Nutrition Research Reviews (2002), **15**, 293–314 DOI: 10.1079/NRR200246

Hormones in international meat production: biological, sociological and consumer issues

Hugh Galbraith

Department of Agriculture and Forestry, University of Aberdeen, 581 King Street, Aberdeen AB24 5UA, UK

Beef and its products are an important source of nutrition in many human societies. Methods of production vary and include the use of hormonal compounds ('hormones') to increase growth and lean tissue with reduced fat deposition in cattle. The hormonal compounds are naturally occurring in animals or are synthetically produced xenobiotics and have oestrogenic (oestradiol-17β and its esters; zeranol), androgenic (testosterone and esters; trenbolone acetate) or progestogenic (progesterone; melengestrol acetate) activity. The use of hormones as production aids is permitted in North American countries but is no longer allowed in the European Union (EU), which also prohibits the importation of beef and its products derived from hormone-treated cattle. These actions have resulted in a trade dispute between the two trading blocs. The major concern for EU authorities is the possibility of adverse effects on human consumers of residues of hormones and metabolites. Methods used to assess possible adverse effects are typical of those used by international agencies to assess acceptability of chemicals in human food. These include analysis of quantities present in the context of known biological activity and digestive, absorptive, post-absorptive and excretory processes. Particular considerations include the low quantities of hormonal compounds consumed in meat products and their relationships to endogenous production particularly in prepubertal children, enterohepatic inactivation, cellular receptor- and non-receptor-mediated effects and potential for interference with growth, development and physiological function in consumers. There is particular concern about the role of oestradiol-17β as a carcinogen in certain tissues. Now subject to a 'permanent' EU ban, current evidence suggests that certain catechol metabolites may induce free-radical damage of DNA in cell and laboratory animal test systems. Classical oestrogen-receptor mediation is considered to stimulate proliferation in cells maintaining receptivity. Mathematical

Abbreviations: ADI, acceptable daily intake; BW, body weight; CYP, cytochrome P450; DHT, 5α-dihydrotestosterone; E_2-17β, oestradiol-17β; EC, European Commission; ER, oestrogen receptor; EU, European Union; IGF, insulin-like growth factor; JECFA, Joint FAO/WHO Expert Committee on Food Additives; MGA, melengestrol acetate; MRL, maximum residue limit in tissues; NOEL, no-observed-effect level; Pr, progesterone; SHBG, sex hormone-binding globulin; T, testosterone; TB, trenbolone; TBA, trenbolone acetate; USFDA, US Food and Drugs Administration; Z, zeranol.

Corresponding author: Dr Hugh Galbraith, fax +44 1224 273731, email h.galbraith@abdn.ac.uk

models describing quantitative relationships between consumption of small amounts of oestrogens in meat in addition to greater concentrations from endogenous production, chemical stoichiometry at cellular level and human pathology have not been developed. Such an approach will be necessary to establish 'molecular materiality' of the additional hormone intake as a component of relative risk assessment. The other hormones, although generally less well researched, are similarly subject to a range of tests to determine potentially adverse effects. The resulting limited international consensus relates to the application of the 'precautionary principle' and non-acceptance by the European Commission of the recommendations of the Codex Alimentarius Commission, which determined that meat from cattle, hormone-treated according to good practice, was safe for human consumers. The present review considers the hormone issue in the context of current international social methodology and regulation, recent advances in knowledge of biological activity of hormones and current status of science-based evaluation of food safety and risk for human consumers.

Hormones: Meat: Human consumers: Cancer: Risk assessment: Oestradiol-17β: Testosterone: Progesterone: Trenbolone acetate: Melengestrol acetate

Introduction

Beef is an important component of the diet of meat-eating human consumers. It is produced from cattle frequently treated with biologically active compounds to alleviate disease or increase efficiency of growth. Among the latter group of chemical compounds are hormonal growth promoters, which have been used extensively in international animal production to improve the efficiency of conversion of food into live-weight gain, reduce fat and increase N retention and lean tissue deposition. Their use continues in certain countries including the United States and Canada, but has not, along with a ban on importation of beef from hormone-treated cattle, been permitted in the European Union (EU) since 1988 (European Commission, 2000). This divergence, in view of implications for world trade, has assumed a major sociological dimension involving national and international regulatory agencies that advise on acceptability of practices affecting the chemical safety of human food. Issues addressed in the present review include concentrations of hormones and metabolites present in food, the role of endogenous hormone supply in normal physiological function and the suggested association of these steroids with formation of certain cancers, particularly for oestrogens. Brief reference will also be made to illegal practices, the role of other endocrine-active chemicals in food and the development of schemes of surveillance and traceability of sources designed to improve assurance of food quality and safety for human consumers.

Implications for international food regulation and trade

Recent background to the dispute concerns the Uruguay Round meeting in 1994, the World Trade Organization agreement and associated 'Agreement on the Application of Sanitary and Phytosanitary Measures' in 1995. Applicable standards for determination of food safety are

https://doi.org/10.1079/NRR200246 Published online by Cambridge University Press

those determined by the Codex Alimentarius Commission (2001), frequently on the basis of recommendations from the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Responses by beef-exporting nations to the EU ban on beef from hormone-treated cattle include separate complaints by the United States and Canada to the Dispute Settlement Body of the World Trade Organization, which concluded that the European Commission (EC) measures did not meet the requirements for risk assessment according to the Agreement on the Application of Sanitary and Phytosanitary Measures (see also Hurst, 1998).

The EC initiated, in 1998, a risk assessment designed to elucidate aspects of toxicology, residues, abuse and environmental impact of the six hormonal compounds, and adopted an Opinion of the EC Scientific Committee of Veterinary Measures relating to Public Health, which provided justification for the ban (Scientific Committee on Veterinary Measures Relating to Public Health, 1999). This Opinion has a major impact on decisions about the acceptability of meat from hormone-treated animals. In turn, the Opinion was reviewed by a sub-group of the UK Veterinary Products Committee (Sub-group of the Veterinary Products Committee, 1999), which identified areas of disagreement and concern.

More recently the Scientific Committee on Veterinary Measures Relating to Public Health (2000, 2002) reviewed specific documents, including that of the Sub-group of the Veterinary Products Committee (1999), and declined to revise its earlier conclusions. In addition, the European Commission adopted Directive 2000/0132 COD and applied the 'precautionary principle' (European Commission, 2001*a*,*b*) to ban indefinitely the use of oestradiol-17β (E_2-17β) and its derivatives in farm animals with the continuation of the provisional prohibition of the five remaining hormones.

Review of scientific information

Literature considering the use, efficacy and safety of hormonal anabolic compounds includes a large international collection of individual publications and reports of scientific working groups and scientific symposia (Coulston & Korte, 1976; Roche & O'Callaghan, 1982; Meissonnier, 1983; Lamming, 1987; European Community, 1996; Owens *et al*. 1997), review papers (for example, see Galbraith & Topps, 1981; Karg & Meyer, 1999; Preston, 1999; Meyer, 2001; Stephany, 2001), the EC-commissioned studies on risk assessment (European Commission, 2001*b*), and JECFA evaluations and technical monographs (for example, see Joint FAO/WHO Expert Committee on Food Additives, 1988, 1989, 1990, 2000*a*,*b*). The present review will focus predominantly on the most recent literature in the context of the Scientific Committee on Veterinary Measures Relating to Public Health's (1999) opinion and the report of the Sub-group of the Veterinary Products Committee (1999), which are science-based documents sociologically central to the current debate.

Active ingredients of growth promoters

The active ingredients of hormonal growth promoters used in beef production and studied in other ruminant and non-ruminant species include naturally occurring testosterone (T), E_2-17β and progesterone (Pr) and the xenobiotic compounds (not produced naturally in animals) trenbolone acetate (TBA), zeranol (Z) and melengestrol acetate (MGA). Apart from MGA, which is given orally, the other compounds are given as commercial implants (silastic rubber or compressed pellets) placed under the skin on the upper surface of the ear (for example, see Scientific Committee on Veterinary Measures Relating to Public Health, 1999), which is then

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required to be discarded at slaughter. Implants variously contain dose levels of 8–20 mg E_2-17β or oestradiol benzoate; 100–200 mg T or T-propionate; 200 mg Pr; 40–300 mg TBA; 36 mg Z, with MGA administered in the diet at 0·25–0·5 mg/d. Effects of combined implants (E_2-17β+Pr; E_2-17β+T; E_2-17β+TBA; Z+TBA) include potentiation of effectiveness of single ingredient treatments, reduction in sex hormone-related behavioural effects and more controlled release of hormone from the implant (for example, see Heitzman, 1976; Brandt, 1997; Galbraith *et al*. 1997). Castrated male cattle generally exhibit the greatest response in growth rate, efficiency of feed conversion, N retention, skeletal muscle hypertrophy and lean tissue deposition occurring in the relative absence of endogenous sex hormones, with smaller responses in post-pubertal heifers and bulls. Oestrogens, in addition to producing sex hormone-related side effects (Galbraith *et al*. 1990, 1997; Zarkawi *et al*. 1991*a*; Wetteman & Lehman, 1997), produce the greatest anabolic responses. These may be enhanced by combination with androgens such as TBA (Preston, 1999) although not T or the β-agonist cimaterol (Galbraith *et al*. 1990, 1997) with responses differing between cattle and sheep.

Development of methodology for assessment

Current practice to determine acceptability of use of a veterinary drug in a meat-producing animal typically includes establishment of values for: (1) acceptable daily intake (ADI); (2) maximum residue limit in tissues (MRL) (for example, see Joint FAO/WHO Expert Committee on Food Additives, 1989; Codex Alimentarius Commission, 2001). An ADI estimates the amount of a drug or residue that may be ingested over a lifetime without appreciable risk. Recommended MRL values in food are those 'without toxicological hazard for human health' and limit food intake to less than the ADI. The Joint FAO/WHO Expert Committee on Food Additives (1990) proposed standard portions for individual animal tissues and products. These were: 300 g meat (muscle tissue); 100 g liver; 50 g kidney; 50 g tissue fat; 100 g egg; 1·5 litres milk, consumed by a 60 kg adult. The endpoints 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 data producing no measurable effect on the parameter chosen for measure. For the sex hormones, the endpoints have generally related to hormonal activity, which may not be appropriate for non-receptor-mediated effects. The Sub-group of the Veterinary Products Committee (1999) has presented separate estimates of food intake by human adults and children.

