HORMONAL GROWTH PROMOTERS IN MEAT PRODUCTION AND DIFFERENCES IN PERCEPTION OF ACCEPTABILITY FOR HUMAN CONSUMERS

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Introduction

Hormonal growth promoters have been used extensively in international animal production throughout the latter half of the 20th Century. Their use continues in beef production in the United States and Canada, but has not been permitted in the European Union (EU) since 1988. The EU also prohibits the importation, from third countries, of meat or meat products derived from animals treated with such growth promoters. The use, efficacy and safety of such compounds have been the subject of a number of scientific symposia (e.g. Coulston and Corte, 1975; Roche and O'Callaghan, 1992; Meissonnier, 1983 and EC, 1995) and reports, including those of the joint FAO/WHO Expert Committee on Food Additives (JECFA, e.g. 1987, 1988, 1989, 1999), Lamming et al (1986), the recently published European Commission "Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health" (SCVPH, 1999) and the "Executive summary and initial evaluation of the scientific reasoning and methods of argument adopted in SCVPH, (1999), by the sub-group of the UK Veterinary Products Committee (SGVPC, 1999). These reports are relevant to the World Trade Organisation 1998 ruling that the EU had not undertaken adequate assessment of risk and that the scientific reports referred to were not adequate in supporting the EU position. Further reports are expected to be published in the future as ongoing exercises in risk analysis and management. This review will focus briefly on recent information selected to support or rebut the acceptability of the use of hormonal growth promoters and on opinions and decisions as to whether meat from hormone-treated animals meets safety criteria laid down by relevant authorities for protection of human consumers. It is clear that the issues involved are complex, involve differences in subjective interpretation of published data and are frequently complicated by the absence of hormonisation of approach and methodology used to obtain the data on which decisions are based.

Active ingredients of growth promoters

The active ingredients of growth promoters used in beef production and studied in other ruminant and non-ruminant species include testosterone (T), oestradiol-17β (E_2-17β) and progesterone (P) which occur naturally and are essential for normal function in animals. Trenbolone acetate (TBA), Zeranol (Z) and melengestrol acetate (MGA) are xenobiotic compounds not produced naturally in animals. Apart from MGA which is given orally, the other compounds are designed to be given as implants (silastic rubber or compressed pellets) placed under the skin on the upper surface of the ear (e.g. SCVPH, 1999), which is then required to be discarded at slaughter. Implants variously contain dose levels of 8-20mg E2-17β or benzoate; 100-200mg T or T-propionate; 200mg P; 40-300mg TBA; 36mg Z with MGA administered in the diet at 0.25-0.5 mg/d. Responses to combined implants (E_2-17β+P; E_2-17β+T; E_2-17β+TBA; Z+TA) include potentiation of effectiveness of single ingredient treatments, reduction in sex-hormone related behavioural effects and more controlled release of hormone from the implant (e.g. Heitzman et al, 1976; Galbraith et al 1997). Castrate male cattle generally exhibit the greatest response in the relative absence of endogenous sex hormones with smaller responses in post-pubertal heifers and bulls. Different species may respond differently, particularly to oestrogens. For example, rodents may exhibit suppression of appetite and growth rate, poultry may eat more and deposit additional fat in tissues; castrate male sheep show greater appetite and lean and frequently fat deposition in the carcass (Galbraith and Topps, 1981; Galbraith et al, 1997).

SCVPH (1999) is an important document with a major current impact on decisions concerning the acceptability of meat from hormone-treated animals for human consumption in the EU. It covers an extensive literature on a range of topics relating to efficacy, residue levels, metabolism and suggested implications for human consumers including consequences following misuse (misplaced implants; "off label" use; black market supplies). The methodology of the approach is essentially in keeping with that described for example by JECFA, (1988) in assessing the safety of veterinary products present in human food. However, the objectives of formalising and hormonising international standards for safe use, and the avoidance of unnecessary technical barriers to international trade, have not yet been achieved in the case of hormonal growth promoters.

Development of methodology for assessment

A major contribution in determining the acceptability of the use of a veterinary drug such as an exogenously applied hormonal growth promoter is the establishment of values in or on a food for (1) maximum residue level (MRL) and (2) acceptable daily intake (ADI), (e.g. JECFA, 1989). Recommended MRL values are focused on residue levels "without toxicological hazard for human health". In practice, JECFA (1989) identified a range of criteria, examples of which are provided below. The amount of food consumed is an important factor in the determination of MRLs. JECFA (1990) proposed standard portions at the upper end of intake data for individual animal tissues and products. These were 300g of meat (muscle tissue); 100g liver; 50g kidney; 50g tissue fat; 100g egg; 1.5ml milk, consumed by a 60kg adult. The end point of toxicological evaluation used to determine ADI values are based on a safety factor (usually 100 or 1000) multiplied by the no-observed-effect-level (NOEL) as the maximum level from toxicological or human data which produces no measurable effect on the parameter chosen for measure. For the sex hormones, the end-points of assessment have generally related to their recognised hormonal activity. It should be noted that SGVPC (1999) presented estimates of food intake by adults and human children, based on dietary surveys and provided data on quantities of food which could be consumed without exceeding the ADI. SCVPH (1999) aimed "to address equally basic scientific principles, but also emerging concerns relating to hormonally-active-substances in the human environment". These included a consideration of effects on human development, increased incidence of sex hormone-related disease such as breast and prostate cancers in addition to allergic and autoimmune diseases. As indicated, aspects of SCVPH (1999) have been the subject of revaluation by SGVPC, (1999). This revaluation addressed aspects "of the scientific reasoning and methods of argument adopted in the key papers cited in the SCVPH

