Risk Assessment of Growth Hormones and Antimicrobial Residues in Meat

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Growth promoters including hormonal substances and antibiotics are used legally and illegally in food producing animals for the growth promotion of livestock animals. Hormonal substances still under debate in terms of their human health impacts are estradiol-17β, progesterone, testosterone, zeranol, trenbolone, and melengestrol acetate (MGA). Many of the risk assessment results of natural steroid hormones have presented negligible impacts when they are used under good veterinary practices. For synthetic hormone-like substances, ADIs and MRLs have been established for food safety along with the approval of animal treatment. Small amounts of antibiotics added to feedstuff present growth promotion effects via the prevention of infectious diseases at doses lower than therapeutic dose. The induction of antimicrobial resistant bacteria and the disruption of normal human intestinal flora are major concerns in terms of human health impact. Regulatory guidance such as ADIs and MRLs fully reflect the impact on human gastrointestinal microflora. However, before deciding on any risk management options, risk assessments of antimicrobial resistance require large-scale evidence regarding the relationship between antimicrobial use in food-producing animals and the occurrence of antimicrobial resistance in human pathogens. In this article, the risk profiles of hormonal and antibacterial growth promoters are provided based on recent toxicity and human exposure information, and recommendations for risk management to prevent human health impacts by the use of growth promoters are also presented.

Key words: Risk assessment, Growth promoters, Growth hormones, Antibiotic feed additives, Human health impact

INTRODUCTION

Veterinary drugs including antibiotics, growth hormones, and antihelminths are used for disease control and growth promotion of livestock animals. However, concerns regarding the safety of livestock products and the prevalence of antimicrobial resistance have grown according to the increased use of veterinary drugs.

Risk assessment is an integrative strategy to assume the probability of human illness caused by the ingestion of livestock products containing residual veterinary drugs. Both antimicrobials and growth hormones used for growth promotion in food-producing animals have provoked much debate on the safety of livestock products for human consumption. Many studies have been performed to estimate the real probability of human health impact. We need to understand the human health risks of antimicrobials and growth hormones under a framework basis composed of assessments of exposure amounts, dose-response relationships, and human illness consequences.

The major goal of this article is to illustrate methods for the risk assessment of growth hormones and antimicrobials and to provide the results of quantitative risk assessments of them based on current scientific knowledge and available data.

PRINCIPLES AND METHODS OF RISK ASSESSMENT FOR VETERINARY DRUGS USED IN FOOD ANIMALS

Veterinary drugs are a type of chemical hazard in foods of animal origin. Many of these substances are evaluated for their potency of human health impacts in case they remain in foods. The approval of veterinary drugs used in food-producing animals can be made after systemic evaluation of efficacy, target animal safety, human health risk, and envi-

ronmental impacts. From the viewpoint of risk management, maximum residue limits (MRLs) 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.

Risk assessments of veterinary drugs consist of assessing their toxicological and microbiological impacts and identifying acceptable consumption levels that the compounds should not exceed. Toxicological impact implies any biological adverse effects caused by direct intake of veterinary drugs, such as body weight change, immune suppression, and various disorders of normal body function. For microbiological impact, the targets of the ingested veterinary drugs are human intestinal microflora rather than the human body. Human intestinal microflora play important roles in the maintenance of human health. Major functions of gut microbiota for human health are: the metabolic fermentation of non-digestible dietary components and endogenous mucus, control of proliferation and differentiation of intestinal epithelial cells, development and homoeostasis of the immune system, and protection against exogenous pathogens (Shenderov, 1998). Microbiological impact needs to be evaluated when the ingested residue compound is antimicrobiologically active, not transformed irreversibly to inactive metabolites, and enters the lower intestine by any administration route (JECFA, 2000a; Jeong *et al*., 2009). Many veterinary antimicrobials are allotted into a class requiring microbiological risk assessment.

A wide range of scientific data is required to ensure certain veterinary drug can be used in a manner that does not have adverse effects on public health. Where appropriate, data should be generated in accordance with national or international Good Laboratory Practice (GLP) guidelines.

The data should cover acute toxicity; short-term toxicity; long-term toxicity; reproductive toxicity; carcinogenicity; genotoxicity; specific organ toxicity including immunotoxicity and neurotoxicity; endocrine disruption effects and impacts on human intestinal microflora; metabolism and depletion in target animals; and environmental fate.

Risk assessments of veterinary drugs residing in foods are performed by following the integrative steps of hazard identification, hazard characterization, exposure assessment, and risk characterization (WHO, 2006). At the step of hazard identification, known or potential adverse health effects in humans are identified, which are induced by a veterinary drug or its metabolites that may be present in a particular food.

Toxicological evaluations, toxicokinetic assessments, and cancer/non-cancer evaluations are mainly performed for hazard identification. At the hazard characterization step, the characteristics of the adverse effects associated with a veterinary drug or its metabolites present in food are demonstrated. In addition, the levels that clearly do not cause any adverse effects on human health are evaluated according to dose-response relationships. NOAELs (no-observed-adverse-effect-levels), ADIs, benchmark doses at lower confidence limits (BMDLs), uncertainty factors, and threshold levels for toxicological concern are drawn from the hazard characterization step. ADI is calculated by dividing the NOAEL with an uncertainty factor. When a veterinary drug or its metabolites are microbiologically active and can enter the lower intestine without inactivation, a microbiological ADI is assessed as well as a toxicological ADI. The lower ADI value is finally selected as the drug's ADI (Table 1). In case the chemical is evaluated as a complete carcinogen, which means a genotoxic carcinogen, it is recommended to