Oestradiol-17β

In evaluating the acceptability of this oestrogen as a growth promoter in beef production the ADI was set by the Joint FAO/WHO Expert Committee on Food Additives (2000*a*) at 0–0·05 μg/kg body weight (BW) (3 μg/60 kg person). The system used an uncertainty factor of 100 on a NOEL level of 0·3 mg/person per d for increased serum corticosteroid-binding globulin concentration in post-menopausal women given a conjugated equine oestrogen preparation. The MRL for E_2-17β residues in muscle, fat, kidney and offal were defined as 'not specified' and unnecessary (Codex Alimentarius Commission, 2001) as residues occurring subsequent to good animal husbandry practice are considered to be safe for consumers. This conclusion disagrees with the view taken by the EC in prohibiting the use of E_2-17β.

In assessing the possible deleterious effects associated with its presence, it is also impor-

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tant to consider mechanisms of interaction with tissues and essential roles in mammalian reproduction, physiological function and development. In brief, E_2-17β and related compounds oestrone and oestriol are 18-carbon steroid hormones synthesised principally in gonadal tissues from 19-carbon steroidal precursors by the cytochrome P450 aromatase complex.

The action of these oestrogenic steroids is produced by interaction with specific intracellular receptors (oestrogen receptor (ER)-α and ER-β and splice variants (Barnes, 2001; Pfaffl *et al*. 2001), forming dimer complexes which, acting as transcription factors and interacting with co-activator proteins and specific nuclear hormone response elements, induce gene expression (Hall *et al*. 2001; Rahman & Sarkar, 2002). The α and β isoforms exhibit 95 % amino acid homology in the DNA-binding domain and about 55 % in the ligand-binding domain of the receptor (Enmark & Gustafsson, 1999) and have been shown to be differentially regulated and expressed in different sites of the body. ER have also been identified in the skeletal muscle of calves (Meyer & Rapp, 1985; Pfaffl *et al*. 2001). These, along with somatotrophin and insulin-like growth factor (IGF)-I, may mediate the oestrogenic growth-promoting response, which remains poorly understood (Breier *et al*. 1988; Preston, 1999).

The development of 'ER knockout' and 'aromatase knockout' mice has provided information on what appears an essential role of oestrogens in male germ-line development and fertility (Joint FAO/WHO Expert Committee on Food Additives, 2000*a*; Nef & Parada, 2000). *In vitro* studies have also shown that E_2-17β ($10^{-9}-10^{-10}$ M) may act as a germ cell survival factor in human testis (Pentikainen *et al*. 2000).

Beneficial effects of oestrogens in managing human osteoporosis (Doren, 2001) and in reducing cardiovascular lesions in post-menopausal women have been associated with receptor-mediation including those on endothelial cell surfaces (Stefano *et al*. 2000) and those involving ER-β in the inhibition of smooth muscle cell proliferation (Gustafsson, 1999).

The aromatic A-ring and presence of the 3-OH group in steroidal oestrogens, which determine ligand-binding activity (Barnes, 2001), are also found in non-steroidal compounds including phyto-oestrogens. These compounds, which are also frequently present in human food, may interact with ER, particularly ER-β in 'non-reproductive' tissues (Pennie *et al*. 1998) and variably influence cardiovascular disease, cancer and osteoporosis (Anderson *et al*. 1999; Ibarreta *et al*. 2001; Sirtori, 2001). There is also evidence that certain dietary phyto-oestrogens may affect synthesis and metabolism of endogenous or exogenously derived steroidal oestrogens by acting as inhibitors of enzymes such as aromatase, sulfotransferase and β-hydroxysteroid dehydrogenases (Kirk *et al*. 2001). These findings and others that indicate different affinities for receptors (Burger, 2000; Nikov *et al*. 2001) and interactions with genomic response elements indicate caution when comparing effects of steroidal and the non-steroidal oestrogens in human biology. Such comparisons are frequently made as a means of defining total 'oestrogen' exposure or load from all sources in the human diet (for example, see Preston, 1997). However, the requirement to metabolise and excrete absorbed steroidal and non-steroidal oestrogenic compounds remains.

Eliminative metabolic pathways frequently include hydroxylation of oestrogens and other hydrophobic steroid and non-steroidal compounds by the cytochrome P450 (CYP) superfamily of proteins (Joint FAO/WHO Expert Committee on Food Additives, 2000*a*). The expression of CYP may involve members of the orphan nuclear receptor superfamily of gene products, which include receptor-transcription factors for steroid hormones (Olefsky, 2001). Of particular interest are the steroid and xenobiotic receptors/pregnenolone X receptor and the constitutive androstane receptor, which have been shown, in the presence of certain steroids and xenobiotic compounds, to activate *CYP3A* and *CYP2B* genes respectively in cultured primary hepatocyte and transgenic rodent model systems (Xie *et al*. 2000; Xie & Evans, 2001). Induction of CYP enzyme activity by some ligands is known to affect metabolism of others (Xie *et al*. 2000), thus

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presenting the possibility that phyto-oestrogens in the human diet may affect metabolism of steroidal oestrogens. Similarly the metabolism of oestrogens by cattle may be affected by other steroids (T, TBA) in combined implants.

The endproducts of metabolism in uncooked animal tissues following treatment with E_2-17β include parent compound, E_2-17α, oestrone and steroidal glucopyranosides in liver with the formation of 2-OH, 16-OH and to a lesser extent 4-OH oestrogens mainly in the liver but also extrahepatically (Joint FAO/WHO Expert Committee on Food Additives, 2000*a*), in addition to non-polar longer-chain fatty acid esters in fatty tissues (for example, see Meissonnier, 1983; MacVinish & Galbraith, 1988, 1993; Hendricks *et al*. 1997, 2001; Preston, 1997; Karg & Meyer, 1999; Joint FAO/WHO Expert Committee on Food Additives, 2000*a*; Lange *et al*. 2001; Maume *et al*. 2001; Paris *et al*. 2001).

Assessment of exposure from consumption of cattle tissues

Although the Scientific Committee on Veterinary Measures Relating to Public Health (1999) has considered quantities of, and exposure to, hormonal residues in raw unprocessed meat and its products, similar concentrations are also suggested to occur in processed meat products also (Hartmann *et al*. 1998). Available information in the Scientific Committee on Veterinary Measures Relating to Public Health (1999) report estimates the theoretical maximum daily intake of excess E_2-17β + oestrone, Pr and T in meat from hormone-treated animals. The data for excess oestrogens indicate consumption of 1–84 ng/person per d (excluding values for pregnant heifers) with the median value of 6·8 ng/person per d. This value approximates to 0·22 % of the maximum ADI of 3 μg/d previously calculated for a 60 kg person. (Joint FAO/WHO Expert Committee on Food Additives, 2000*a*).

The Scientific Committee on Veterinary Measures Relating to Public Health (1999) also addressed the approach taken by the US Food and Drugs Administration (USFDA) in determining the acceptable level of exposure to hormonal compounds based on assumption of absence of physiological effect of consumption of 1 % or less of the daily synthesis in prepubertal human males (E_2-17β and Pr) or females (T), as appropriate. Minimum acceptable levels of E_2-17β determined as increments above those occurring in uncooked edible tissues of untreated heifers, steers or calves (Code of Federal Regulations, 2001) are as follows (ng/kg): muscle, 120; liver, 240; kidney, 360; fat, 480. Based on the JECFA methodology of determining intake of hormones from 500 g meat (300 g muscle; 100 g liver; 50 g kidney; 50 g fat), these USFDA values represent a currently acceptable daily consumption of E_2-17β of up to 102 ng. This value approximates to 1–2 % of the currently used values for calculated daily production rates of E_2-17β in prepubertal boys of 6 μg. It is interesting to note that Hartmann *et al*. (1998) suggest a much greater production rate including oestrone, of 100 μg.

Maume *et al*. (2001), using MS, have recently reported total concentrations ranging from 40–142 ng/kg for E_2-17α and E_2-17β in tissues of male castrated cattle untreated or implanted at 200 kg live weight with one or 'off-label' overdose of two or four implants containing 20 mg E_2-17β and 200 mg TBA. They calculated the contribution to the maximum ADI of 0·2 % for untreated animals, which increased to 3·9 % and 4·7 % for animals receiving two or four times the normal dose level. Lange *et al*. (2001) have reported responses to 3- and 10-fold the normal prescribed dose of oestradiol benzoate-containing implants, which exceeded USFDA increments (differences between oestradiol concentrations in implanted and non-implanted animals) by up to 1·2 μg/kg in liver and kidney samples. Values (ng/kg) for E_2-17β for the single 'approved' commercial implant averaged 3·0, 30, 70, 20 for loin, liver, kidney and peri-renal fat

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respectively. Based on the application of standard portions for these three tissues (Joint FAO/WHO Expert Committee on Food Additives, 1990), intakes of E_2-17β can be estimated as 9 ng/person per d, which is of a similar order to the median value of 6 ng identified for intake of E_2-17β and oestrone (Scientific Committee on Veterinary Measures Relating to Public Health, 1999). Daxenberger *et al*. (2001) have reported values of theoretical maximum daily intakes of 20 ng E_2-17β following good practice.

Andersson & Skakkebaek (1999) have argued that daily production and metabolic clearance rates of oestrogens (and androgens) derived originally for adult women and used for children including prepubertal boys should be corrected for differences in body size or surface area to avoid a suggested 2–3-fold overestimation in values. Greater sex hormone-binding globulin (SHBG) concentrations in children were also suggested to reduce metabolic clearance rates further.