report" and was "unable to support the conclusion that risks associated with consumption of meat from hormone treated cattle may be greater than previously thought". SGVPC (1999) also provides useful information on hormone residues in tissues and consequences of these for indices of intake by human consumers. A brief review of evidence presented for each hormonal compound in the context of SCVPH (1999), SGVPC (1999) and other relevant information in the literature is presented below.

Oestradiol-17β

SGVPC (1999) has summarised basic information on the evaluation of acceptability of E_2-17β as a growth promoter in beef production. The ADI, (0.00005 mg/kg body weight; 3μg/60kg person) was set by JECFA (1999) using an uncertainty factor of 100 on a NOEL level of 0.3mg/person/day for increased serum corticosteroid binding globulin concentration in post-menopausal women given a conjugated equine oestrogen preparation. MRLs for E_2-17β residues in muscle, fat, kidney and offal were 'not specified' "as residues occurring subsequent to recommended use of the drug are considered to be safe for consumers. Oestradiol-17β is an 18-carbon steroid hormone synthesised principally, in gonadal tissues from 19-carbon steroidal precusors. Its presence along with related steroids, oestrone and oestriol, is essential for normal sexual development and function in female animals. The action of these oestrogenic steroids is produced by interaction with specific intracellular (α- and β-) receptors forming dimer complexes which interact with specific nuclear DNA sequences resulting in gene expression. More recently, potentially beneficial affects of oestrogens in reducing cardiovascular lesions in post-menopausal women has been associated with receptors on the cell surface of endothelial cells (Stefano et al, 2000). The aromatic A-ring and presence of the 3-OH group are known to be important determinants of ligand binding activity in addition to disposition of groups at position 17. These A-ring characteristics are also found in a range of non-steroidal compounds including phyto-oestrogens which may (a) produce oestrogenic responses in receptor-containing tissues and (b) have potential benefits for humans health (Anderson et al, 1999). The mechanism of growth-promoting action of E_2-17α has not been fully elucidated but may involve growth hormone, increased sensitivity of the liver to growth hormone and elevated concentrations of IGF-I (Breier at al, 1988). Major metabolites of injected radio-labelled E2-17β in cattle include E2-17α and oestrone, with conjugates of E_2-17β and oestrone also present in urine, glucopyranosides in liver, and E_2-17α, E_2-17β and oestrone present in faeces (SCVPH, 1999). Attention is drawn to the question of possible formation of the 2-OH, 4-OH and 16α-OH oestrogens in cattle. Considerable information is available elsewhere on the major metabolites in meat animal tissues following treatment with E_2-17β (e.g.Meissonnier, 1983; Henricks et al, 1997; MacVinish and Galbraith, 1988; 1993).

Assessment of exposure from consumption of cattle tissues

While SCVPH (1999) indicates that no data are available about the "absorption, biotransformation and elimination of E_2-17β, oestrone and E_2-17α from meat and meat product "nor about the affects of routine cooking or other processing", "data" are presented concerning estimation of excess exposure of human consumers to oestrogens in meat from treated animals. SCVPH (1999) presents data derived from JECFA (1999) which estimates the theoretical maximum daily intake of excess E_2-17β + oestrone, P and T in meat from hormone-treated animals. The data for excess oestrogens indicate consumption of 1 to 84ng/person/day (excluding values for pregnant heifers) with the median value of 6.8ng/person/day. These values are considerably less than the ADI of 3μg/d previously calculated for a 60kg person. However, SCVPH (1999) questions the validity of the figures used on the grounds of inadequate evidence of peer review and appropriateness of the oestrogen used in the post-menopausal women test system.