Table 1. Comparison of toxicological and microbiological risk assessments

Item and procedure	Toxicological risk assessment	Microbiological risk assessment
Target	• Human (usually extrapolated from animals)	• Human intestinal flora
Evaluation	• General oral toxicity (acute, short-term, and long-term toxicity) • Genotoxicity • Carcinogenicity • Reproductive toxicity • Developmental toxicity • Immunotoxicity • Other specific toxicities • Observations in humans	• Emergence of antimicrobial resistance in human intestinal normal microflora • Disruption of barrier composed of human intestinal normal microflora • Change of metabolic microbiological activity
Endpoint	No-observed-adverse-effect-level (NOAEL) of the most sensitive toxic effect	No-observed-adverse-effect-concentration (NOAEC) of the most sensitive human gut flora
Uncertainty factor	• Inter-species differences: 10 • Intra-species differences: 10 • Nature of toxic effects, data quality: 2~10	• Data quality: 2~10 • Type of most sensitive human flora
Calculation of ADI	NOAEL (µg/kg bw/day)/uncertainty factor	{MIC50 (µg/g) × Mass of colonic content (g)}/{Fraction of bioavailable dose × Uncertainty factor × Body weight (kg)}

operate a policy of prohibition and control levels "as low as reasonably practicable" (FAO/WHO, 2004).

During exposure assessment, the likely intake amount of a veterinary drug or its metabolites pertaining to toxicological concerns along with exposures from other sources where relevant is estimated. The Estimated Daily Intake (EDI) amount is calculated using a nationally or internationally accepted approach. The EDI value of a veterinary drug is drawn by the sum of all values that are calculated by multiplying the median residue levels in food components with estimates of dietary intake. When groups of high risk consumers or sensitive populations responding to a specific veterinary drug are identified, the exposure amount is assessed more carefully. The approval of the usage of veterinary drugs is determined by comparing the EDI with the ADI. If the EDI is higher than the ADI, approval may not be possible or withdrawn.

At the step of risk characterization, estimations of the severity and occurrence of known potential adverse health effects in a given population are estimated based on hazard identification, hazard characterization, and exposure assessment. Evaluation of the risk caused by consumption in highly sensitive population groups can be performed when required in the viewpoint of public health. The margin of exposure (MOE), margin of safety (MOS), level of protection (LOP), and MRLs are determined at this step. A large margin of safety is generally posed, which leads to a level well below that which causes any toxic effects in animal studies. The actual intake of an approved substance is entirely lower than the estimated level for human health concerns because it is extremely unlikely that each and every item of consumed food over an individual's lifetime will have been treated with a particular compound.

For risk management, it is the ADI not MRL that refers to human health-related risks by consuming a certain amount of veterinary drug via food intake. Data on toxicity and residue have been assessed and conditions are placed on their registration to ensure MRLs are not exceeded before the approval of veterinary drugs.

Inspection and surveillance of residues of veterinary drugs are very important regulation tools to secure food safety. However, it is not practical to monitor all items of suspected compounds in foods. Risk scoring for setting priorities in veterinary drug residue monitoring is recommended as an efficient strategy for risk management such that more intensified monitoring is applied for components having higher scores of potential risk. Risk is scored according to toxicological thresholds, estimated exposure amounts, the occurrence of antimicrobial resistance, violation frequency, and so forth.

Newly developed approaches including BMDL and MOE evaluations, EDI calculations, and mode of action (MOA) assessments provide more scientific and practical ways for risk assessment. The endeavor to make more reasonable

and sound directions in the authorization of veterinary drugs, establishment of ADIs and/or MRLs, and scoring of risk priority may guarantee food safety via state-of-the-art health risk assessments and risk management advice, optimally attuned to consumer needs.

POTENTIAL HUMAN HEALTH IMPACTS OF GROWTH HORMONES USED IN FOOD ANIMALS

The hormonal substances used for growth promotion in cattle are the naturally occurring steroids: estradiol-17β, progesterone, and testosterone, as well as synthetic compounds such as zeranol, which has high affinity for estrogen receptors, trenbolone acetate, which has affinity to androgen receptors, and melengestrol acetate, which has similar activity to progestins.

Estradiol. Estradiol-17β, alone or in combination with other hormonally active substances, is administered to cattle by a subcutaneous implant, usually in the ear, to improve rates of weight gain and feed efficiency (Wagner, 1983). The amount of estradiol benzoate treated per animal is 10~28 mg, which is equivalent to 8~24 mg of 17β-estradiol. The release rate from one type of commercial implant is approximately 60 μg per animal daily (JECFA, 2000b).

Estradiol exerts its biological effects largely by binding with intracellular receptors, and in females it induces growth and development of the reproductive tract and breasts and the appearance of secondary sex characteristics after binding with the receptors.

In general, orally administered estradiol is inactive because it is metabolized and conjugated in the gastrointestinal tract and liver (Moore *et al.*, 1982). Fine-particle formulations of estradiol given orally for contraception or hormone replacement therapy in menopausal women show bioavailability of 5% of that of a dose administered intravenously (Kuhnz *et al.*, 1993). Estrogen did not exert teratogenic effects in a human study of approximately 7,700 infants whose mothers took oral contraceptives while pregnant (Rothman and Louik, 1978). Estradiol has genotoxic potential by inducing micronuclei, aneuploidy, and cell transformation *in vitro*, and oxidative damage to DNA and DNA single-strand breakage *in vivo* (IARC, 1979, 1999). In long-term studies of carcinogenicity in mice and in rats, increased incidences of tumors were found in mammary and pituitary glands; the uterus, cervix, vagina, and testicles; and lymphoid organs and bone (IARC, 1979). Malignant kidney tumors occurred in intact and castrated male hamsters and in ovariectomized female hamsters. IARC (1987) also concluded that estradiol-17β is a Group I human carcinogen that has sufficient evidences for carcinogenicity to humans. The carcinogenicity of estradiol is found to be a result of its interaction with hormonal receptors because tumors largely occur in tissues possessing high levels of hormone receptors. Overall, estra-

diol is evaluated as a genotoxic carcinogen, however, it is necessary to consider that estradiol is a natural hormone synthesized in the human body and used as a human medicine.