The Scientific Committee on Veterinary Measures Relating to Public Health (1999) also addresses the question of measurement of 'true' concentrations of E_2-17β by reference to the development of a recombinant yeast bioassay (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 average values of 0·08 pg/ml for twenty-three prepubertal boys (mean age, 9·4 years) and 0·6 pg/ml for twenty-one prepubertal girls (mean age, 7·7 years). The results are used to suggest that concentrations of E_2-17β in blood of boys and prepubertal girls may be 12- and 100-fold less respectively than those derived from radioimmunoassay analysis and that intakes of oestrogen (which were measured by radioimmunoassay analysis) from oestradiol-treated animals may be greater than daily endogenous production rates, particularly in the context of USFDA values given earlier. In contrast, the Sub-group of the Veterinary Products Committee (1999) gives estimates of exposure to free and conjugated E_2-17β and oestrone based on radioimmunoassay methodology. Exposure for boys, for example, is given (μg/person per 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 Pr). This value, in excess of 100 μg, compares with an ADI for a 40 kg child of 2·0 μg (Joint FAO/WHO Expert Committee on Food Additives, 2000*a*).

Further and unresolved issues include the sensitivity of prepubertal children to low concentrations of oestrogen whether of endogenous or exogenous origin and differences between male and females (Andersson & Skakkebaek, 1999). Variation in oral bioavailability between, for example, ethinyl oestradiol and E_2-17β (60 and 10 %) is also indicated as important in highlighting the combined effects of absorption from the gastrointestinal tract and hepatic metabolism producing relative inactivation of the absorbed naturally occurring steroid. In considering the Scientific Committee on Veterinary Measures Relating to Public Health's (1999) opinion, the Sub-group of the Veterinary Products Committee (1999) has expressed concern about the inappropriateness of comparing values derived by differing analytical methodology. It is quite apparent that analytical methodology should be considered further (Zacharewski, 1997) to achieve the uniformity recommended by the Joint FAO/WHO Expert Committee on Food Additives (1990).

Genotoxicty, carcinogenicity and effects on growth, development and immunity

The Scientific Committee on Veterinary Measures Relating to Public Health (1999) has 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 to form chemical 'adducts' and cause effects outwith normal interaction with receptors. Studies cited in animal and cell culture models include those of Thibodeau *et al*. (1998),

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Hodgson *et al*. (1998) and Rajah & Pento (1995). The interpretation of these studies is questioned by the Sub-group of the Veterinary Products Committee (1999), which indicates that the tests used were not standard and had serious defects in methodology. In addition, the Sub-group of the Veterinary Products Committee (1999) cites a number of well-validated assays that gave negative results for genotoxicity of E_2-17β. More recently Russo *et al*. (2001, 2002) have provided evidence that E_2-17β, although less effective than the carcinogen benz(a)pyrene or diethylstilboestrol, produced genotypic changes in the cultured human breast epithelial (MCF-10F) ER-negative cell line. Although catechol-oestrogen formation was not demonstrated, the data indicate non-receptor-mediated effects on nuclear DNA, which produced chromosomal changes at loci similar to those observed in hyperplasia and carcinoma of breast tissues. These include loss of expression of tumour-suppressing or apoptosis-inducing gene products normally expected to induce death in cells affected by chemical alteration of nucleotide bases. The concentrations of E_2-17β used ranged from 0·007–0·25 nM and may be compared, for example, with the highest concentration of bioavailable E_2-17β plus oestrone (estimated at 2 % of total) of 0·040, 0·022 nM in young adult woman in menstrual follicular and luteal phase, respectively, and 0·006 nM in post-menopausal women (vom Saal & Finch, 1988).

Liehr (2000, 2001), in reviewing evidence for genotoxicity and mutagenicity of oestradiol following hydroxylation, has described chromosomal abnormalities, gene amplification, microsatellite changes and gene point mutations in cell culture or in the oestrogen-sensitive Syrian hamster kidney model *in vivo*. Mitosis of genetically damaged cells is suggested to be stimulated by classical hormone receptor–gene interactions such as those involving ER normally absent in proliferative cells but apparently expressed in pre-malignant cells, which also express a range of growth factors including epidermal growth factor, fibroblast growth factors and transforming growth factor-β, their receptors and certain prostaglandins (Richards *et al*. 2002). While many cells became ER unresponsive, responses in oestrogen-dependent cancers may be regulated by the relative 'oestrogenic' activity of individual oestrogens and metabolites (Hoogenboom *et al*. 2001). Utilising the human T47D breast cancer cell line the activities of E_2-17β and oestrone were shown to be similar and those for E_2-17α, the synthetic 17-hydroxy benzoate ester, 17-hydroxy palmitate ester of oestrone and 17-oleate ester of E_2-17β, less active by factors of 100, 3, 25 and 200 in comparison with E_2-17β. The 4-hydroxy metabolites of E_2-17β were 25–500 times more active than the 2-hydroxy metabolites, with the 4-methoxy-ester of oestrone having a 700-fold reduced potency. This latter result is also of interest since methylation of 4-OH E_2-17β by the enzyme catechol-*o*-methyl tranferase blocks entrance of this metabolite to the redox cycling pathway, preventing interaction with nucleotide bases of DNA (Joint FAO/WHO Expert Committee on Food Additives, 2000*a*). The presence of antioxidants such as ascorbic acid (Liehr & Wheeler, 1983) and phyto-oestrogens (Wiseman & Duffy, 2001) may also reduce the free radical induction by E_2-17β of tumours in sensitive tissues. Similarly, the presence of indole-3-carbinol, a component of Brassica vegetables may also decrease the incidence of mammary tumours by reducing production of 16α-hydroxy-oestrone, inhibiting expression of cyclin-dependent kinase 6 (Cram *et al*. 2001) and altering expression of genes regulating cell proliferation and apoptosis (Rahman & Sakar, 2002). The reduced catechol oestrogen formation of the synthetic oestrogen 17α-ethinyl oestradiol has also been associated with a decreased carcinogenic activity (Liehr, 2001).

Models that describe the non-receptor- and receptor-mediated activities of E_2-17β and its metabolites in cancer production and promotion have also been used to study the potentially endocrine-disrupting effects of exogenous oestrogens, frequently at supraphysiological doses, on reproduction and fertility. For example, prenatal treatment of mice with injected diethylstilboestrol (0·01–100 μg maternal BW per d; Newbold, 2001) and 17α-ethinyl oestradiol (up to

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2·0 μg/kg BW per d; Thayer *et al*. 2001) reduced fertility in female offspring and prostate growth and sperm production in male offspring, respectively. These results are consistent with reports of lesion development in reproductive tissues of daughters and, transgenerationally, small numbers of grandsons of women treated with orally active diethylstilboestrol during pregnancy (Klip *et al*. 2002). These effects were produced in response to dose levels prescribed during pregnancy as great as 150 mg/d (for example, see Dieckman *et al*. 1953).

In addition to activity in cell-based models, E_2-17β has been classified as carcinogenic in laboratory rodents and man (International Agency for Research on Cancer, 1987, 1999). The potential carcinogenicity of oestrogens is discussed by the Scientific Committee on Veterinary Measures Relating to Public Health (1999) and the Joint FAO/WHO Expert Committee on Food Additives (2000*a*) in terms of high dose levels and long periods of exposure arising from medical use in human subjects, for example, oral contraception, post-menopausal hormone replacement therapy and administration during pregnancy. Richards *et al*. (2002) have estimated that 60–70 % of human breast cancers may have a sex-hormone-related aetiology with approximately 60 % of those oestrogen-dependent. While epidemiological studies have associated breast cancer incidence positively with endogenous oestrogen concentrations, Hilakivi-Clarke *et al*. (2002) have recently suggested that the timing of exposure and effectiveness of DNA repair mechanisms are also important in determining whether oestrogens may increase, decrease or not affect the risk of cancer development. In determining assessment of risk the Joint FAO/WHO Expert Committee on Food Additives (2000*a*) preferred reference to oestrogens when used for hormone replacement therapy than for oral contraception. Oestrogen alone was considered to increase the incidence of cancer of the endometrium and breast, effects that were reduced by co-provision of progestagens. Typical dose levels effectively designed to replace endogenous production are given as 0·625 mg conjugated equine oestrogens/d or 1–2 mg E_2-17β/d.

The incidence of oestrogen-related cancers in the human population is a particularly serious matter and is a major reason given for the EU ban on the use of E_2-17β and derivatives in animal production (European Commission, 2001*a*). The contribution to the incidence of such cancers by consumption of 6–20 ng oestrogen/d in meat from oestrogen-treated animals is not known. In considering quantitatively the relative exposure of human consumers to oestrogens from all sources including meat, endogenous concentrations, including those occurring prepubertally and those arising from voluntary consumption of exogenous oestrogenic hormones such as in oral contraceptives or hormone replacement therapy (Joint FAO/WHO Expert Committee on Food Additives, 2000*a*), would appear to be of greater concern. However, it is also clearly undesirable for individuals with oestrogen-dependent tumours to consume exogenous oestrogens from whatever source.

The role of oestrogens in immune function in man and animals is briefly considered by the Scientific Committee on Veterinary Measures Relating to Public Health (1999) with no conclusion provided in relation to consumption of meat containing E_2-17β and its residues.

Testosterone

The Joint FAO/WHO Expert Committee on Food Additives (2000*a*) has summarised information on the use of T as a growth promoter and implications for its presence in the food of human consumers. The ADI is set at 0–2 μg/kg BW (140 μg/70 kg person) using an uncertainty factor of 1000 on a NOEL of 100 mg/person per d in a study of five eunuchs. The Scientific Committee on Veterinary Measures Relating to Public Health (1999), apparently in error, cites a different and lower ADI value of 0·2 μg/kg and 14 μg/70 kg person. All of these values are considerably in excess of the value of 189 ng/person highest excess exposure to T from hormone-

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treated beef (Scientific Committee on Veterinary Measures Relating to Public Health, 1999). MRL values are recommended as 'not specified' for T residues in bovine muscle, fat, kidney and liver, since they are considered safe for human consumers (Codex Alimentarius Commission, 2001). This conclusion is at variance with that of the Scientific Committee on Veterinary Measures Relating to Public Health (1999).

