Hashimoto, Uezono, and Ueta 2012). The role of oxytocin in the milk-let down process is well understood. Basal
Oxytocin was approved by the FDA in 1972 for therapeutic use in several animal species, specifically for inducing uterine contractions and contractions of smooth muscle cells of the mammary gland for milk letdown.

Disturbed milk ejection can be caused by unfamiliar surroundings, lack of routine, and a multitude of different stressors, associated with a reduced release or complete absence of oxytocin from the pituitary (central inhibition) which impairs milk ejection.

Current FDA regulations allow the use of injected (intravenous, subcutaneous, or intramuscular) oxytocin in livestock.

Oxytocin is not currently approved for widespread use in U.S. dairies, but is permitted for cows with milk difficulty under veterinary supervision.

Circulating levels of oxytocin are low (1–5 pg/ml), but oxytocin is naturally released into blood circulation after 0.5–3 min of tactile stimulation of the teat or nipple (e.g., suckling of the offspring or pre-stimulation during milking) in quantities ranging from 10 to 100 pg/ml of plasma. A minimum of 10 pg/ml is enough to elicit a milk-ejection response (Schams and Karg 1986). Within the mammary gland, oxytocin binding to its receptor causes myoepithelial cells surrounding the alveoli to contract and consequently, the milk stored in the alveoli travels into the mammary ducts, the gland cistern and finally out of the gland (Akers and Lefcourt 1984). Oxytocin has a short half-life in the blood (0.55–3.6 min), meaning that it is rapidly broken down and cleared from circulation. Therefore, the active removal of milk must be closely timed with stimulation of the teat or nipple. More than 80% of the milk is stored in the alveolar fraction of the gland and will only be available after milk ejection is induced by the release of oxytocin (Bruckmaier and Blum 1998).

Oxytocin was approved by the FDA in 1972 for therapeutic use in several animal species, specifically for inducing uterine contractions and contractions of smooth muscle cells of the mammary gland for milk letdown. In humans, oxytocin injection (brand name Pitocin®) is used for its ability to stimulate labor and milk ejection. The initial dose of Pitocin® is set at 0.5–1 milliunits/min (mU/min). Afterwards, the dose can be gradually increased in increments of 1–2 mU/min until the desired effect is established. Exogenous administration of this hormone has been widely used in veterinary and clinical medicine, including companion animals. In dairy cows, complete removal of the alveolar milk at each milking is crucial to remove inhibitors of lactation built up in milk and maintain milk synthesis and secretion throughout a lactation. To achieve this, a calm routine is crucial for successful milking. Disturbed milk ejection can be caused by unfamiliar surroundings, lack of routine, and a multitude of different stressors, associated with a reduced release or complete absence of oxytocin from the pituitary (central inhibition) which impairs milk ejection (Bruckmaier, Schams, and Blum 1993). This not only causes milk losses, primarily in primiparous and late lactation cows, but also is associated with an increased risk of mastitis. To overcome this issue, a synthetic form of oxytocin can be exogenously administered intramuscularly to dairy cows before each milking to aid with milk ejection-reflex (Beloand Bruckmaier 2010). An alternative to oxytocin injections is vaginal or cervical stimulation to promote oxytocin release and trigger a milk ejection response, however this practice is time-consuming, laborious, and not practical in most dairy farms’ milking parlors or robotic milking settings.

Current FDA regulations allow the use of injected (intravenous, subcutaneous, or intramuscular) oxytocin in livestock. Each milliliter of injection formulation contains 20 U.S.P. units of oxytocin. The recommended dosage for obstetrical use is 5 ml in cows and horses and 1.5–2.5 ml in ewes and sows, however it is most commonly used to assist in milk letdown in cows (0.5–1.0 ml) and sows (0.25–1.0 ml). The plasma concentrations of oxytocin achieved by injecting the recommended doses are higher than plasma concentrations of un-injected controls at milking. Oxytocin is also used in dairy cows for mastitis therapy (Knight et al. 2000) or chronically to increase milk production (Nostrand et al. 1991), even though oxytocin is listed only for use in “postparturition therapeutic applications” which presumably does not include prolonged use to increase dairy milk production. In fact, oxytocin is not currently...
approved for widespread use in U.S. dairies, but is permitted for cows with milk difficulty under veterinary supervision. The chronic use of oxytocin reduces the responsivity of the mammary gland to oxytocin. Consequently, withdrawal can be detrimental for milk ejection, complete udder emptiness, intra-mammary infections, and milk synthesis (Macuhová, Tancin, and Bruckmaier 2004). Oxytocin is currently included on the National List of Allowed and Prohibited Substances as a synthetic substance allowed for use in organic livestock production.