SCVPH (1999) also addressed the approach taken by the US Food and Drugs Administration in determining the acceptable level of exposure to oestradiol. Acceptable levels of parent hormone levels in beef (*Ref: Code of Federal Regulations (CFR) 21, Part 556, Tolerances for residues of new animal drugs in food*) are given as (ng/kg) for muscle, 120; liver, 240; kidney, 360; fat, 480. Based on the JECFA methodology of determining intake of hormones from 500g meat (300g muscle; 100g liver; 50g kidney; 50g fat), these FDA values represent a currently acceptable daily consumption of E_2–17β of 102ng and represents 1-2% of the currently used calculated daily production rates of pre-pubertal children. For natural steroids, the FDA concludes that "no physiological effect will occur in individuals chronically ingesting animal tissues that contain an increase of endogenous steroid equal to 1% or less of the amount in micrograms produced by daily synthesis in the segment of the population with the lowest daily production. In the case of estradiol and progesterone, pre-pubertal boys synthesise the least; in the case of testosterone, pre-pubertal girls synthesise the least". The daily production rate of E_2-17β in boys was estimated to be 6μg. It is interesting to note that SGVPC (1999) cites the paper of Hartman et al 1998, which suggests a value including oestrone of 100μg.

SCVPH, (1999) drawing heavily on the paper by Andersson and Skakkabaek (1999), questions the basis for determination of daily production rates of oestrogens (and androgens) in pre-pubertal children. These authors present arguments suggesting that production rates and metabolic clearance rates (MCRs) have not been accurately measured for healthy pre-pubertal children. They suggest that MCRs derived for adult women and used for pre-pubertal boys should be corrected for differences in body size or surface area to avoid a suggested 2-3 fold overestimation in values, and that greater sex-hormone binding globulin concentrations in children may also reduce MCRs further (suggested reductions in concentrations of free steroid may also be expected to decrease uptake and undesirable responses in sensitive target issues).

The question of measurement of 'true' concentrations has been further addressed by the development of a recombinant yeast bioassay (RCBA), (Klein et al, 1994) with a high specificity for E_2-17β, and a detection limit of <0.02 pg/ml, which compares with 2 pg/ml in existing assays. The assay gave mean values of 0.08 pg/ml for 23 pre-pubertal boys (aged 9.4 ± 2.0 years) and 0.6 pg/ml (aged 7.7 ± 1.9 years) for 21 pre-pubertal girls. These results are used in SCVPH (1999) as indicating that blood E_2-17β concentrations in pre-pubertal girls and pre-pubertal boys may be 12 and 100 fold less respectively than these derived from RIA analysis and that intakes of oestrogen from oestradiol-treated animals may be considerably in excess of daily endogenous production rates, particularly in the context of USFDA values given above. SGVPC (1999) gives estimates of exposure to free and conjugated E_2-17β and oestrone based on RIA methodology. Exposure for boys, for example, is given (μg/person/d) as 100 (endogenous) 0.08 (normal diet) and 0.0086 (from consuming meat from tissues of a steer treated with a combination of E_2-17β and P). This compares with an ADI of 2.0μg (for a 40kg child).

In addition to the method of analysis of sex steroids, further issues discussed by Andersson and Skakkebaek (1999) are the possible sensitivity of pre-pubertal children to low concentrations of oestrogen whether of endogenous or exogenous origin and gender differences in male and females. Variation on oral bioavailability between, for example, ethinyl oestradiol and E_2-17β (60% and 10%) are also indicated as important in highlighting the combined effects of absorption from the GIT and hepatic metabolism producing relative inactivation of the absorbed steroid. In considering SCVPH (1999), (SGVPC, 1999) has expressed concern about the yeast based-RCBA for E_2-17β. SGVPC (1999) which cites extensively the paper of Hartman et al (1998) relating intakes of steroid hormones in food to estimates of endogenous production of hormones by adults and children has advised caution in interpreting results due (a) to the possibility of competitive binding caused by cross-reaction with unspecified compounds (suggesting the need for chromatographic separation) (b) the acceptability of current RIA methods (c) the absence of validation in publications by other groups. SGVPC also suggested that SCVPH (1999), acted inappropriately in comparing residue concentrations in meat derived from RIA methodology with those for human plasma in which the RCBA was used (a point also made by Andersson and Skakkebaek, (1999). It is quite apparent that further studies on analytical methodology should be undertaken to achieve the uniformity recommended by JECFA (1989).

Genotoxicty, carcinogenicity and effects on growth, development and immunity

SCVPH (1999) considered the potential genotoxicty of oestradiol based on oxidative metabolism to 2-OH or 4-OH catechol forms, which can transform to semiquinone and quinone forms. These may react with DNA and cause effects outwith normal interaction with receptors. Redox cycling of catechol oestrogens is also considered by SCVPH (1999), although the connection is not made with the potentially beneficial effects of phytoestrogens which may also have related properties in exhibiting anti-oxidant activity (Anderson et al, 1999). Additional studies cited include (1) those of Thibodeau et al, (1998) in which it was suggested that E_2-17β, 2-OH-E_2-17β, 4-OH-E_2-17β and 16α-OH-oestrone, induced methotrexate resistance in a breast carcinoma cells (2) the finding of microsatellite instability in oestrogen-induced tumours (Hodgson et al, 1998) possibly caused by oestrogen-induced oxidative DNA damage and the observations by Rajah & Pento (1995) that 10^{-10}M E_2-17β (but not 10^{-8} or 10^{-9}M) caused a 2.4 fold increase in mutation at the hprt locus in V79 cells. The interpretation of these results is questioned by SGVPC (1999) which indicates that the tests are not standard and had serious defects in methodology.