T is a 19-C steroid produced in testicular Leydig cells, thecal cells of the ovary and in the adrenal cortex. It has well-recognised androgenic properties, has an essential role in male animal reproduction (Joint FAO/WHO Expert Committee on Food Additives, 2000*a*) and can be converted to 5α-dihydrotestosterone (DHT), a more potent androgen, in certain tissues by the action of 5α-reductase with other metabolites formed following the action of other dehydrogenases and CYP. It also acts as a precursor for oestrogen synthesis following aromatisation and C19 demethylation. T and DHT form intracellular androgen-receptor complexes that affect nuclear gene expression in diverse tissues, such as the gonads and the dermis of the hair follicle (for example, see Rilianwati *et al*. 2000; Zhu *et al*. 2000). The majority (97–98 %) of transport in blood is effected by binding to carrier proteins serum albumin and SHBG, which may interact with SHBG receptors on target cell surfaces and mediate androgen–cell interactions (Joint FAO/WHO Expert Committee on Food Additives, 2000*a*). Its mode of action in altering skeletal muscle turnover in favour of greater anabolism remains only partially understood but may involve direct receptor mediation and/or indirect effects produced by somatotrophin and IGF-I with reductions in glucocorticoid receptor levels (Meyer, 2001) observed in cattle although not in sheep (Galbraith & Berry, 1994).

Assessment of exposure from consumption of cattle tissues

Post-pubertal males are considered to have the highest endogenous production rates (6500 μg/d) with values of between 140–240 μg/d for adult women and 65 and 32 μg/d for prepubertal boys and girls respectively (Hartmann *et al*. 1998). Orally administered T is known to have limited bioavailability in man because of rapid hepatic metabolism and excretion in bile or urine (Baird *et al*. 1969; Joint FAO/WHO Expert Committee on Food Additives, 2000*a*). The Scientific Committee on Veterinary Measures Relating to Public Health (1999) tabulates concentrations present in tissues following treatment of heifers and female calves with 200 mg implants of T. Concentrations were generally ranked fat>kidney>liver>muscle. Residues in treated animals were greater than those in untreated (for example, heifer muscle at day 30 gave values (ng/kg) of 102 *v.* 19·6 respectively) but were less than those present in muscle from untreated bulls (535 ng/kg) and pregnant heifers at 240 d gestation (418 ng/kg). Lange *et al*. (2001) have recently described maximum residues (630 ng/kg) in uncooked kidney and fat of Holstein x Friesian heifers treated 8 weeks before slaughter with 10-fold greater than normal 'off-label' dose levels of T-propionate plus E_2-benzoate. Consumption of, for example, 0·5 kg of these tissues represents an intake of 315 ng, which is less than the maximum ADI value of 140 μg for a 70 kg person by a factor in excess of 400. The Sub-group of the Veterinary Products Committee (1999) has calculated that the maximum daily excess exposure due to consumption of beef from cattle treated with 20 mg E_2-benzoate and 200 mg T-propionate is 9·2 ng for girls, which compares with total amounts in a normal diet of 40 ng and estimated endogenous production of 32 μg. Tolerance levels (values above those occurring in untreated animals) (μg/kg) for T in uncooked tissues of heifers and established by the USFDA (Code of Federal Regulations, 2001) are: muscle, 0·64; liver, 1·3; kidney, 1·9; fat, 2·6. Using these levels, consumption of the 'standard' quantities of 300 g muscle, 100 g liver, 50 g kidney and 50 g fat approximates to 600 ng/person per d exposure to exogenous T, which although greater than the maximum estimated excess exposure to T in meat from treated cattle of

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189 ng/person per d described by the Scientific Committee on Veterinary Measures Relating to Public Health (1999) is considerably below the maximum ADI of 140 μg. The USFDA acceptable maximum value of 600 ng/d represents 1–2 % of the estimated daily production rate of T of 32 μg/d for prepubertal girls. Over-estimation of the endogenous production as suggested for E_2-17β by Andersson & Skakkebaek (1999) increases the percentage of intake above that considered acceptable by USFDA. The nanogram values for intake of T from beef are small in comparison to milligram quantities of exposure effected by certain male contraceptive preparations. These include, for example, weekly subcutaneous injections of 200 mg testosterone enanthate and implants of up to 800 mg of T with or without a depot progestin. These are designed to release 6–9 mg/d to induce azoospermia (Handelsman *et al*. 1996).

Genotoxicity, carcinogenicity and effects on growth, development and immunity

The Scientific Committee on Veterinary Measures Relating to Public Health (1999) addresses the same questions of toxicology for T as for E_2-17β. T is considered a possible carcinogen in man (International Agency for Research on Cancer group 2A) based on convertibility to E_2-17β and its metabolites, which are considered to be genotoxic (Joint FAO/WHO Expert Committee on Food Additives, 2000*a*; Liehr, 2001; Russo *et al*. 2001, 2002). The Joint FAO/WHO Expert Committee on Food Additives (2000*a*) concluded that there was only limited evidence for carcinogenic effects in man. Androgens are also considered generally, but not exclusively, to have inhibitory effects on oestrogen, or chemically induced mammary tumours following reports of pro-cancer synergy (Liao & Dickson, 2002). Quantitative relationships between dose level of chemical agents and hormones in the production of these responses have not been determined.

Elevations in CYP19 aromatase expression have been observed in or close to human breast tumour sites (Richards *et al*. 2002) suggesting an increased capacity to synthesise oestrogens from androgen precursors. Studies by these authors of a range of growth factors in a number of normal and oestrogen-responsive and -unresponsive breast cancer cell lines demonstrated variable elevation of aromatase activity and cyclo-oxygenase-1 and -2 gene expression in breast epithelial and stromal cells. The results suggested the involvement of prostaglandins such as prostaglandin E_2 in the autocrine and paracrine regulation of oestrogen synthesis in epithelial and stromal cells respectively.

Effects of T on growth and reproduction are predictable on the basis of knowledge of its role in man and animals. These include effects on the hypothalamic–pituitary–gonadal axis, muscle, bone mass and behaviour (Joint FAO/WHO Expert Committee on Food Additives, 2000*a*). There are inadequate data to suggest any deleterious effects on immune function (Scientific Committee on Veterinary Measures Relating to Public Health, 1999). T and its derivatives are also used by human athletes to enhance skeletal muscle accretion (Bhasin *et al*. 2001).

Progesterone

The maximum ADI for Pr was set at 30 μg/d using an uncertainty factor of 100 and lowest observable effect level of 200 mg/person per d (3·3 mg/kg BW per d at 60 kg BW) in a human uterine test system for a fine-particle preparation of the steroid (Joint FAO/WHO Expert Committee on Food Additives, 2000*a*). MRL values were set as ‘not specified’ for bovine muscle, fat, kidney and liver indicating the conclusion of the committee that residues did not present a concern for the health of human consumers.

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Pr is a 21-C steroid hormone. It has an obligate role in the regulation of the female reproductive cycle and, in association with oestrogens, preparation for, and maintenance of, pregnancy in man and animals (Joint FAO/WHO Expert Committee on Food Additives, 2000*a*). It acts to decrease the sensitivity of the uterus to oxytocin. Production rates from tissues such as gonad, placenta and adrenal cortex have been estimated: (a) in pre-menopausal women at 418 μg/d in the follicular phase of the reproductive cycle and 94 000 μg/d in late pregnancy; (b) in post-pubertal men at 416 μg/d. The action of Pr is mediated by both 'classical' and 'non-classical' receptor–nuclear gene interactions (El Hefnawy *et al*. 2000) with the additional possibility of inhibition of the oxytocin receptor by a direct binding mechanism (Grazzini *et al*. 1998).

Assessment of exposure from consumption of cattle tissue

Residues in cattle include the parent compound (frequently at highest concentrations) and a range of metabolites. The Scientific Committee on Veterinary Measures Relating to Public Health (1999) compares concentrations (μg/kg, 60 d after implantation) of residual Pr in steers either untreated or treated with 200 mg Pr respectively, as follows: 0·27 *v*. 0·4; 0·26 *v*. 0·35; 0·17 *v*. 0·20 and 2·48 *v*. 3·40 for muscle, liver, kidney and fat respectively with values for pregnant heifers much greater at 10·1, 3·42, 6·19 and 239. Maximum permitted concentrations (μg/kg) of Pr in uncooked tissues of steers and calves above those of untreated animals and according to USFDA (Code of Federal Regulations, 2001) are as follows: muscle, 3; liver, 6; kidney, 9; fat, 12. Applying the 'standard' values for an intake of 500 g beef/d results in an estimated exposure to Pr of 2·6 μg/person per d. This quantity is 1–2 % of the estimated daily production rate for prepubertal boys of 150 μg/d (although this value may be an overestimate according to Andersson & Skakkebaek (1999)), and approximates to 8·7 % of the maximum ADI of Pr arising from consumption of meat from hormone-treated cattle. The Sub-group of the Veterinary Products Committee (1999) also provides examples of the maximum exposure of a range of human consumers to Pr with values (μg/d) for boys given as 150 (endogenous); 8·9 (normal diet); 0·024 (residues in beef) and with up to 600 mg/d for women in contraception or hormone replacement therapy (Joint FAO/WHO Expert Committee on Food Additives, 2000*a*). In man, oral Pr is inactivated in the digestive tract or rapidly absorbed, transported in blood by corticosteroid-binding globulin and albumin (2–3 % bioavailable), metabolised extrahepatically and hepatically by CYP and other enzymes, mainly to more polar forms such as 5β-pregnane-3α-o1–20α-diol-glucuronide, and excreted in urine (Joint FAO/WHO Expert Committee on Food Additives, 2000*a*).

Genotoxicity, carcinogenicity and effects on growth, development and immunity

Pr is associated with increased incidence of tumours of the mammary gland and reproductive tract of laboratory animals (Joint FAO/WHO Expert Committee on Food Additives, 2000*a*). However, this committee considered that Pr was not mutagenic and, on balance, had no genotoxic potential. The Scientific Committee on Veterinary Measures Relating to Public Health (1999) considered that evidence for carcinogenicity in man was inadequate despite widespread usage of Pr-containing or progestogen-only contraceptive preparations. On the basis of laboratory animal data, the International Agency for Research on Cancer allocated progestogen-only contraceptives to group B (possibly carcinogenic to man). More recent

studies, based on knowledge of cellular signal transduction pathways, have implicated a role for both Pr and oestrogen receptors in the development of breast cancer and the change from a hormone-dependent to -independent state (Rahman & Sarker, 2002). Effects of Pr on reproduction may be expected on the basis of known biological effects on the hypothalamic–pituitary–gonadal-axis. There is evidence to suggest immunosuppression in animals (for example, during pregnancy), but insufficient to assess the effects of Pr residues from meat of treated animals based on these parameters (Scientific Committee on Veterinary Measures Relating to Public Health, 1999).