JECFA (2000b) determined the NOAEL (No-observed-adverse-effect level) of estradiol-17β as 5 μg/kg bw/day based on human epidemiological data rather than animal toxicity data. The value was calculated from 0.3 mg/day of estradiol administered orally to women (60 kg mean body weight), which did not relieve any symptoms of menopause and there were no changes in serum concentrations of corticosteroid-binding globulin (CBG). The ADI of 0~50 ng/kg bw/day was determined by dividing the NOAEL of 5 μg/kg bw/day with an uncertainty factor 100.

Estradiol-17β occurs naturally in all mammals. Background levels vary with the age and sex of each animal species. The highest natural levels are found in pregnant animals. The normal daily production of estradiol-17β is 6.5 μg in prepubertal boys, 48 μg in men, and 37.8 mg in pregnant women (Angsusingha *et al.*, 1974). Estradiol is used as a growth promoter in cattle and may produce two-fold to several ten-fold increases in levels, reaching peaks in the liver and fat of steers and calves (Paris, *et al.*, 2006). The amounts of estradiol in the muscle tissue of treated veal calves, heifers, and steers were 11~280 ng/kg, whereas 3~35 ng/kg were detected in non-treatment groups. The intake amount of estradiol via the meat of treated animals (0.0045~0.180 μg per 500 g portion of meat) is approximately forty times to thousands of times lower than the amount of human daily production of the hormone (Table 2). In addition, estradiol becomes inactivated when administered orally due to gastrointestinal and/or hepatic metabolic functions. JECFA (2000b) concluded that the amount of exogenous 17β-estradiol ingested via meat from treated cattle would be incapable of exerting any hormonal effects in human beings

Table 2. Comparison of the amounts of steroid hormones produced daily in the human body and ingested via the diet from hormone-treated animals

Hormones	Total daily production (μg/day) (JECFA, 2000b; EFSA, 2007)	Residue in muscle from non-treated animals (μg/kg) (Paris et al., 2006)	Residue in muscle from treated animals (μg/kg) (Paris et al., 2006)	Ingested amount via intaking muscle from treated animals* (μg/day)
Estradiol	< 14 (prepubertal boys) 10~24 (prepubertal girls) 27~68 (adult men) 30~470 (adult women)	0.003~0.035	0.011~0.28	0.0033~0.084
Progesterone	150~250 (prepubertal children) 416~750 (adult men, premenopausal women)	0.0~0.9	0.23~0.77	0.069~0.231
Testosterone	30~100 (prepubertal children) 210~480 (adult female) 2100~6900 (adult male)	0.006~0.029	0.031~0.360	0.0093~0.108

*: calculated considering a person intakes 300 g of muscle per day

Table 3. Toxicological endpoints and regulatory limits of hormonal growth promoters

Compound	Toxicological endpoint	NOAEL (μg/kg bw/day)	ADI (μg/kg bw/day)	MRLs (μg/kg) for cattle tissues	Ref.
17β-estradiol	No relief of the symptoms of menopause and changes in the serum concentrations of corticosteroid-binding globulin	5	0~0.05	unnecessary	JECFA, 2000b
Testosterone	Androgenic effects	1,700	0~2	unnecessary	JECFA, 2000b
Progesterone	Changes in the human uterus	3,300 (LOAEL)	0~30	unnecessary	JECFA, 2000b
Zeranol	Estrogenic effects	50	0~0.5	2 (muscle), 10 (liver)	JECFA, 1988
Melengestrol acetate	Changed menstrual cycle	5	0~0.03	1 (muscle), 10 (liver) 2 (kidney), 18 (fat)	JECFA 2006c
Trenbolone acetate	Androgenic effects	2	0~0.02	2 (muscle, β-trenbolone) 10 (liver, α-trenbolone)	JECFA, 1988

since bioavailability is very low in the case of orally administered estradiol, and even when absorbed into the circulatory system, circulating estradiol is in the inactive form mainly bound to sex hormone-binding globulin (Fotherby, 1996). JECFA recommended that establishing MRLs is unnecessary because exogenous estradiol is structurally identical to that produced endogenously in human beings, showing great variation in levels according to age and sex (Table 3).

Progesterone. Progesterone is administered to cattle in combination with estradiol benzoate at a ten to one ratio (progesterone 100~200 mg with estradiol 10~20 mg) as an ear implant, to increase rates of weight gain and feed efficiency. Progesterone is also used to synchronize estrus in lactating and non-lactating dairy cows and goats via an intravaginal sponge. Exogenously administered progesterone is structurally identical to the progesterone produced in animals and humans. Progesterone is poorly absorbed by oral ingestion and inactivated in the gastrointestinal tract and/or liver, which makes its bioavailability less than 10% after oral administration (Simon *et al.*, 1993). Orally administered micronized progesterone for hormone replacement therapy in women reaches a peak plasma concentration within 4 h and returns to baseline by 6 h (Nahoul *et al.*, 1993; Sitruk-Ware *et al.*, 1987).