In addition, SGVPC (1999) cites a number of well-validated assays which gave negative results for the genotoxicity of E_2-17β, and concludes that E_2-17β "does not induce mutagenic changes in any of the standard mutagenic assay which can be predicted to detect any increase in genetic damage produced by oxidative reactions". The potential carcinogenicty of oestrogens is discussed by SCVPH (1999) in terms of high dose levels and long periods of exposure arising from medical use in humans, e.g. oral contraception, post-menopausal hormone replacement therapy and administration during pregnancy. While no data are available currently on the effects of low dose exogenous estrogens, a number of oestrogen-related genetoxic effects are described which are suggested to occur independently of the presence of hormone receptors. Oestradiol has been classified as carcinogenic in animals (for example, in certain tissues in mice) (IARC, 1987). Evidence from epidemiological studies was presented which associated endogenous oestrogen concentrations with the risk of breast cancer. However, SGVPC (1999) concludes that the SCVPH (1999) evaluation is based on weak evidence and does not consider factors such as fat consumption, dietary changes and the relative exposure to hormonally-active substances in the diet and those produced endogenously. SCVPH (1999) also considers a number of reports describing the effects of oestrogens on growth and reproductive processes in animals and man. The report concludes that environmental E_2-17β could exert deleterious effects on fertility in men and women by acting through various direct and indirect mechanisms. In contrast SGVPC (1999) states that there is no evidence of low level environmental exposure to exogenous sex hormones affecting human reproduction or development. The role of oestrogens in immune function in humans and animals is briefly considered by SCVPH (1999) with no conclusion provided in relation to consumption of meat containing oestradiol residues.

Testosterone

SGVPC (1999) has summarised basic information on the use of testosterone as a growth promoter. The ADI is set at 2μg/kg bw using an uncertainty factor of 1000 on a NOEL of 100 mg/person/d in a study of 5 eunuchs. SCVPH (1999) cites a different and lower ADI value of 0.2μg/kg bw which calculates to 14μg/70kg person which is stated to be considerably in excess of the value of 189 ng/person highest excess exposure to testosterone from hormone-treated beef. MRL values are recommended as "not specified" for testosterone residues in bovine muscle, fat, kidney and liver, since they are considered safe for human consumers.

Testosterone is a naturally-occurring endogenously-produced 19-carbon steroid hormone with well recognised andogenic properties and an essential role is the reproductive system of male animals. It can be converted to 5α-dihydrotestoserone (DHT), a more potent androgen in certain tissues. It also acts as a precursor for oestrogen synthesis following aromatisation of the steroid A-ring and removal of the methyl groups at position 19. Testosterone and DHT form complexes with a specific androgen receptor and affect nuclear gene expression in diverse tissues such as in the reproductive system and the dermis of the hair follicle. Its mode of action remains only partially understood. The question of why larger circulating concentrations of androgens compared with oestrogens are present in sexually entire male animals compared with E_2-17β in females and why larger doses of exogenous androgens are needed for growth promotion has not been explained. SCVPH (1999), (source not given) tabulates concentrations present in muscle, liver, kidney and fat following treatment of heifers and female calves, with 200mg implants of testosterone and in comparison with untreated non-pregnant and pregnant heifers. Concentration were generally ranked fat> kidney> liver> muscle. Residues in treated animals were greater than those untreated (for example, heifer muscle at day 30 gave values (ng/kg) of 102 vs 19.6 respectively). However, these were considerably less than endogenous residues present in muscle tissue from bull (535ng/kg) and pregnant heifer at 240 days gestation (418ng/kg). Orally administered testosterone is known to have limited bioavailability in humans because of rapid hepatic metabolism and biliary excretion in bile or urine. MCR values for men and women are cited as 516 and 304l/d respectively.