Trenbolone acetate

TBA, the 17-acetylated ester of trenbolone (TB)-17β-OH (17β-hydroxyestra 4, 9, 11 triene-3, one), is a synthetic xenobiotic steroid. Risk assessment requires consideration of the presence of any residues in meat or meat products. The maximum temporary ADI value for TB-17β-OH has been set at 0·01–0·02 μg/kg BW per d (1·4 μg/kg bovine meat per 70 kg person) based on an uncertainty factor of 100 and NOEL of 2 μg/kg BW per d for sex-related indices in male pigs given TBA orally (Joint FAO/WHO Expert Committee on Food Additives, 1988, 1989). MRL (μg/kg) were set at 2 (TB-17β-OH) and 10 (TB-17α-OH) for muscle and liver respectively. Major metabolites include TB-17α-OH, suggested to have 10 % of the hormonal activity of the 17β-OH isoform and excreted in bile, and conjugated glucuronides also excreted in urine and bile. Neuman (1975) estimated that TBA had a 15–30 times greater androgenic and about 50 times greater anabolic activity than T in the castrated male rat. Bauer *et al*. (2000) have recently shown that TB-17β-OH had a similar binding affinity to DHT for the recombinant human androgen receptor, with values for metabolites TB-17α-OH and trendione approximating to less than 5 % of the 17β-OH isoform. Similarly, TB-17β-OH was shown to have a greater affinity for the bovine Pr receptor than Pr (1:1·37) and to compete poorly with DHT for the human SHBG.

Given alone, TBA had similar limited anabolic activity to T in stimulating growth and carcass protein deposition in castrate male sheep although TBA did exhibit up to 50 % greater apparent androgenic activity in increasing weight of sex hormone-sensitive tissues (Galbraith *et al*. 1990, 1997*)*. Combinations of T and TBA treatment showed additive effects for those androgenic indices. TBA also variably reduced the 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. In addition TB-17β-OH has also been shown to exhibit progestagenic activity (Karg & Meyer, 1999), compete for corticosteroid receptors (Hancock *et al*. 1991) and to reduce glucocorticoid receptor binding capacity (an effect different to that produced by T or nandrolone) (Sharpe *et al*. 1986; Galbraith & Berry, 1994). These latter effects may be associated with reductions in protein turnover rates in skeletal muscle as first observed in a rodent model (Vernon & Buttery, 1978). Treatment of steers with E_2-17β combined with TB-17β-OH has been shown to increase IGF-1 and IGF-2 concentrations in serum, which in turn stimulates proliferation of skeletal muscle satellite cells *in vitro* (Preston, 1999).

Assessment of exposure from consumption of cattle tissues

The Sub-group of the Veterinary Products Committee (1999) gives examples of maximum extractable concentrations of TB-17α-OH in tissues of steers implanted with 40 mg E_2-17β and

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200 mg 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. Concentrations of a similar order were reported by Hendricks *et al*. (2001) in tissues of steers treated with 200 mg TBA, 15 or 30 d before slaughter. Safe concentrations (μg/kg) for total TB residues in uncooked tissues of cattle as determined by USFDA (Code of Federal Regulations, 2001) are 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 (maximum ADI of 0·7 μg at 70 kg BW and intake of 500 g meat) (Joint FAO/WHO Expert Committee on Food Additives, 1988) and calculate to a maximum intake of about 43 μg/d using the standard values for intake of muscle, liver, kidney and fat. Lange *et al*. (2001) variously describe the exceeding of threshold values of TB-17α-OH, TB-17β-OH and trendione in liver in heifers given an 'off-label' 10-fold normal dose of TBA and following a 3-fold normal dose in one animal. Interaction of TBA with beef tissues is well recognised to produce non-extractable covalently bound residues, which may be water-solubilised following treatment with proteolytic enzymes (Karg & Meyer, 1999).

Genotoxicity, carcinogenicity and effects on growth, development and immunity

Lamming (1987) considered methodology to evaluate the safe use for human consumers of the synthetically produced xenobiotic compounds TBA and Z in meat production systems. The Scientific Committee on Veterinary Measures Relating to Public Health (1999) has summarised mutagenicity and genotoxicity data for TB-17β-OH and TB-17α-OH that gave similar results in a range of cellular and sub-cellular tests. Karg & Meyer (1999) drew attention to the predominance of negative findings, but also to those, for example, of Schiffmann *et al*. (1985) in which positive effects were recorded. The consequences for human consumers of consumption of covalently bound residues including chemical association to DNA has not been fully evaluated (Karg & Meyer, 1999). High doses (0·9–9 mg/kg BW per d) of TBA have also been shown to produce liver hyperplasia and tumours in mice with some evidence of pancreatic abnormalities in rats (Joint FAO/WHO Expert Committee on Food Additives, 1988) and hepatic abnormalities in guinea-pigs (Zarkawi *et al*. 1991*b*). However, a 2-year carcinogenicity bioassay study in rats apparently did not confirm evidence of carcinogenicity of TB-17β-OH (Schiffmann *et al*. 1988; Scientific Committee on Veterinary Measures Relating to Public Health, 1999). The Scientific Committee on Veterinary Measures Relating to Public Health (1999) concluded that current evidence is insufficient to complete a quantitative assessment of risk. A more recent report by Metzler & Pfeiffer (2001) reviewed current information on the genotoxic potential of TB-17β-OH and metabolites using a range of *in vitro* tests. Negative results were obtained for the hypoxanthine phosphoribosyl transferase system in V79 cells and at the *LACl* gene locus in *Escherichia coli*. Marginally positive results were obtained for micronuclei induction in V79 cells (50–125 μM concentration) and in DNA adduct formation (3–30 μM concentration) in rat hepatocytes. Results for TB-17α-OH were also reviewed by these authors with no clear conclusion presented for this metabolite also. The Scientific Committee on Veterinary Measures Relating to Public Health (1999) also summarised a range of studies that described effects on reproduction typical of those expected from a compound with androgenic sex hormone activity. The Scientific Committee on Veterinary Measures Relating to Public Health (1999) concludes that there are insufficient data to relate dose level of TB-OH residues to level of increased risk for human consumers, including those to the immune system.

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Zeranol

The maximum ADI value for Z is 0·5 μg/kg BW based on a NOEL in ovariectomised female cynomolgus monkeys of 0·05 mg/kg BW per d and safety factor of 100 (Joint FAO/WHO Expert Committee on Food Additives, 1988). MRL of 2 and 10 μg/kg respectively were set for bovine muscle and liver (Codex Alimentarius Commission, 2001).

Z (α-zearalanol) is a non-steroidal resorcylic acid lactone derivative of the fungal product zearalenone. It is not endogenously produced in animals but may be ingested in contaminated feed. It has well-recognised oestrogenic activity. The relative binding to ER has been shown as up to 20 % that of the E_2-17β with metabolites zearalenone and taleranol less active than Z (Lamming, 1987) and of a similar order to diethylstilboestrol (Fitzpatrick *et al*. 1989). The oestrogenic potency of Z and a range of other oestrogens has been recently described using a novel recombinant yeast bioassay expressing human or rainbow trout ER and a carcinoma (uterine) Ishikawa cell line (Le Guevel & Pakdel, 2001).

Assessment of exposure from consumption of cattle tissues

The Scientific Committee on Veterinary Measures Relating to Public Health (1999) summarised residue concentrations (μg/kg) determined by radioimmunoassay 70 d after implantation of 36 mg Z in male and female cattle as follows: kidney, 0·13–0·16; liver, 0·20–0·30; muscle, 0·13–0·72; fat, 0·073–0·184. It was concluded that mean residue levels (μg/kg) as Z equivalents and following good practice did not exceed 0·2 in muscle, 10·0 in liver, 2·0 in kidney and 0·3 in fat. Lange *et al*. (2001) have reported dose-dependent concentrations in tissues of heifers, which were greatest (at 8·1 μg/kg) in liver from animals treated 8 weeks before slaughter with an 'off-label' 10-fold greater dose than normally prescribed. This value is less than the acceptable tolerance value of 10 μg/kg for Z in bovine liver (Joint FAO/WHO Expert Committee on Food Additives, 1988). The mean value for single dose treatment was considerably less at 0·6 μg/kg.

'Safe' concentrations (μg/kg) set by the USFDA (Code of Federal Regulations, 2001) for residues of Z in uncooked tissues of cattle are as follows: muscle, 150; liver, 300; kidney, 450; fat, 600. On this basis and applying standard values for intake, the maximum acceptable daily ingestion of Z approximates to 128 μg/person per d and exceeds the Joint FAO/WHO Expert Committee on Food Additives (1988) ADI value of 35 μg for a 70 kg person, by a factor approaching 4. The Sub-group of the Veterinary Products Committee (1999) suggests that the ingestion of standard portions of dietary components by adults produces an intake of 0·169 μg/person per d, which is 0·56 % of the ADI.

Genotoxicity, carcinogenicity and effects on growth, development and immunity

The Scientific Committee on Veterinary Measures Relating to Public Health (1999) concluded that there was inadequate information to evaluate the mutagenicity and genotoxicity of Z. Some evidence was presented for a positive response in a DNA-repair recombination assay in *Bacillus subtilis*, but two other tests were negative. Other workers (Coe *et al*. 1992) have reported the production of hepatic tumours in Armenian hamsters following subcutaneous implantation of Z at high dose levels approximating to 360 mg/kg BW or 36 mg/animal. This dose is typical of that given to cattle. More recently, evidence has been reviewed for the genotoxicity of Z and metabolites in a range of systems including induction of hypoxanthine phos-

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phoribosyl transferase and *LAC1* mutations in cultured V79 cells and *E. coli* respectively, induction of micronuclei in V79 cells and adduct formation in cultured primary rat hepatocytes (Metzler & Pfeiffer, 2001). A 'marginally positive' response for Z and its major metabolites α-zearalanone and taleranol in the micronucleus induction test was reported at concentrations approaching the cytotoxic. Z is known to exert typical oestrogenic effects on reproductive parameters in animals (Joint FAO/WHO Expert Committee on Food Additives, 1988). For example, exposure prepartum of male mice to Z (150 μg/kg maternal BW at day 9 and 10 of gestation) has been shown to cause testicular abnormality postpartum (Perez Martinez *et al*. 1997). The Scientific Committee on Veterinary Measures Relating to Public Health (1999) considered that no estimate could be made of a dose–response relationship either for reported effects or for the possible risks of exposure to Z residues in meat or meat products.