The main function of progesterone is to regulate the female reproductive cycle for the preparation and maintenance of pregnancy in association with estrogens (JECFA, 2000b). Progesterone also induces increases of plasma cholesterol and low-density lipoprotein, decreases of high-density lipoprotein, and sodium excretion. The major metabolites found in plasma are pregnanediol 3a-glucuronide, 17-hydroprogesterone, and 20a-dihydroprogesterone. Most of the progesterone in blood is bound to CBG or albumin, where approximately 17% of serum progesterone is bound to CBG and 80% to albumin, and 2.5% is in the free form (Ribinson *et al.*, 1985).

The amount of progesterone produced in the human body varies according to physiological status, such as 418 µg/day in premenopausal women in the follicular phase of the reproductive cycle, 94,000 µg/day during late pregnancy, and in post-pubertal men production is 416 µg/day (Table 2) (Galbraith, 2002).

In humans, progestogens are mainly used for contraception and for hormone replacement therapy. The therapeutic dose of fine-particle progesterone is 400 mg/day for 10 days in women, and a dose of 300 mg/day for 10 days per month is well tolerable (JECFA, 2000b). In a human study to explore the effects of oral micronized progesterone on endometrial maturation, healthy menopausal women orally given 300 mg/day micronized progesterone for 14 days after estrogen priming for 30 days showed incomplete conversion of the uterus to full secretory activity of uterus with

significant increase of glandular glycogen by 124%. However, the group receiving 600 mg/day showed full secretory conversion of uterus with 291% increase of glandular glycogen. Nuclear estrogen receptor content in the stroma of the endometrium was decreased by both doses of progesterone, but the group given 300 mg/day did not reach significance (Kim *et al.*, 1996).

In other human studies, post menopausal women were given 200 or 300 mg/day of progesterone orally for the last 14 days of percutaneous estradiol treatment of 1.5 or 3 mg/day for 21 of 28 days for one or five years. There was no evidence of endometrial hyperplasia or carcinoma after five years of estradiol and progesterone treatment (Moyer *et al.*, 1993). Oral fine-particle progesterone treatments of 200 or 300 mg/day in sixty women with oligomenorrhea or amenorrhoea showed effects of withdrawal bleeding with unchanged lipid concentrations (Shangold *et al.*, 1991). Furthermore, there were no adverse effects in women receiving 200 mg/day of fine-particle progesterone orally for hormone replacement therapy, which induced minor changes in plasma lipoprotein profiles in some subjects but not all, and no changes in haemostatic parameters (Sitruk-Ware *et al.*, 1987).

In a study using female BALB/cfC3H/Crgl mice, 100 µg of progesterone was administered subcutaneously alone for five days beginning 36 h after birth causing ovary-dependent, persistent vaginal cornification and hyperplasia in the vaginal and cervical epithelia, and significantly higher incidence of mammary tumours in mammary tumour-virus bearing mice (Jones and Bern, 1977). Progesterone increased the incidences of ovarian, uterine, and mammary tumours in mice as well as mammary gland tumours in dogs (IARC, 1979), and these effects were regarded as hormone activity related. The IARC concluded that there is limited evidence for the carcinogenicity of progesterone in experimental animals and no evaluations of its carcinogenicity in humans can be made in the absence of epidemiological data (IARC, 1979, 1987). Progesterone has shown no evidence of genotoxicity (IARC, 1987; Seraj *et al.*, 1996). In addition, progesterone did not induce any adverse effects on fertility and development in rats and rhesus monkeys (Wharton and Scott, 1964).

In comparison studies for concentrations of progesterone in edible tissues from non-treated and treated veal calves, heifers, and steers, ranges of progesterone were not different between the groups; however, in the treated animals, amounts of progesterone in adipose tissue (3.20~8.66 µg/kg) were several times higher than amounts found in the control animals (0.87~1.60 µg/kg) (Table 2) (Paris *et al.*, 2006). This increased amount is about a thousand times lower than daily production amount in adult men and women of normal status.

For changes in the human uterus, JECFA established the ADI of progesterone as 0~30 µg/kg bw based on a LOAEL of 200 mg/day (equivalent to 3.3 mg/kg bw/day). One-hun-

dred as an uncertainty factor was allotted as 10 for extrapolation from the LOAEL to the NOAEL and 10 for individual variations. MRL was recommended to be unnecessary because it is identical to endogenous progesterone and the amount of estimated daily intake via food consumption is negligible comparing the level of daily production in human beings (JECFA, 2000b) (Table 3).

Testosterone. Testosterone propionate (200 mg) in combination with estradiol benzoate (20 mg) is administered to cattle as an ear implant for growth promotion. Orally administered testosterone is mainly inactivated during digestion and hepatic metabolism. The bioavailability of orally treated testosterone is approximately 3.6% of the administered dose. In an earlier study, the plasma half-life was 10 min after intravenous administration and about 90% of the administered dose was excreted into the urine (Tauber *et al.*, 1986).

Testosterone is synthesized in testicular Leydig cells, ovarian thecal cells, and the adrenal cortex, and it exerts activity via binding with androgen receptors. Testosterone is a precursor of other steroid hormones. The active metabolite of testosterone is dihydrotestosterone (DHT), which is metabolized to androsterone, androstanedione, and 3α- and 3β-androstanediol. The physiological concentration of circulating testosterone is 3~10 ng/ml in men (Miyamoto *et al.*, 1998). The major functions of testosterone are pubertal development for spermatogenesis, regulation of the differentiation of the prostate, stimulation of erythropoietin production in the kidney and stem cells of the haematopoietic system, and the acceleration of growth during puberty in conjunction with growth hormone.