Assessment of exposure from consumption of cattle tissues

Post-pubertal males are considered to have the highest endogenous production rates (6,500 μg/d) with values of between 140-240 μg/d for adult women and 65 and 32 μg/d for pre-pubertal boys and girls respectively (Hartman et al, 1998). SGVPC (1999) has

calculated that maximum excess exposure due to consumption of beef from cattle treated with 20mg E_2-benzoate and 200mg testosterone propionate is 0.0092μg for girls which compares with total amounts in a normal diet and endogenous production of 0.04 and 32μg respectively. SCVPH (1999) cites tolerance levels (μg/kg) for testosterone in uncooked tissues of steers and calves established by the FDA (*Ref: Code of Federal Regulations) (CFR) 21, Part 556, Tolerances for residues of new animal drugs in food*) as: muscle - 0.64; liver - 1.3; kidney - 1.9; fat - 2.6. Using these levels, consumption of the 'standard' quantities of 300g muscle, 100g liver, 50g kidney and 50g fat approximates to 600ng/person/day exposure to exogenous testosterone, and 330% of the maximum estimated excess exposure to testosterone in meat from treated cattle of 189ng/person/d. The FDA acceptable maximum value of 600ng/d represents 1-2% of the estimated daily production rate of testosterone of 32 μg/day for pre-pubertal girls. Given the argument and information produced by Andersson and Skakkabaek (1999) for E_2-17β (see above) and applied to testosterone, of possible overestimation of production rates, SCVPH (1999) considers that excess testosterone intake could "at best exceed the 1% FDA safety margin and at worst be greater than that naturally present". This view is subject to the same questions concerning analytical methodology as described above for oestrogens.

Genotoxicity, carcinogenicity and effects on growth, development and immunity

SCVPH (1999) addresses the same questions of toxicology for testosterone as for E_2-17β and concludes that no genotoxity has been reported for testosterone and no information is available for testosterone-produced DNA damage. However, indirect effects may occur following aromatisation to E_2-17β and its metabolites which is considered to be genotoxic (see above). Evidence is presented for carcinogenic effects from studies of feeding testosterone in experimental animals, but evidence for effects in humans including those produced by testosterone-related residues is limited. However, based on convertibility to E_2-17β and epidemiological data, testosterone is considered as a possible carcinogen in humans (IARC group 2A). Effects of testosterone on growth and reproduction are predictable on the basis of knowledge of its role in human beings and animals, (effects on the hypothalamic-pituitary-gonadal axis, muscle and bone mass and behaviour). There are inadequate data to suggest any deleterious effects on immune function (SCVPH, 1999). Testosterone and its derivatives are also used for performance enhancement by human athletes.

Progesterone

Using an uncertainty factor of 100 and lowest observable effect level (LOEL) of 200mg/person/d (3.3mg/kg bw/d at 60kg bw) in a human uterine test system for fine-particle progesterone, the ADI was set at 30μg/d, (SGVPC, 1999 based on JECFA, 1999). MRL values were set as "not specified" for progesterone residues in bovine muscle, fat, kidney and liver. Progesterone is a 21-carbon steroid hormone differing from testosterone by the presence of a 2-carbon side chain at the 17-carbon position. It has an obligate role in the regulation of the female reproductive cycle preparation for, and maintenance of, pregnancy in humans and animals in association with oestrogens. Production rates (JECFA, 1987) have been estimated in pre-menopausal women at 418 μg/d in the follicular phase of the reproductive cycle and 94,000 μg/d in late pregnancy and in post-pubertal men to be 416 μg/d. The action of progesterone in animals is mediated by receptor-nuclear gene interactions. Residues in cattle include the parent compound (frequently at highest concentrations) and a range of metabolites. SCVPH (1999) give concentrations (μg/kg) residual progesterone for an untreated versus 200mg-progesterone-treated steer at 60 days after implantation as follows: 0.27 vs 0.4; 0.26 vs 0.35; 0.17 vs 0.20 and 2.48 vs 3.40 for muscle, liver, kidney and fat. These values compare with the much greater concentrations in tissue of pregnant heifers of 10.1, 3.42, 6.19 and 239 (μg/kg).

Assessment of exposure from consumption of cattle tissue

SCVPH (1999) cites tolerance levels (μg/kg) for progesterone levels in uncooked tissues of steer and calves according to USFDA (*Ref : Code of Federal Regulations (CFR) 21, Part 556, Tolerances for residues of new animal drugs*) as follows : muscle - 3; liver - 6; kidney - 9; fat - 12. Applying the 'standard' values for intake of 500g/d of beef results in an estimated exposure to progesterone of 2.6 μg/person/d. This quantity calculates at 1-2% of the estimated daily production rate for pre-pubertal boys considered to be most at risk (150 μg/d) and approximates to 0.3% of the maximum excess exposure to progesterone arising from consumption of meat from hormone-treated cattle. SCVPH (1999) again expresses concern at the validity of estimates for endogenous production as discussed by Andersson and Skakkabaek (1998) and indicates that the FDA 1% safety margin may be readily exceeded. SGVPC (1999) also provide examples of the maximum exposure of a range of human consumers to progesterone with values for boys given (μg/d) as 150 (endogenous); 8.9 (normal diet); 0.024 from residues in beef. In humans, oral progesterone is rapidly absorbed and metabolised to inactive forms such as 5β-pregnane-3α-o1-20α-diol-glucuronide (SCVPH, 1999).