Public concerns over the use of this hormone in dairy livestock not only to aid milk ejection but to routinely boost milk output resulted in studies to address oxytocin levels in the milk, potential milk alterations, or transfer to human consumers. After exogenous administration of high doses of oxytocin, milk and plasma oxytocin levels were comparable to those of untreated cows during milk ejection (Prakash et al. 2009). Even though these authors reported that oxytocin is stable at different pasteurization temperatures, the milk is considered safe for human consumption. Although a few short-term experiments using oxytocin report slight changes in milk fat, chronic use of oxytocin at milking does not alter milk fat content and protein percentages. Moreover, chronic use of this hormone in dairy cows at milking causes an overall greater milk yield between 3 and 10%, and consequently total quantity of milk fat and milk protein, with no apparent effect on health (Ballou et al. 1993; Nostrand et al. 1991). It is important to note that regardless of the source, whether secreted endogenously or administered exogenously, oxytocin physiological effects are observed within minutes and it is metabolized rapidly, leading to inactive products which are the same amino acids used to make all proteins. Because this drug metabolizes rapidly there is no apparent risk of residues occurring in milk.

Use of hormones to improve cow health

Granulocyte colony stimulating factor

The dynamic, metabolic, and physiologic changes occurring as a cow transitions from pregnancy into lactation predispose the cow to increased mastitis susceptibility alongside other metabolic problems. Mastitis is the most common disease in dairy cattle characterized by various degrees of severity. Although mastitis can occur from physical injuries, it is primarily caused by biological agents such as bacteria introduced either during the milking process or from environmental contact. Lactating dairy cows with mastitis produce lower milk quantity and quality relative to healthy cows (Wilson et al. 2004), leading to significant economic losses (Hogeveen, Huijps, and Lam 2011; Rollin, Dhuyvetter, and Overton 2015). Currently, antimicrobial treatment is indispensable to maintain bovine udder health, animal welfare, and dairy farm economics. Emergence and spread of antimicrobial resistance is an urgent matter of public interest, and consequently, antimicrobial usage in production livestock is a critically discussed subject (Krömker and Leimbach 2017). Maintaining good management practices as well as stimulating immunocompetence and disease resistance of cows by proper use of immunomodulators can help to reduce antibiotic use in dairy farms (Trevisi et al. 2014).
Another immune system activator in mammals is a protein (cytokine) known as granulocyte colony-stimulating factor (G-CSF) which is naturally produced by white blood cells (immune cells). This protein stimulates the precursors of immune cells such as neutrophils, macrophages, monocytes, eosinophils, and basophils in a process called myeloid cell proliferation. Humans taking G-CSF supplements have increased levels of circulating immune cells (Karawajczyk et al. 1997), and it has been used to aid in recovering depleted immune cells following chemo/radiation therapy and hematopoietic stem cell transplantation (reviewed by Elfenbein and Sackstein 2004). Human granulocyte colony stimulating factor (rhG-CSF) was administered to dairy cows with the goal of activating the function of peripheral blood granulocytes and protecting the mammary gland from a Staphylococcus aureus challenge (Nickerson et al. 1989). These authors observed a 46.7% reduction in new infections in quarters of treated cows compared with controls. More recently, a polyethylene glycol-conjugated bovine granulocyte colony-stimulating factor (PEG-bG-CSF) treatment, was the first product of its kind to be approved by the FDA for the reduction in the incidence of clinical mastitis in the first 30 days of lactation in periparturient dairy cows.

Since the bioavailability of PEG-bG-CSF after oral exposure is negligible, the FDA concluded that because of the negligible oral bioavailability of PEG-bG-CSF there is no need to establish an ADI. Furthermore, since the oral bioavailability of PEG-bG-CSF is negligible the ingestion of potential residues of PEG-bG-CSF in animal tissues or products does not pose a risk to consumers (Rhodes 2018).