In a human study, 400 mg of fine-particle testosterone administered orally for 21 days was well tolerated without any significant side effects in healthy male volunteers (Johnsen *et al.*, 1974, 1976). Increases of prostate gland weight and volume as well as amounts of serum testosterone, DHT, androstenedione, and estradiol were found by intramuscular injections of 200 mg of testosterone enanthate (equivalent to 8 mg/kg bw) for 28 weeks in adult male baboons (Karr *et al.*, 1984). Many studies on genotoxicity have shown that testosterone alone has no genotoxic potential (Han *et al.*, 1995; Ho and Roy, 1994; Lasne *et al.*, 1990; Seraj *et al.*, 1996; Tsutsui *et al.*, 1995). Testosterone (10 mg) induced resorption of embryos in female SD rats when it was treated subcutaneously on day 10 of gestation (Sarkar *et al.*, 1986). For the carcinogenic potential of testosterone, the IARC (1979) determined that it is reasonable, for practical purposes, to regard testosterone as if it presented a carcinogenic risk to humans due to an absence of adequate data in humans, but there is sufficient evidence for its carcinogenicity in experimental animals.

In human medicine, testosterone is used to treat deficient testicular function in men, and to replace hormones in postmenopausal women in combination with estrogen (Sands and Studd, 1995). Orally administered testosterone undecanotate induced the progression of virility and testicular growth, and the acceleration of growth associated with puberty in delayed boys at 40 mg per day for 15-21 months without any side-effects (Butler *et al.*, 1992). In a human study with eunuchs, 25 and 100 mg of testosterone administered orally did not exert any effects; however, 400 mg exerted effects such as sexual desire, erection, ejaculation, and general well-being (Johnsen *et al.*, 1974). In another study, oral administration of testosterone at 100 mg/day restored sexual function slightly (Foss and Camb, 1939).

JECFA (2000b) established the ADI of testosterone as 0~2 µg/kg bw based on a NOAEL of 100 mg/day (equivalent to 1.7 mg/kg bw/day) and an uncertainty factor of 1000 based on the study of eunuchs. Paris *et al* (2006) reported that the residue level of testosterone in muscle of implanted veal calves or heifers is 0.031~0.360 µg/kg, while that of non-treated animals is 0.006~0.029 µg/kg. When comparing the ADI value, the amount of testosterone via beef intake from hormone-treated animals is thousands of times lower than the ADI. The MRL of testosterone in beef is not necessary because of the same reasons for the cases of estradiol and progesterone (Table 3).

Zeranol, melengestrol, and trenbolone. Zeranol, melengestrol, and trenbolone are all synthetic xenobiotic growth promoters. Zeranol is a non-steroidal anabolic agent administered subcutaneously as an ear implant in cattle and shows estrogenic activity (Katzenellenbogen *et al.*, 1979). Zeranol is metabolized to zearalenone and taleranol and tissue residue levels of zeranol are in the range of 0.01~1.21 µg/kg with peak levels in liver tissue (Paris *et al.*, 2006). Orally administered zeranol showed weak estrogenic effects in long-term toxicity studies using rats, dogs, and monkeys through changes in mammary glands and reproductive organs (Davis *et al.*, 1977; Everett *et al.*, 1987; Revuelta *et al.*, 1997; WHO, 2000b). Zeranol and its metabolites, zearalenone and taleranol, were negative in several *in vitro* and *in vivo* genotoxicity assays (Bartholomew and Ryan, 1980; Ingerowski, *et al.*, 1981; Scheutwinkel *et al.*, 1986; Williams, 1984). In carcinogenicity studies of rats and mice, only the mice showed a higher incidence of tumors in the anterior lobe of the pituitary gland compared to a control group, but this effect was regarded as being due to the estrogenic properties of zeranol (Everett, *et al.*, 1987; Gardner, 1941; JECFA, 1988). In an uterotropic assay using sexually immature rats, orally administered zeranol, zearalanone, and taleranol presented estrogenic potencies 1/150, 1/400, and 1/350 that of estradiol-17ß, respectively (Everett *et al.*, 1987). In ovariectomized female cynomolgus monkeys, zeranol given orally for 13 weeks induced the maturation of vaginal epithelial cells at 0.5 and 5 mg/kg bw/day, and the NOAEL was evaluated as 0.05 mg/kg bw/day based on the estrogenic effects of zeranol (JECFA, 1988). JECFA recom-

mended its ADI to be 0~0.5 μg/kg bw/day by applying an uncertainty factor of 100 for interspecies and individual differences (JECFA, 1988). The MRLs are settled as 2 μ/kg for muscle and 10 μ/kg for liver in beef (Table 3).

Melengestrol is a synthetic progestogen administered orally as a feed additive to improve feed efficiency. The approved feeding doses are in a range of 0.25~0.50 mg/heifer per day during the fattening and finishing periods (Neidert *et al.*, 1990). Its activity is revealed via a high affinity for progesterone receptors as well as increases in prolactin secretion and the activation of estrogen receptors (Perry *et al.*, 2005). Melengestrol acetate (MGA) was metabolized to 2β,15β-dihydroxy methyl MGA, 6-hydroxy methyl-MGA, 15β-hydroxy-MGA, and 2β-hydroxy MGA in a vitro system prepared from cattle, and the most active metabolite among them was 2β-hydroxy MGA showing 9-times less potency than MGA (WHO, 2004). The residue level found in Canadian beef heifers treated with MGA at a rate of 0.40 mg/animal per day during 1982~1984 was 2.8 pg/kg as a mean value (ranging < 2 to 28.7 pg/kg), and 4.6% of all samples had MGA residues of more than 10.0 pg/kg of fat (Neidert *et al.*, 1990).