Genotoxicity, carcinogenicity and effects on growth, development and immunity

No information is reported to be available on mutagenicity and genotoxicity of progesterone, although JECFA (1999) is cited as referring to unreferenced statements on interactions with DNA. Evidence for carcinogenicity of progesterone is cited in the context of increased tumour evidence in laboratory animals in tissues of the mammary gland and reproductive tract. Evidence in humans is considered inadequate despite widespread usage of progesterone or progestogen-only contraceptive preparations. Taking into account the laboratory animal data, IARC allocated progestogen-only contraceptives in group B (possibly carcinogenic to humans). Effects on reproduction may be expected on the basis of known biological effects on the hypothalamic-pituitary-gonadal-axis. There is evidence to suggest immuno-suppression in animals (for example, during pregnancy), but unsufficient to assess the effects of progesterone residues from meat of treated animals on these parameters (SCVPH, 1999).

Trenbolone Acetate

Trenbolone acetate (TBA) is a synthetic steroid. It is the 17-acetylated ester of trenbolone-17β-OH (17β-hydroxyestra 4, 9, 11 triene-3, one). Risk assessment therefore differs from that applied to the use of compounds which occur endogenously in animals. For TBA and other xenobiotic compounds the presence of any residues in meat or meat products has to be assessed. However, considerable information is available to estimate risk characteristics. The ADI value is set at 0.02 μg/kg bw/d based on an uncertainty factor of 100 and HOENL of 2μg/kg bw/d for sex-related indices in male pigs given TBA orally (SGVPC, 1999). (SCVPH (1999) indicates a temporary ADI of 0.01μg/kg bw/d) and a 'temporary' acceptable residue level of 1.4 μg/kg bovine meat

for TB-17β-OH based on 500g meat consumption by a 70kg person. TBA is normally hydrolysed to active TB-17βOH, with major metabolites identified as the TB-17αOH excreted in bile with TB-17βOH the major free residue in muscle. Conjugated glucuronides are also excreted in urine and bile. SCVPH (1999) quotes values indicating an 8-10 times greater (anabolic) potency than testosterone (Neuman, 1975). This report indicates that the TBA may have 15-30 times greater androgenic and about 50 times greater anabolic activity than testosterone in the castrated male rat. More recent studies in sheep (Galbraith et al, 1994, 1997) not cited in either SCVPH (1999) or SGVPC (1999) suggest that TBA given alone had similar anabolic activity to testosterone in stimulating growth and carcass protein deposition in castrate males although TBA did exhibit up to 50% greater apparent androgenic activity in stimulating increased weight of sex-hormone sensitive tissues. Combinations of T and TBA treatment showed additive effects for those androgenic indices. TBA also reduced variably weight of the adrenal and thyroid glands and consistently, thymus weight (also noted in TBA-treated veal calves) an effect reversed by combined treatment with either T or E_2-17β - responses which appear not to have been considered by regulatory agencies. The data comparing TBA and E_2-17β alone or combined in castrate male sheep also clearly demonstrated a growth response to the oestrogen with limited or no response to TBA alone. This result is achieved at plasma concentrations for E_2-17β ranging from 40 to 85 ng/l and 1.1 to 2.7 μg/l for TB-17βOH (Galbraith et al, 1997). Growth and/or carcass responses have also been noted in female cattle (e.g. Heitzman, 1976) and sheep (Sulieman et al, 1992) treated with TBA. In addition TB-17βOH has also been shown to exhibit progestogonic activity (Karg and Meyer, 1999) and to reduce glucocorticoid receptor binding capacity (an effect different to that produced by testosterone or nandrolone) (Sharpe et al, 1986 and Galbraith & Berry, 1994). These latter effects are possibly associated with effects in reducing protein turnover rates in skeletal muscle. A major feature of the interaction of TBA with beef tissues is the formation of bound non-extractable residues which, following treatment with proteolytic enzymes, became water soluble, indicating the presence of polar compounds. Karg & Meyer (1998) consider that residues covalently bound to protein and DNA should be investigated as part of a further evaluation of safety for human consumers.

Assessment of exposure from consumption of cattle tissues

SCVPH (1999) cites values (μg/kg) set by FDA (*CFR 21, part 556, Tolerance for residues of new animal drugs*) in uncooked tissues of cattle as follows; muscle-50; liver-100; kidney-150; fat-200. These values are consistently greater than the temporary acceptable residue concentrations of 1.4 μg/kg (ADI of 0.7μg at 70kg bw) and calculate to an intake of ca. 43μg/d using the standard values for intake of muscle, liver, kidney and fat (SCVPH, 1999). SGVPC (1999) give an example of maximum TB-17αOH concentrations in steers implanted with 40mg E_2-17β and 200mg TBA in muscle, liver, kidney and fat as (μg/kg) of 0.014, 1.871, 0.347 and 0.018 respectively. These are calculated to give a dietary intake for adults of 0.21 μg/d which approximates to 17.5% of the ADI. Residues of free TB-17βOH, were determined at concentrations (μg/kg) of 0.139 (muscle); 0.328 (liver); 0.494 (kidney); and 0.247 (perinephric fat) in male castrate sheep 60 days following implantation of 35mg TBA + 5mg OE_2-17β with results of a similar order obtained following implantation of TBA in mature female sheep (MacVinish & Galbraith, 1988, 1993). Mean values for conjugated TB-17βOH were consistently lower than those for extractable free TB-17βOH.