Melengestrol acetate

MGA is a synthetic progestagenic compound that has been used mainly in North America to promote growth in female cattle. Since the publication of the Scientific Committee on Veterinary Measures Relating to Public Health's (1999) opinion, the Joint FAO/WHO Expert Committee on Food Additives (2000*b*) have reviewed many aspects of its biochemistry and toxicology and established 'temporary' maximum residue levels (μg/kg) of 2·0 for liver and 5·0 for fat. The maximum ADI value was set at 0·03 μg/kg BW based on the minimum effective dose of 5 μg/kg BW in a female cynomolgus monkey model system and a safety factor of 200. A tolerance level of 25 μg/kg in fat tissue has been applied by USFDA (Code of Federal Regulation, 2001). The major effect of MGA is the suppression of oestrus in female cattle. It is considered to maintain tonic luteinising hormone secretion and to act with follicle-stimulating hormone on the ovarian follicle to stimulate oestrogen production and the growth-promoting effect. Hageleit *et al*. (2000) have determined responses in heifers to oral doses of MGA ranging from 0·5–5·0 mg/d (normal inclusion level is 0·25–0·5 mg/d).

Concentrations in blood of Pr were shown to decrease at all dose levels and those for E_2-17β-OH, on the 0·5 mg/d dose, increased from 1 to 5 ng/l and were decreased by higher dietary inclusion levels. Three-fold increases of E_2-17β-OH in fat were described following MGA treatment.

Based on earlier studies including inhibition of menses in normal ovulating women (7·5–10 mg/d effective; 5 mg/d, not effective), a minimum effective daily dose level of 0·7 mg and no effect daily dose of 0·4 mg were calculated for MGA in women (Lauderdale *et al*. 1977). Data on pharmokinetics and biotransformation of MGA to a number of unidentified metabolites in cattle and human subjects have been established (Joint FAO/WHO Expert Committee on Food Additives, 2000*b*).

Genotoxicity, carcinogenicity and effects on growth, development and immunity

The data on mutagenicity and carcinogenicity are considered by the Scientific Committee on Veterinary Measures Relating to Public Health (1999) to be inadequate to assess carcinogenic potential of MGA and to be affected by lack of application of contemporary methodology (Joint FAO/WHO Expert Committee on Food Additives, 2000*b*). The effects of MGA in inhibiting normal menstrual and oestrous cycles are well characterised. Studies on the binding characteristics of MGA for receptors and carrier proteins by Bauer *et al*. (2000) showed a 5·3-fold greater

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affinity of MGA than Pr for the bovine progestin receptor with the relative affinity values for major metabolites ranging from 28 % to 85 % that of Pr. MGA also exhibited a low affinity for the human SHBG in comparison with DHT. Metzler & Pfeiffer (2001) have recently reported the absence of genotoxic potential of MGA in recognised *in vitro* test systems, but described the presence of an apoptosis-inducing contaminant in a commercial MGA formulation.

Discussion

The Scientific Committee on Veterinary Measures Relating to Public Health (1999, 2002) and the Sub-group of the Veterinary Products Committee (1999) refer to the importance of good veterinary practice in assessing the risk to consumers of beef from hormone-treated animals. Any deviation from good practice whether from wrongly sited implants, incorrect dose, or any other illegal application must render the meat endproduct unacceptable for human consumption. It is a conclusion of the present review that a culture of strict quality assurance should be applied to satisfy regulatory bodies and human consumers concerned about the quality of their food. Delivery of quality assurance necessitates effective surveillance involving random testing of meat samples, competent analysis of residues and traceability schemes such as currently applied under EU Regulation (EC)1760/2000 (for example, see Department for Environment, Food and Rural Affairs, 2002). The application of a Hazard Analysis Critical Control Points system in the beef production process along with the development of more sophisticated biotechnological systems such as DNA profiling (Cunningham, 2000) would also improve surveillance methodology. The opportunities for improved methodology are complicated by commercial interests and differences in regulatory framework and surveillance in the international trading blocs. Other issues include the apparent presence of a 'black market' in the EU but not in the US where legal compounds are available (Stephany, 2000, 2001). Elimination of hazards associated with misuse may permit greater focus on science-based evaluation of intrinsic health risk for human consumers.

Major concerns about the EC ban following contemporary science-based risk assessment include the small concentrations of hormonal residues present in meat in comparison with ADI values established by JECFA and the Codex Alimentarius Commission and, particularly for E_2-17β, endogenous concentrations and production rates (Sub-group of the Veterinary Products Committee, 1999). Stephany (2000, 2001), for example, has compared the concentration levels of E_2-17β in 250 g steaks from 'hormone-free' and E_2-17β-treated cattle (less than 2·5 and 5 ng respectively) with 6·5 ng contained in 50 g hen egg. Much of the toxicological research on hormones and residues has not assessed responses in test systems that relate quantitatively to exposure of human consumers to the chemically complex endproducts of meat digestion, absorption and systemic transport. Factors frequently not taken into account include enterohepatic elimination and post-absorptive partitioning into concentrations of 'free' hormone available for uptake into tissues and less-available protein- or lipid-bound fractions. The recent compelling evidence for the genotoxic and tumourigenic activities of E_2-17β has not yet been placed in a context where quantitative assessment of risk can be made. For example, concentrations of E_2-17β and hydroxylated metabolites have not been measured and related to formation of adducts with DNA according to principles of chemical stoichiometry. Knowledge on the effectiveness of antioxidants, naturally occurring anti-tumour agents and DNA repair mechanisms in reducing genotoxicity also appears to be heavily qualitative at present. The question of whether pg or ng quantities of hormone in meat can induce effects in addition to those produced by μg quantities already present could also usefully be considered in the context of stoichiometry and underly-

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ing biology. Such an approach would assist in elucidating the 'molecular materiality' of the additional hormonal supply, remove associated uncertainty and support the application of the 'precautionary principle' on the basis of relative risk.

The difference in beef production practice between North America and the EU means that consumers of North American-derived beef have greater likelihood of exposure to residues of hormonal growth-promoting compounds than European counterparts. Given the 14-year time period since the original EU ban there is opportunity for further analysis to determine the incidence of endocrine-related cancers in appropriately selected cohorts of population in the two geographical regions. Such an approach is likely to be complicated by the presence of other hormonally-active 'endocrine disrupters' in food and water of human consumers, including steroidal excretory products of human and animal metabolism (Jégou, 2001; Schiffer *et al*. 2001) and perhaps even in topically-applied soap manufactured from beef tallow. Analysis of blood samples using methodology that compared endocrine characteristics of meat-eating men *v*. vegans (Allen & Key, 2000) could also determine profiles of steroid hormones in consumers of hormone- and non-hormone-treated beef.

In conclusion, it is apparent that the subject of hormone growth promoters in meat is complex with deficiencies in knowledge of biology and chemistry, differences in interpretation of conventional toxicological evaluation, and concerns for human health and implications for trade, contributing to the absence of a sociological consensus for consumers internationally.

Acknowledgements

The author acknowledges the helpful comments from Dr David Miller and Dr Naomi Hepburn in the preparation of this manuscript.

References

Allen NE & Key TJ (2000) The effects of diet on circulating sex hormone levels in men. *Nutrition Research Reviews* **13**, 159–184.

Anderson JJB, Anthony M, Messina M & Garner SC (1999) Effects of phyto-oestrogens in tissues. *Nutrition Research Reviews* **12**, 75–116.

Andersson A-M & Skakkebaek NE (1999) Exposure to exogenous oestrogens in food: possible impact on human development and health. *European Journal of Endocrinology* **140**, 477–485.

Baird DT, Horton R, Longcope C & Tait JF (1969) Steroid dynamics under steady state conditions. *Recent Progress in Hormone Research* **25**, 611–664.

Barnes S (2001) Oestrogens and their promiscuous receptors: confronting reality. *Biochemical Society Transactions* **29**, 231–236.

Bauer ERS, Daxenberger A, Petri T, Sauerwein H & Meyer HHD (2000) Characterisation of the affinity of different anabolics and synthetic hormones to the human androgen receptor, human sex hormone binding globulin and to the bovine progestin receptor. *APMIS* **108**, 838–846.

Bhasin S, Woodhouse L & Storer TW (2001) Proof of effect of testosterone on skeletal muscle. *Journal of Endocrinology* **170**, 27–38.

Brandt RT (1997) Factors affecting release rates and blood levels of hormones from steroidal implants. In *Impact of Implants on Performance and Carcass Value of Beef Cattle*, pp. 34–69 [FN Owens, DR Gill, G Dolezal, JB Morgan and GW Horn, organisers]. Stillwater, OK: Oklahoma State University.

Breier BH, Gluckman PD & Bass JJ (1988) The somatotrophic axis in young steers: influence of nutritional status and oestradiol-17β on hepatic high-and low-affinity somatotrophic binding sites. *Journal of Endocrinology* **116**, 169–177.

Burger HG (2000) Selective oestrogen receptor modulators. *Hormone Research* **53**, Suppl. 3, 25–29.