Melengestrol acetate was found to be a low acute toxic chemical in rodents after oral administration. Melengestrol acetate was not a genotoxic chemical in a full range of *in vitro* and *in vivo* assays, including bacterial and mammalian cellular gene mutation assays, unscheduled DNA synthesis assay, and micronuclei test in mice. In a tumor study, a higher incidence of mammary tumors was found in C3Han/f mice, but this was caused by the increased release of prolactin rather than direct action of MGA (JECFA, 2000c). Orally administered MGA induced reproductive toxicity as impaired pregnancy and parturition and greater pup loss in beagle dogs, and the NOAEL for reproductive toxicity was set at 2 μg/kg bw/day (JECFA, 2000c; Lauderdale, 1977). MGA exerted embryotoxic, fetotoxic, and teratogenic effects including resorption, dead fetuses, visceral malformation, and incomplete skeletal ossification in rabbits, in which the NOAEL was 0.4 mg/kg bw/day (JECFA, 2000c). The most appropriate end-point for MGA is a progestational effect such as changed menstrual cycles of female cynomolgus monkeys with a NOAEL of 5 μg/kg bw/day (JECFA, 2000c). An ADI of 0~0.03 μg/kg bw/day was established by applying an uncertainty factor 200 to the NOAEL. The MRLs recommended by JECFA are 1, 10, 2, and 18 μg/kg for cattle muscle, liver, kidneys, and fat, respectively (JECFA, 2006c) (Table 3).

Trenbolone acetate (TBA) is a synthetic anabolic steroid administered to cattle as a subcutaneous implant in the ear to increase feed efficiency either alone or in combination with estradiol-17β or zeranol (Metzler and Pfeiffer, 2001; Pottier *et al.*, 1973). TBA exerts its anabolic effects via binding to androgen and glucocorticoid receptors (Sillence and Rodway, 1990). The approved dose is 200 mg/implant per heifer or steer 60~90 days before slaughter (Heitzman and Hardwood, 1977). Major metabolites of TBA are the stereoisomers 17α- and 17β-trenbolone (Hoffman *et al.*, 1984; Pottier *et al.*, 1973). 17β-trenbolone is mainly found in muscle tissue, whereas 17α-trenbolone occurs mainly in the liver and bile excreta (JECFA, 1988). Its binding affinity to the androgen receptor is similar to that of dihydrotestosterone, but it has a stronger affinity to the progesterone receptor than progesterone (Hoffman *et al.*, 1984). 17α-trenbolone and the other metabolites of TBA have lower binding affinities to androgen and progesterone receptors (Bauer *et al.*, 2000). When TBA is co-administered with estradiol-17β, TBA delays estradiol excretion (Heitzman, 1983). TBA is a weak toxic chemical with an oral LD50 of 1,000~1,500 mg/kg bw. The genotoxicities of TBA, 17α-trenbolone, and 17β-trenbolone were negative in various *in vitro* and *in vivo* assays (Ingerowski *et al.*, 1981; Lutz *et al.*, 1988; Schiffman *et al.*, 1988). In carcinogenicity studies, TBA given by feeding induced liver hyperplasia in mice at 0.9~9 mg/kg bw/day and islet-cell tumours of the pancreas in rats at 1.85 mg/kg bw/day, as a consequence of the hormonal activity of TBA (Schiffman *et al.*, 1985, 1988). At a higher level of 2 μg/kg bw/day in pigs, TBA induced hormonal effects involving decreased testosterone levels in the serum of male pigs; reductions in weights of the testes, ovaries, and uteri; atrophy of testicular interstitial cells; suppression of cyclic ovarian activity; absence of glandular development of the uterine endometrium; and lack of alveolar development and secretion in the mammary glands (JECFA, 1988; van Leeuwen, 1993). Orally given ß-trenbolone induced antigonadotropic activity in castrated male rhesus macaque monkeys aged 8~17 years by the maintenance of seminal vesicle morphology and serum levels of testosterone and estradiol. The no-hormonal-effect level was evaluated as 2 μg/kg bw/day in this study (Wilson *et al.*, 2002). JECFA (1988) recommended the ADI of TBA to be 0~0.02 μg/kg bw/day according to a no-hormonal-effect-level of 2 μg/kg bw/day, based on hormonal effects observed in pigs and castrated monkeys, and an uncertainty factor 100. The MRLs of TBA are 2 μg/kg of β-trenbolone in cattle muscle and 10 μg/kg of α-trenbolone in cattle liver (Table 3).

POTENTIAL HUMAN HEALTH IMPACTS OF ANTIBIOTICS USED IN FOOD ANIMALS

The name 'antibiotic growth promoters' comes from the growth promoting effects of antibiotics, which were first discovered in the 1940s when chickens fed by-products of tetracycline fermentation grew faster than those not fed such by-products (Dibner and Richards, 2005). The modes of action of antibiotics inducing growth promoting effects are mainly through antibacterial activity and via direct metabolic effects (Butaye *et al.*, 2003). The suppression of spe-

cific toxin-producing organisms and the sparing of feed nutrients, particularly urea and amino acids, by antibacterial agents are modes of actions inducing growth promoting effects (Dibner and Richards, 2005). By suppressing disease-causing organisms, including toxin producers, in an animal's environment, antibiotics may reduce the incidence of clinical and subclinical diseases that hinder animal performance. The nutrient sparing effects of antibiotics come from their growth enhancement of intestinal organisms that synthesize nutrients required by the animals. Such organisms may provide vitamins and amino acids and digest cellulose to end products that are useful to the animals. In addition, they depress the growth of organisms that compete with host animals for nutrients and reduce wall thickness, implying the potential for improved absorption and explaining the nutrient sparing effects (Corpet, 2000).