Genotoxity, carcinogenicity and effects on growth, development and immunity

Lamming et al (1987) considered methodology to evaluate the safe use for human consumers of the synthetically-produced compounds TBA and Z in meat production systems. SCVPH (1999) has summarised mutagenicity/genotoxicity data for TB-17βOH and-17-αOH which gave similar results in a range of cellular and sub-cellular tests. Karg and Meyer (1999) drew attention to the predominance of negative findings, but also to those for example, of Schiffman et al. (1985) in which positive effects were recorded. Reference to covalent binding of TB-17βOH to whole cell and sub-cellular preparations of rat hepatocytes is made by SCVPH (1999) indicating the formation of DNA adducts under the conditions described. High doses of TBA have also been shown to produce liver hyperplasia and tumours in mice with some evidence of pancreatic abnormalities in rats and hepatic abnormalities in guinea pigs (Zarkawi et al, 1991). However, a 2-year carcinogenicity bioassay study in rats apparently did not produce evidence of carcinogenicity of TB-17βOH. SCVPH (1999) concluded that current evidence is insufficient to complete a quantitative assessment of risk. SCVPH (1999) also summarised a range of studies which described effects on reproduction typical of those expected from a compound with androgenic sex hormone activity. Effects included female virilization and interference with reproductive cycles. SCVPH (1999) concludes that the data available "do not allow a realistic assessment of a dose response relationship" which may be of value in assessing risk for human consumers of TBOH residues in animal tissues. Similarly, insufficient evidence is available to determine effects of low levels of TB-17βOH on the immune system of human beings.

Zeranol

The ADI value for zeranol is 0.5μg/kg bw based on a HNOEL in ovariectomised female cynomolgus monkeys of 0.05mg/kg bw/d and giving an ADI value of 0.5μg/kg bw (35μg/70kg bw), (SCVPH, 1999). MRL values were set for bovine muscle and liver of 2 and 10μg/kg respectively. Zeranol (α-Zearanol) is a non-steroidal resorcylic acid lactone derivative of the myoestrogen zearalenone. It has been shown to occur naturally but is not endogenously produced in animals but may occur as a result of ingestion of contaminated feed. Lamming et al (1987), for example, indicate that the relative binding to oestrogen receptors is up to 20% that of the E_2-17β with zearalenone and taleranol less active than zeranol. SCVPH (1999) also summarised the results of a number of studies which showed that zearalenone and taleranol have been demonstrated as residues in cattle. Additional metabolites, suggested to be monohydroxlated derivates, have been detected in a rat liver microsome system. Following 36mg zeranol implantation in steers values determined by RIA (μg/kg) at 70 days in a number of tissues were as follows: kidney 0.13 to 0.16; liver, 0.20 to 0.30; muscle, 0.13 to 0.72 and fat, 0.073 to 0.184 (SCVPH, 1999). It was concluded that the mean residue levels (μg/kg) as zeranol equivalents did not exceed 0.2μg/kg in muscle, 10μg/kg in liver, 2μg/kg in kidney and 0.3μg/kg in fat. One study in humans has suggested that zeranol has a half-life in blood of 22h, with the possibility of accumulation of parent compound or of metabolites (Migdalof, 1983)

Assessment of exposure to Zeranol from consumption of cattle tissues

SCVPH (1999), cites values (µg/kg) set by the FDA for residues of zeranol in uncooked tissues of cattle. (*Ref: CFR 21, Part 55, Tolerance for residues of new animal drugs in food*). These are; muscle - 150; liver - 300; kidney - 450; fat - 600. On this basis and applying standard values for intake, the acceptable daily ingestion of zeranol approximates to 128µg/person/day, and exceeds the ADI, of 35µg for a 70kg person by a factor approaching 4. SGVPC (1999) presents data which suggest that the ingestion by adults, of standard portions of dietary components gives rise to an intake of 0.169µg/person/d which is 0.56% of the ADI.