### ADI, MRL and safe levels for hormones in foods

#### Risk Estimates of Residues in Food

Risk assessment is an integrative strategy to assume the probability of human illness caused by the ingestion of livestock products containing residual veterinary drugs. Risk assessments for use of antimicrobials in livestock production have largely involved the risk for development of antibiotic resistance of microbes while risk assessment for hormones have largely assessed potential risks for impact on human growth, development, and disease. Hormone and hormone-like products used for livestock production are regulated in the United States by the FDA. Internationally, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is a committee of the Food and Agriculture Organization (FAO) of the United Nations and the WHO. One of JECFA’s roles is to perform risk assessments on residues of veterinary drugs in food. Both the FDA and the JECFA establish residue levels tolerances or Maximum Residue Limits (MRL). From the viewpoint of risk management, MRL are regarded as a monitoring tool for compliance to the approved conditions of use, and the ADI level is a decision point for human health impacts (Jeong et al. 2010).

#### Human exposure to hormone residues

Endogenous steroid hormones are produced mainly by the ovaries (estrogen, progesterone) and testes (testosterone), throughout the lifetime of humans and mammals. These hormones are required for proper physiological functions,
such as growth, function of the reproductive organs, and development of secondary sex characteristics. The existence of these naturally occurring hormones in humans and food-producing animals documents the human consumer is exposed throughout his/her lifetime to rather large quantities of these steroid hormones through his/her own daily production and, too much lesser quantities, from untreated food-producing animals.

**Analytical methods for residues**

The objective of the total residue and metabolism study is to develop information on the amount, persistence, and chemical nature of the total residues, and the metabolic fate of the new animal drug in the treated target animals. Products requiring an interval between use and reduction of residues to a safe concentration must have a practical method for determining the quantity of the product in edible animal tissue ingested. This practical method is employed to continuously monitor samples by the USDA-FSIS for muscle, kidney, liver, and fat, and by the FDA for milk to ensure that the proposed use of the product does not pose any human health harm.

For analogs (xenobiotics) of the natural hormones, studies are required by the FDA-CVM to adequately address: (1) toxicology including mutagenicity, 90-day feeding, reproductive, teratology, and special as needed per drug studies, (2) metabolism in the target animal and comparative metabolism in rodents, (3) identification of the tissue(s) containing the greatest concentration of residues (target tissue), (4) identification of the residue that would be measured to determine when the residue(s) were below a safe concentration (marker residue), and (5) time post-treatment when the residue(s) are depleted in the tissues.

Data on residues of xenobiotic hormones that occur are used to establish a no observed effect level (NOEL) which is accepted as the no observed adverse effect level (NOAEL).

A safety factor is applied to the NOEL to obtain an allowable daily intake (ADI). A safety factor (SF) of 100 fold (10-fold for extrapolating animal data to humans and 10-fold for variability in sensitivity to the toxicity of the product among humans as well as humans of lower body weights) is used unless a product is believed to have potential to be a carcinogen, which uses a SF of 1000 or greater.

Once a final ADI, safe concentration, target tissue, and marker residue are selected, a tolerance for the new animal drug can be determined.

\[
\text{ADI} = \frac{\text{NOAEL}}{\text{SF}}. \\
\text{Safe Concentration (SC)} = \frac{\text{ADI} \times 60 \text{ kg}}{\text{CF}}. \\
\]

Once a final ADI, safe concentration, target tissue, and marker residue are selected, a tolerance for the new animal drug can be determined. The tolerance is the maximum concentration of a marker residue, or other residue indicated for monitoring, that can legally remain in a specific edible tissue of a treated animal. The FDA-CVM may assign the tolerance by harmonizing with previously

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**Data on residues of xenobiotic hormones that occur are used to establish a no observed effect level (NOEL) which is accepted as the no observed adverse effect level (NOAEL).**

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**Once a final ADI, safe concentration, target tissue, and marker residue are selected, a tolerance for the new animal drug can be determined.**
established international MRL. Tolerances and MRL are both numbers that describe the limits of residues. However, tolerances and MRL are not derived in the same manner and are used for different purposes. At times, it may be appropriate to harmonize the tolerance with an already established MRL.