However, many scientists, activists, regulators, and politicians have expressed urgent concerns on using antibiotics in food animals, since it could cause resistant strains of bacteria that harm human health. WHO (2002) recommended that the use of antimicrobials for disease prevention can only be justified when it can be shown that a particular disease is present on the premises or is likely to occur. Meanwhile, the control of subclinical diseases and therapeutic interventions for recognized clinical bacterial diseases by using antibacterial agents is frequently the only practical option, and therapy creates burdens in both economic and humane perspectives when disease-prevention measures fail (Phillips *et al.*, 2004; Snary *et al.*, 2004). Many of the concerns on the usage of antimicrobial growth promoters are focused on the contamination of food with bacteria that are resistant to antimicrobials. However, there is a continuing debate on the impact of antimicrobial use in animal husbandry and the risk of resistance transmission to human pathogens.

Presi *et al.* (2009) studied risk scoring for setting priorities in the monitoring of antimicrobial resistant bacteria in chicken, pork, beef, and veal meat distributed in four different product categories as fresh meat, frozen meat, dried raw meat products, and heat-treated meat products. They provided data that fresh and frozen chicken meat contributed 6.7% of the overall risk in the highest category, and fresh and dried raw pork meat contributed 4.0%. The contributions of beef and veal were only 0.4% and 0.1%, respectively. Hurd and Malladi (2008) revealed very low risk impacts of antimicrobial feed additives used in food-producing animals on human health by quantitative risk assessment. That is, the predicted risk of suboptimal human treatment of *Campylobacter coli* infections from swine is only 1 in 82 million; with a 95% chance it could be as high as 1 in 49 million for macrolides, and the risk of *Campylobacter jejuni* in poultry or beef is even less. In the case of penicillin, Cox *et al.* (2009) noted that the true risk could well be zero, providing their calculation that "not more than

0.037 to 0.18 excess mortalities per year might be prevented in the whole U.S. population if current use of penicillin drugs in food animals were discontinued and if this successfully reduced the prevalence of antibiotic-resistant *E. faecium* infections among intensive care unit (IUC) patients." For streptogrammins, banning virginiamycin has been estimated to prevent from 0 to less than 0.06 statistical mortalities per year in the entire U.S. population (Cox and Popken, 2004).

For the purpose of risk assessment of antimicrobials used in food-producing animals, categorizations are made based on the importance of each drug class to human health: fluoroquinolones, glycopeptides, streptogramines, etc. are allocated into category I indicating very high importance; aminoglycosides, microlides, lincosamides, etc. are placed in category II indicating high importance; tetracyclines and sulphonamides are medium importance drugs in category III; and bacitracin and inophores are low importance drugs in category IV (Health Canada, 2002). When antimicrobials are proposed for usage in food-producing animals, much data are required on: the relationship between antimicrobial use in animals and the occurrence of antimicrobial resistance in human pathogens; antimicrobial resistance in animal pathogens/commensals and human health consequences; proportions of human infections caused by resistant bacteria versus susceptible bacteria; genetic aspects of antimicrobial resistance, host specificity, virulence, and the spread in animal and human populations. International bodies such as CODEX, WHO, and OIE developed several guidelines for the management of antimicrobials used in food-producing animals.

Antimicrobial resistance is a multi-dimensional public health issue with broad implications. Because it requires an integrated evidence-based approach of risk management, further development of appropriate risk analysis methodologies is crucial to assess the human health impact of antimicrobial use in animals.

Regulatory approval of antibiotic applications for growth promotion in livestock has been based on demonstrable target animal safety, residual drug safety, edible tissue clearance and avoidance, and environmental safety, as well as measurable growth promoting effects. The establishment of NOAELs and ADIs of antimicrobial growth promoters are based on toxicological and microbiological evaluations. The lowest NOAEL value for the most sensitive adverse impact on human health is selected as a point of departure in risk assessment. A decision tree for the determination of adverse microbiological effects of residues of antimicrobial drugs in food-producing animals has been provided by JECFA (2000a). Emergencies of antimicrobial resistance, barrier disruption effects, and changes in specific metabolic microbiological activities are evaluated for residues of antimicrobial drugs in foods when the antimicrobials, including their metabolites, have antimicrobial properties, the drugs enter the

lower bowel by any route, the ingested residues are not transformed irreversibly to inactive metabolites, the ADI derived from toxicological data is not sufficiently low to protect the intestinal microflora, and finally, the data from the therapeutic use of the drug class in humans or from *in vitro* or *in vivo* model systems indicate effects could occur in the gastrointestinal microflora.

Table 4 presents the risk assessment results for representative feed additives including bacitracin, tetracyclines, penicillins, streptomycin, bambermycin (or flavomycin), tilmicosin, lincomycin, tiamulin, avilamycin, tylosin, colistin, and erythromycin along with their ADIs and MRLs.

Table 4. Toxicological or microbiological endpoints and regulatory limits of antimicrobial growth promoters