Codex Alimentarius Commission (2001) *Codex Alimentarius: Codex Standards for Veterinary Drug Residues in Food*. http://www.codexalimentarius.net/index-en.stm

Code of Federal Regulations (2001) *Title 21 – Food and Drugs. Part 556 – Tolerances for Residues of New Animal Drugs in Food*. http://www.access.gpo.gov/nara/cfr/waisidx_01/21cfr556_01.html

Coe JE, Ishak KG, Ward JM & Ross MJ (1992) Tamoxifen prevents induction of hepatic neoplasia by zeranol, an estrogenic food contaminant. *Proceedings of the National Academy of Science USA* **89**, 1085–1089.
Coulston F & Korte F (1976) *Anabolic Agents in Animal Production*. Stuttgart: Georg Thieme Publishers.
Cram EJ, Liu BD, Bjeldanes LF & Firestone GL (2001) Indole-3-carbinol inhibits CDK6 expression in human MCF-7 breast cancer cells by disrupting Sp1 transcription factor interactions with a composite element in the CDK6 gene promoter. *Journal of Biological Chemistry* **276**, 22332–22340.
Cunningham EP (2000) Traceability from farm to consumption. In *Proceedings of the 46th International Congress of Meat Science and Technology*, pp. 798–801 [J Lasta, editor]. Argentina: Buenos Aires.
Daxenberger A, Ibarreta D & Meyer HHD (2001) Possible health impact of animal oestrogens in food. *Human Reproduction Update* **7**, 340–355.
Department for Environment, Food and Rural Affairs (2002) *Compulsory Origin Labelling from 1 January 2002*. http://www.defra.gov.uk/foodrin/beeflab/beeflab.htm
Dieckman WJ, Davis ME, Rynkiewicz SH & Pottinger RE (1953) Does administration of diethylstilbestrol during pregnancy have therapeutic value? *American Journal of Obstetrics and Gynecology* **66**, 1062–1075.
Doren M (2001) Prevention of osteoporosis – role of hormonal replacement therapy and selective oestrogen receptor modulators. *Actuelle Rheumatologie* **26**, 187–194.
European Commission (1996) *Scientific Conference on Growth Promotion in Meat Production*. Proceedings. Brussels and Luxembourg: EC Commission D-G VI.
European Commission (2000) *Proposal for a Directive of the European Parliament and of the Council*. http://europa.eu.int/comm/food/fs/him/him0l_en.pdf???
European Commission (2001*a*) *Hormones – The Precautionary Principle – No to Cattle Growth Promoters*. http://www.fst.rdg.ac.uk/foodlaw/news/eu-01–20.htm
European Community (2001*b*) *Hormones in Bovine Meat*. http://europa.eu.int/comm/dgs/health_consumer/library/press/press57_en.pdf
El-Hefnawy T, Manna PR, Luconi M, Baldi E, Slotte JP & Huhtaniemi I (2000) Progesterone action in a murine Leydig tumour cell line (mLTC-1) possibly through a non classical receptor type. *Endocrinology* **141**, 247–255.
Enmark E & Gustafsson JA (1998) Estrogen receptor β – a novel receptor opens up new possibilities for cancer diagnosis and treatment. *Endocrine-Related Cancer* **5**, 213–222.
Fitzpatrick DW, Picken CA, Murphy LC & Buhr M (1989) Measurement of the relative binding affinity of zearalenone, α-zearalenol and β-zearalenol for uterine and oviduct estrogen receptors in swine, rats and chickens: an indicator of estrogenic potencies. *Comparative Biochemistry and Physiology* **94C**, 691–694.
Galbraith H & Berry AD (1994) Effect of naturally occurring and synthetic androgens on growth, body composition and muscle glucocorteroid receptors in wether lambs. *Animal Production* **58**, 357–364.
Galbraith H, Hatendi PR, Alderson EM & Scaife JR (1990) The effect of cimaterol and oestradiol-17β alone or combined on growth and body composition of wether lambs. *Animal Production* **51**, 311–320.
Galbraith H, Singh SB & Scaife JR (1997) Response of castrated male sheep to oestrogenic and androgenic compounds implanted alone or in combination. *Animal Science* **64**, 261–269.
Galbraith H & Topps JH (1981) Effect of hormones on the growth and body composition of animals. *Nutrition Abstracts and Reviews* **51B**, 521–540.
Grazzini E, Guillon G, Mouillac B & Zingg HH (1998) Inhibition of oxytocin receptor function by direct binding of progesterone. *Nature* **392**, 509–512.
Gustafsson JA (1999) Estrogen receptor β – a new dimension in oestrogen mechanism of action. *Journal of Endocrinology* **163**, 379–383.
Hageleit M, Daxenberger A, Kraetzl W-D, Kettler A & Meyer HHD (2000) Dose-dependent effects of melengestrol acetate (MGA) on plasma levels of oestradiol, progesterone and luteinizing hormone in cycling heifers and influences on oestrogen residues in edible tissues. *APMIS* **108**, 847–854.
Hall JM, Crouse JF & Korach KS (2001) The multifaceted mechanisms of estradiol and estrogen receptor signaling. *Journal of Biological Chemistry* **276**, 36869–36872.
Hancock DL, Wagner JF & Anderson DC (1991) Effect of estrogens and androgens on animal growth. In *Growth Regulation in Farm Animals*, pp. 255–298 [AM Pearson and TR Dutson, editors]. London: Elsevier Applied Science.
Handelsman DJ, Conway AJ, Howe CJ, Turner L & Mackey MA (1996) Establishing the minimum effective dose and additive effects of depot progestin in suppression of human spermatogenesis by a testosterone depot. *Journal of Clinical Endocrinology and Metabolism* **81**, 4113–4121.
Hartmann S, Lacorn M & Steinhart H (1998) Natural occurrence of steroid hormones in food. *Food Chemistry* **62**, 7–20.
Heitzman RJ (1976) The effectiveness of anabolic agents in increasing rate of growth in farm animals: Report on experiments in cattle. In *Anabolic Agents in Animal Production*, pp. 89–98 [F Coulston and F Korte, editors]. Stuttgart: George Thieme.
Hendricks DM, Brandt RT Jr, Titgemeyer EC & Milton CT (1997) Serum concentrations of trenbolone-17β and oestradiol-17 β and performance of heifers treated with trenbolone acetate, melengestrol acetate or oestradiol-17β. *Journal of Animal Science* **75**, 2627–2633.
Hendricks DM, Gray SL, Owenby JJ & Lackey BR (2001) Residues from anabolic preparations after good veterinary practice. *APMIS* **109**, 273–283.
Hilakivi-Clarke L, Cabanes A, Olivios, Kerr L, Bouker KB & Clarke R (2002) Do estrogens always increase breast cancer risk? *Journal of Steroid Biochemistry and Molecular Biology* **80**, 163–174.

Hodgson AV, Ayala-Torres S, Thompson EB & Liehr JG (1998) Estrogen-induced microsatellite DNA alterations are associated with Syrian hamster kidney tumorigenesis. *Carcinogenesis* **19**, 2169–2172.
Hoogenboom LAP, De Haan L, Hooijerink D, Bor G, Murk AJ & Brouwer A (2001) Estrogenic activity of estradiol and its metabolites in the ER-CALUX assay with human T47D breast cells. *APMIS* **109**, 101–107.
Hurst DR (1998) *Hormones: European Communities – Measures Affecting Meat and Meat Products*. http://www.ejil.org/journal/Vol9/No1/sr1g.html
Ibarreta D, Daxenberger A & Meyer HHD (2001) Possible health impact of phytoestrogens and xenoestrogens in food. *APMIS* **109**, 161–184.
International Agency for Research on Cancer (1987) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, supplement no. 7. Overall evaluations of carcinogenicity: an updating of IARC monographs volumes 1 to 42. Lyon: International Agency for Research on Cancer.
International Agency for Research on Cancer (1999) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, vol. 72. Hormonal contraception and post menopausal hormone therapy. Lyon: International Agency for Research on Cancer.
Joint FAO/WHO Expert Committee on Food Additives (1988) Evaluation of certain veterinary drug residues in food. *WHO Technical Report Series*, no. 763. Geneva: WHO.
Joint FAO/WHO Expert Committee on Food Additives (1989) Evaluation of certain veterinary drug residues in food. *WHO Technical Report Series*, no. 788. Geneva: WHO.
Joint FAO/WHO Expert Committee on Food Additives (1990) Evaluation of certain veterinary drug residues in food. *WHO Technical Report Series*, no. 799. Geneva: WHO.
Joint FAO/WHO Expert Committee on Food Additives (2000*a*) Toxicological evaluation of certain veterinary drug residues in food. *WHO Food Additive Series*, no. 43. Geneva: WHO.
Joint FAO/WHO Expert Committee on Food Additives (2000*b*) Toxicological evaluation of certain veterinary drug residues in food. *WHO Food Additive Series*, no. 45. Geneva: WHO.
Jégou B (2001) General discussion. Existing guidelines for the use of meat hormones and other food additives in Europe and USA. *APMIS* **109**, Suppl. 103, S551–S556.
Karg H & Meyer HHD (1999) Update evaluation of trenbolone acetate, zeranol and melengesterol acetate as growth promoters (considerations concerning the 'hormone issues' between the EU and USA at the WTO). *Archiv für Lebensmittel Hygiene* **50**, 28–37.
Kirk CJ, Harris RM, Wood DM, Waring RH & Hughes PJ (2001) Do dietary phytoestrogens influence susceptibility to hormone-dependent cancer by disrupting the metabolism of endogenous oestrogens? *Biochemical Society Transactions* **29**, 209–216.
Klein KO, Baron J, Colli JH, McDonnel DP & Cutler GB (1994) Estrogen levels in childhood determined by ultra-sensitive recombinant cell bioassay. *Journal of Clinical Investigation* **94**, 2475–2480.
Klip H, Verloop J, van Gool JD, Koster META, Burger CW & van Leeuwen FE (2002) Hypospadias in sons of women exposed to diethylstibestrol in utero: a cohort study. *Lancet* **359**, 1102–1107.
Lamming GE (1987) Scientific report on anabolic agents in animal production. *Veterinary Record* **121**, 389–392.
Lange IG, Daxenberger A & Meyer HHD (2001) Hormone contents in peripheral tissues after correct and off-label use of growth promoting implants in cattle: Effect of the implant preparations Finaplix-H, Ralgro, Synovex-H and Synovex Plus. *APMIS* **109**, 53–65.
Lauderdale JW, Goyings LS, Krzeminski LF, Zimbelman RG (1977) Studies of a progestogen (MGA) as related to residues and human consumption. *Journal of Toxicology and Environmental Health* **3**, 5–33.
Le Guevel R & Pakdel F (2001) Assessment of oestrogenic potency of chemicals used as growth promoter by in-vitro methods. *Human Reproduction* **16**, 1030–1036.
Liao DH & Dickson RB (2002) Roles of androgens in the development, growth and carcinogenesis of the mammary gland. *Journal of Steroid Biochemistry and Molecular Biology* **80**, 175–189.
Liehr JG (2000) Is estradiol a genotoxic mutagenic carcinogen? *Endocrine Reviews* **21**, 40–54.
Liehr JG (2001) Genotoxicity of the steroidal oestrogens oestrone and oestradiol: possible mechanism of uterine and mammary cancer development. *Human Reproduction Update* **7**, 273–281.
Liehr JG & Wheeler WJ (1983) Inhibition of estrogen induced renal carcinoma in Syrian hamsters by vitamin C. *Cancer Research* **43**, 4638–4642.
MacVinish LJ & Galbraith H (1988) The effect of implantation of trenbolone acetate and oestradiol-17β in wether lambs at two initial live weights on concentrations of steroidal residues and blood glucose, urea and thyroid hormones. *Animal Production* **47**, 75–85.
MacVinish LJ & Galbraith H (1993) A note on the concentrations of steroidal residues in tissues of mature female sheep implanted with trenbolone acetate. *Animal Production* **56**, 277–280.
Maume D, Deceuninck Y, Pouponeau K, Paris A, Le Bizec B & André F (2001) Assessment of estradiol and its metabolites in meat. *APMIS* **109**, 32–38.
Meissonnier E (1983) *Anabolics in Animal Production*. Paris: Office International des Epizooties.
Metzler M & Pfeiffer E (2001) Genotoxic potential of xenobiotic growth promoters and their metabolites *APMIS* **109**, 89–95.
Meyer HHD (2001) Biochemistry and physiology of anabolic hormones used for improvement of meat production. *APMIS* **109**, 1–8.
Meyer HHD & Rapp M (1985) Estrogen receptor in bovine skeletal muscle. *Journal of Animal Science* **60**, 294–300.