The FDA cannot approve a compound for use as a new animal drug in food-producing animals when the compound or any of its metabolites has been found to cause cancer in animals or humans, unless: (1) the compound will not adversely affect the animals for which it is intended, and (2) no residue of the compound will be found by approved regulatory methods in any edible tissues. For products that have been identified by regulatory agency personnel to not become a component of food at concentrations considered unsafe, a regulatory method is not required.

The JECFA has established MRL for xenobiotic compounds (i.e., trenbolone acetate and zeranol), and has set an average ADI for natural compounds, such as estradiol-17β and testosterone propionate. Ensuring food safety to the public is based upon calculations from the no-hormonal effect level (NHEL). Once the NHEL is established, a safety factor of 100-fold is assigned and the resulting value is the ADI in µg/kg of body weight. The determination of the NHEL, ADI, MRL all occur during the new animal drug application process and prior to use in commercial practice in order to ensure minimal risk for environmental exposure to humans. The reason there is no MRL for natural compounds is because implanted animals rarely have differing residue levels from non-implanted intact animals following harvest.

The non-synthetic beef implants, bST, and GH in transgenic salmon do not have tolerances since these hormones are in milk, fish flesh, and beef naturally and exogenous administration does not result in residues that are outside of the normal range. Moreover, GH from salmon and cattle are species limited and when ingested it is digested. The tolerances for synthetic implants are in Table 6.

<table>
<thead>
<tr>
<th>Name</th>
<th>FDA (US 21 CFR 556)</th>
<th>JECFA (JECFA 2014) (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melengestrol acetate</td>
<td>Fat: 25 µg/kg</td>
<td>Muscle: 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver: 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kidney: 20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fat: 18</td>
</tr>
<tr>
<td>Trenbolone acetate</td>
<td>Cattle edible tissue (excluding milk): NR¹</td>
<td>Muscle: 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver: 10</td>
</tr>
<tr>
<td>Zeranol</td>
<td>Cattle edible tissue: NR¹</td>
<td>Muscle: 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver: 10</td>
</tr>
</tbody>
</table>

Table 6. Tolerances for residues of synthetic growth enhancing technologies in animal tissues. ‘MRL is not required.'
Some products such as pharmaceuticals, marketed for use in animals have withdrawal periods established for use of the product. The withdrawal period or the milk discard time is the interval between the time of the last administration of a new animal drug and the time when the animal can be safely harvested for food or the milk can be safely consumed. Withdrawal times are the intervals between the last product administration and when residues are depleted below the concentration deemed to be safe for human consumption. Withdrawal times are conservative statistical estimates to assure the animal products are safe for human consumption.

The ADI may need to be partitioned across edible tissues based on the expected use of the new animal drug and the food consumption values for each tissue. For products not used in lactating dairy cows, the ADI can be allocated to edible muscle, liver, kidney, or fat. For products approved in lactating dairy cows or for products approved in lactating dairy cows and other meat-producing species, a part of the ADI would be reserved for milk. The remaining part of the ADI would be partitioned among edible tissues.

Summary

Use of endocrine regulators to improve efficiency of growth, lactation and reproduction has contributed significantly to providing a safe and low-cost food supply to American consumers.

Adverse health events in humans associated with correct label use of approved products is virtually unknown and when these events are reported in other countries they are always associated with illegal or off-label use of products that are contaminated with other compounds or were given at dosages not allowed in the United States.

Hormones are naturally present in milk, meat and eggs, and the FDA has concluded that no physiological effect could be expected in consumers eating animal products containing additional amounts of the hormone that is less than or equal to 1% of the amount produced by the human daily and would be digested like other proteins and steroids found in milk and meat. None of the approved naturally occurring hormones in use produce residues in animal products that surpass this target level. For synthetic compounds, the use of production enhancing technologies is heavily regulated in the United States and across the world by multiple agencies resulting in an extremely safe food supply. Correct use of supplemental hormone products in food animals according to their labels is safe, having no adverse effects on human health.

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