Compound	Toxicological or microbiological endpoint	NOAEL	ADI	MRLs (µg/kg) for edible tissues	Ref.
Bacitracin	Microbiological endpoint: Inhibition of gram positive strains isolated from human gut flora	5.7 (µg/ml)	0~3.9 (µg/kg bw/day)	For rabbits: 150 (muscle, fat, liver, kidney) 100 (milk)	EMA, 2003
Tetracyclines	Microbiological endpoint: The selection of resistant Enterobacteriaceae of human intestinal microflora	33 (µg/kg bw/day)	0~30 (µg/kg bw/day)	For cattle, pigs, poultry and sheep: 200 (muscle), 600 (liver), 120 (kidney) 100 (milk), 400 (eggs)	JECFA, 1998a
Penicillins	Toxicological endpoint: Hypersensitivity reactions in human	30 (µg/kg bw/day)	0~30 (µg/kg bw/day)	For cattle, chicken and pigs 50 (muscle, liver, kidney) 4 (milk)	JECFA, 1998b
Streptomycin	Toxicological endpoint: Decrease in body weight gain	5 (mg/kg bw/day)	0~50 (µg/kg bw/day)	For cattle, chicken, pigs, sheep: 600 (muscle, liver, fat), 1000 (kidney), 200 (milk)	JECFA, 1998c
Bambermycin (Flavomycin)	Microbiological endpoint: Inhibition of Fusobacterium of human intestinal microflora	0.25 (µg/ml)	0~1 (µg/kg bw/day)	Not recommended	Jeong, 2009
Tilmicosin	Toxicological endpoint: Decrease of body weight gain and increase of heart rate	4 (mg/kg bw/day)	0~40 (µg/kg bw/day)	For chicken: 150 (Muscle), 2400 (liver), 600 (kidney), 250 (skin/fat) For turkey: 100 (Muscle), 1400 (liver), 1200 (kidney), 250 (skin/fat) For cattle and sheep: 100 (muscle, fat), 1000 (liver), 300 (kidney) 50 (sheep milk) For pigs: 100 (muscle, fat), 1500 (liver), 1000 (kidney)	JECFA, 2009a
Lincomycin	Microbiological endpoint: Inhibition of Gram positive bacteria of human gastrointestinal flora	2.5 (mg/kg bw/day)	0~30 (µg/kg bw/day)	For chicken: 200 (muscle), 500 (liver,kidney), 100 (fat) For pigs: 200 (muscle), 500 (liver), 1500 (kidney), 100 (fat) 150 (milk)	JECFA, 2000d
Tiamulin	Toxicological endpoint: Change of electrocardiogram and increase of serum alanine aminotransferase and lactate dehydrogenase	3 (mg/kg bw/day)	0~30 (µg/kg bw/day)	For pigs: 100 (muscle), 500 (liver) For chicken: 100 (muscle, skin/fat) 1000 (liver) 1000 (eggs) For turkey 100 (muscle, skin/fat) 300 (liver)	EMA, 2008
Avilamycin	Toxicological endpoint: No significant adverse effect in ling term toxicity study	150 (mg/kg bw/day)	0~2 (mg/kg bw/day)	For pigs, chicken, turkey and rabbits: 200 (muscle, kidney, skin/fat), 300 (liver)	JECFA, 2009b

Table 4. Continued

Compound	Toxicological or microbiological endpoint	NOAEL	ADI	MRLs (μg/kg) for edible tissues	Ref.
Tylosin	Microbiological endpoint: Inhibition of Gram positive bacteria of human gastrointestinal microflora	1.698 (μg/ml)	0~30 (μg/kg bw/day)	For cattle, pigs and chicken: 100 (Muscle, liver, kidney, fat) 100 (milk), 300 (eggs)	JECFA, 2009c
Colistin	Microbiological endpoint: Inhibition of *E. coli* of human gastrointestinal microflora	1 (μg/ml)	0~7 (μg/kg bw/day)	For cattle, sheep, goat, pig, chicken, turkey and rabbit 150 (muscle, liver, fat) 200 (kidney) 50 (milk), 300 (eggs)	JECFA, 2006a
Erythromycin	Microbiological endpoint: Inhibition of *Bifidobacterium* of human gastrointestinal microflora	0.1 (μg/ml)	0~0.7 (μg/kg bw/day)	For chicken and turkey 100 (muscle, liver, kidney, fat) 50 (eggs)	JECFA, 2006b

As a whole, it is important to develop appropriate risk analysis methodologies for the assessment of the human health impacts of antimicrobial use in animals. One needs to bear in mind that the discontinuation of any antimicrobial used in food-producing animals without a full quantitative risk assessment may be unnecessary and even harmful to both animal and human health. Good hygiene practices should be insisted on farms, in abattoirs, during the distribution and marketing of foods, and during food preparation by consumers, and efforts concentrating on minimizing the transmission of all food-borne pathogens regardless of their antibiotic susceptibility are very important.

CONCLUSION

The use of hormonal growth promoters and antimicrobial growth promoters in food-producing animals has provoked many concerns on their human health impacts. A better understanding of human health risks posed by the use of such drugs is essential for making regulatory decisions and programs that support the prudent nonhuman use of hormonal drugs and antimicrobials. Risk assessments play a key role in the security of food safety. By following through with hazard identifications, hazard characterizations, exposure assessments, and risk characterizations, we attain more scientific background for decisions on risk management options in the protection of public health.

Recent results of risk assessments on hormonal substances including estradiol-17β, progesterone, testosterone, zeranol, trenbolone, and melengestrol acerate (MGA) indicate that natural steroid hormones have negligible human health impact when they are used under good veterinary practices, and for synthetic hormone-like substances, ADIs and MRLs are provided for the protection of human health.

Antimicrobials are used for growth promotion effects by adding them to feedstuffs at a dose lower than the therapeutic dose. The induction of resistant bacteria and the disruption of normal human intestinal flora are major concerns of human health for antimicrobial growth promoters. In many countries, impacts on normal human intestinal flora induced by residual antimicrobials or their metabolites are fully assessed, and microbiological ADIs and MRLs are established based on microbiological impacts prior to the approval of antimicrobials. However, risk assessment of antimicrobial resistance requires multi-dimensional information, including the relationship between antimicrobial use in animals and the occurrence of antimicrobial resistance in human pathogens, and the genetic aspects of antimicrobial resistance in animals and human populations, etc. Given the complexity of assessing antimicrobial resistance, the development of more appropriate risk assessment methodologies is crucially required to better understand the human health impact of antimicrobial use in animals.

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