Genotoxicity, carcinogenicity and effects on growth, development and immunity

SCVPH (1999) concluded that there was inadequate information to evaluate the mutagenicity and genotoxicity of zeranol. Some evidence was presented for a positive response to an unspecified concentration in a rec-assay in *Bacillus subtilis*, but two other tests were negative. Karg and Meyer (1998) indicate that results for a long term *in-vitro* rat study at an oral dose rate of 1.25mg/kg bw/d did not produce carcinogenic effects. SCVPH (1999) cites a low incidence of production of hepatic and renal tumours of hamsters but conclude that no assessment can be made on the possible carcinogenicity of zeranol and that there are no data available connecting zeranol residues in meat to risk of cancer for humans. Zeranol has been reported to exert typical oestrogenic effects on reproductive parameters in animals (JECFA, 1988). SCVPH (1999) also cites evidence of effects of exposure of male mice *pre-partum* to zeranol which caused testicular abnormalities *post-partum*. SCVPH (1999) considered that no estimate could be made of a dose response relationship for reported effects nor of the possible risks of exposure to zeranol residues in meat or meat products.

Melengestrol Acetate

Melengestrol acetate (MGA)is a synthetic compound with progestogenic activity. No ADI values have been set for melengestrol acetate although a tolerance level of 25µg/kg in the fat tissue has been applied by USFDA (*Code of Federal Regulations, CFR 21, Part 556*) (SCVPH, 1999). Its major effects are the suppression of oestrus in female cattle, while producing tonic increases in LH, but inhibiting the surge needed for ovulation. It is considered to act with FSH to stimulate oestrogen production by the ovarian follicle, which in turn is thought to produce the growth-promoting effect frequently observed following oral treatment. Based on studies including inhibition of menses in normal ovulating women, (7.5 to 10mg/d effective; 5mg/d, not effective) a minimum effective daily dose level of 0.7mg and no effect daily dose of 0.4mg were calculated for MGA in women. Using values for residue levels in beef muscle and fat (less than 10µg/kg after 48h withdrawal), it was calculated that a daily intake of ca. 45kg MGA-fed heifer beef would not exceed the no-effect dose. Data on pharmokinetics and bio-transformation of MGA in (a) cattle and (b) humans have been established, (SCVPH, 1999). For example, cattle fed radio-labelled MGA are reported to excrete ca. 87% in faeces and 13% in urine, with ca. 15% excreted unmetabolised. Karg and Meyer, (1998) highlighted the importance of residual MGA in fat tissue of treated animals.

Genotoxicity, carcinogenicity and effects on growth, development and immunity

The data on mutagenicity and carcinogenicity is considered by SCVPH (1999) to be inadequate to assess carcinogenic potential of MGA. The effects of MGA in inhibiting normal menstrual and oestrus cycles are well characterised. However, SCVPH (1999) considers that no systematic data are available to assess risk from consuming beef from MGA-treated animals on growth, reproductive and other toxicological parameters in human beings.

Additional comments

Karg and Meyer (1998) have drawn attention to a number of recent issues which include the presence of non-polar residues of, for example, E_2-17β which forms esters of long chain fatty acids which persist in fatty tissues. These authors consider that the possibility of formation of similar esters of trebolone and zeranol which should be investigated. Implications for the use of animal fat for non-food purposes such as 'tallow' used in soap manufacture may also be considered. Relevant also is the need to increase in understanding of interactions between the two classes of oestrogen receptor, endogenous oestrogens, and phytoestrogens with potentially beneficial effects on human health from, for example, anti-oxidant activity (Anderson et al, 1999). Zearalenone is considered to require further investigation in the context of apparently increased environmental exposure to oestrogenic compounds. The possible role of "orphan nuclear receptors" in mediating the effects of sex hormone action is also highlighted by Karg and Meyer (1999). SCVPH (1999) and SGVPC (1999) also refer to the importance of good practice in assessing the risk to consumers of beef from hormone-treated animals. Any deviation from this whether from wrongly sited implants with large residue potential, incorrect dose, or an any other aspect of illegal use will render unacceptable the meat end-product. It is apparent that a culture of strict quality assurance concerning these deviations, in addition to those concerning normal useage, continues to be needed to satisfy regulatory bodies and increasingly discerning human consumers.

It is useful to consider finally the main conclusions of SGVPC (1999). These are summarised as: (a) likely levels of consumer exposure to residues of growth promoters were very low in comparison with acceptable daily intakes and amounts of hormone produced naturally "by the bodies of some people"; (b) SCVPH (1999) did not provide adequate evidence to substantiate mutagenic/genetoxic properties of E_2-17β nor for the other compounds; (c) the yeast RCBA method for analysis of E_2-17β was not adequately validated; (d) conclusions on aspects of immunity, links with common cancers and on aspects of human development and reproduction did not adequately address the issue of low level residues (including that of compounds already subjected to inactivation and reduction in activity in target animals) and ignored certain reports in the literature. It is apparent that a number of questions remain to be answered and further consideration is needed if an improved consensus is to be achieved in a consumer environment which increasingly requires well-founded answers of clarity and authority.

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