Nef S & Parada LF (2000) Hormones in male sexual development. *Genes and Development* **14**, 3075–3086.
Neuman F (1975) Pharmacological and endocrinological studies on anabolic agents. In *Anabolic Agents in Animal Production*, pp. 253–264 [F Coulston and F Korte, editors]. Stuttgart: Georg Thieme Publishers.
Newbold RR (2001) Effects of developmental exposure to diethylstibestrol (DES) in rodents: clues for other environmental estrogens. *APMIS* **109**, Suppl. 103, 261–271.
Nikov GN, Eshete M, Rajnarayanan RV & Alworth WL (2001) Interactions of synthetic oestrogens with human estrogen receptors. *Journal of Endocrinology* **170**, 137–145.
Olefsky JM (2001) Nuclear receptor mini review series. *Journal of Biological Chemistry* **276**, 36863–36864.
Owens FN, Gill DR, Dolezal G, Morgan JB & Horn GW (organisers) (1997) *Symposium: Impact of Implants on Performance and Carcass Value of Beef Cattle*. Stillwater, OK: Oklahoma State University.
Paris A, Goutal I, Richard J, Bécret A & Guéraud F (2001) Uterotrophic effect of a saturated fatty acid 17-ester of estradiol-17β administered orally to juvenile rats. *APMIS* **109**, 365–375.
Pennie WD, Aldridge TC & Brooks AN (1998) Differential activation by xenoestrogens of ERα and ERβ when linked to different response elements. *Journal of Endocrinology* **158**, R11–R14.
Pentikainen V, Erkkila K, Suomalainen L, Parvinen M & Dunkel L (2000) Estradiol acts as a germ cell survival factor in the human testis in vitro. *Journal of Clinical Endocrinology and Metabolism* **85**, 2057–2067.
Perez Martinez C, Ferreras Estrada MC, Garcia Iglebias MJ, BravoMoral AM, Espinosa Alvarez J & Escudero Diez A (1997) Effects of in utero exposure to nonsterosteroidal estrogens on mouse testis. *Canadian Journal of Veterinary Research* **61**, 94–98
Pfaffl MW, Lange IG, Daxenberger A & Meyer HHD (2001) Tissue specific expression pattern of estrogen receptors (ER): Quantification of ERα and ERβ mRNA with real-time RT-PCR. *APMIS* **109**, 345–355.
Preston RL (1997) Rationale for the safety of implants. In *Impact of Implants on Performance and Carcass Value of Beef Cattle*, pp. 199–203 [FN Owens, DR Gill, G Dolezal, JB Morgan and GW Horn, organisers]. Stillwater, OK: Oklahoma State University.
Preston RL (1999) Hormone containing growth promoting implants in farmed livestock. *Advanced Drug Delivery Reviews* **38**, 123–138.
Rahman KM & Sarkar FH (2002) Steroid hormone mimics: molecular mechanisms of cell growth and apoptosis in normal and malignant mammary epithelial cells. *Journal of Steroid Biochemistry and Molecular Biology* **80**, 191–202.
Rajah TT & Pento JT (1995) The mutagenic potential of antioestrogens at the HPRT locus in V79 cells. *Research Communications in Molecular Pathology and Pharmacology* **89**, 85–92.
Richards JA, Petrel TA & Brueggemeier RW (2002) Signaling pathways regulating aromatase and cyclooxygenases in normal and malignant breast cells. *Journal of Steroid Biochemistry and Molecular Biology* **80**, 203–212.
Rilianwati, Kero J, Paukku T & Huhtaniemi I (2000) Long term testosterone treatment prevents gonadal and adrenal tumorigenesis of mice transgenic for the mouse inhibin α promoter/simian virus T-antigen fusion gene. *Journal of Endocrinology* **166**, 77–85.
Roche JF & O'Callaghan D (1982) *Manipulation of Growth in Farm Animals*. Maryland: Martinus Nijhoff Publishers.
Russo J, Hu YF, Tahin Q, Mihaila D, Slater C, Lareef MH & Russo IH (2001) Carcinogenicity of estrogens in human breast epithelial cells. *APMIS* **109**, 39–52.
Russo, J, Lareef MH, Tahin Q, Hu Y-F, Slater C, Ao X & Russo IH (2002) 17β-estradiol is carcinogenic in human breast epithelial cells. *Journal of Steroid Biochemistry and Molecular Biology* **80**, 149–162.
Schiffer B, Daxenberger A, Meyer K & Meyer HHD (2001) The fate of trenbolone acetate and melengestrol acetate after application as growth promoters in cattle: environmental studies. *Environmental Health Perspectives* **109**, 1145–1151.
Schiffmann D, Hieber L, Schmuck G, Pecham R, Metzler M & Henschler D (1988) Trenbolone induces micronucleus formation and neoplastic formation in Syrian hamster fibroblasts but not in mouse C3H10TI/2 cells. *Archives of Toxicology* **62**, 49–53.
Schiffmann D, Metzler M, Neudecker T & Henschler D (1985) Morphological transformations of Syrian hamster embryo fibroblasts by the anabolic agent trenbolone. *Archives of Toxicology* **58**, 59–63.
Scientific Committee on Veterinary Measures Relating to Public Health (1999) Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health. Assessment of Potential Risks to Human Health from Hormone Residues in Bovine Meat and Meat Products. European Commission D-G XXIV. http://europa.eu.int/comm/food/fs/sc/scv/out21_en.pdf
Scientific Committee on Veterinary Measures Relating to Public Health (2000) Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health on 'Review of Specific Documents Relating to the SCVPH Opinion of 30 April 99 on Potential Risks to Human Health from Hormone Residues in Bovine Meat and Meat Products (adopted on 03 May 2000)'. http://europa.eu.int/comm/food/fs/sc/scv/out33_en.pdf
Scientific Committee on Veterinary Measures Relating to Public Health (2002) Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health on 'Review of Previous SCVPH Opinions of 30 April 1999 and 3 May 2000 on the Potential Risks to Human Health from Hormone Residues in Bovine Meat and Meat Products (adopted on 10 April 2002)'. http://europa.eu.int/comm/food/fs/sc/scv/out50_en.pdf
Sharpe PM, Buttery PJ & Haynes NB (1986) The effect of manipulating growth in sheep by diet or anabolic agents on plasma cortisol and muscle glucocorticoid receptors. *British Journal of Nutrition* **56**, 289–304.
Sirtori CR (2001) Risks and benefits of soy phytoestrogens in cardiovascular disease, cancer, climacteric symptoms and osteoporosis. *Drug Safety* **24**, 665–682.
Stefano GB, Prevot V, Beauvillain J-C, Cadet P, Fimiani C, Welters I, Fricchione GL, Breton C, Lassalle P, Salzet M &

Bilfinger TV (2000) Cell surface estrogen receptors mediate calcium dependent nitric oxide release in human endothelia. *Circulation* **101**, 1594–1597.

Stephany RW (2000) Hormones Found in Meat Samples from Regular Controls Within the European Union and from US Imports. http://www.chemical-awareness.com/news.php?nid=13&aid=286

Stephany RW (2001) Hormones in meat: different approaches in the EU and in the USA. *APMIS* **109**, Suppl. 103, s357–s364.

Sub-group of the Veterinary Products Committee (1999) Executive Summary and Critical Evaluation of the Scientific Reasoning and Method of Argument Adopted in SCVPH (1999). London: MAFF.

Thayer KA, Ruhlen RL, Howdeshell KL, Buchanan DL, Cooke PS, Preziosi D, Welshons WV, Haseman J & vom Saal FS (2001) Altered prostate growth and daily sperm production in male mice exposed prenatally to subclinical doses of 17α-ethinyl oestradiol. *Human Reproduction* **16**, 988–996.

Thibodeau PA, Bissonnette N, Bedard SK, Hunting D & Paquette B (1998) Induction by estrogens of methotrexate-resistance in MCF-7 breast cancer cells. *Carcinogenesis* **19**, 1545–1552.

Vernon BG & Buttery PJ (1978) Protein turnover in rats treated with trenbolone acetate. *British Journal of Nutrition* **36**, 575–579.

vom Saal FS & Finch CE (1988) Reproductive senescence: phenomena and mechanisms in mammals and selected vertebrates. In *The Physiology of Reproduction*, p. 2365 [E Knobil and J Neill, editors]. New York: Raven Press.

Wetteman RP & Lehman F (1997) Buller steers – causes and control. In *Impact of Implants on Performance and Carcass Value of Beef Cattle*, pp. 123–128 [FN Owens, DR Gill, G Dolezal, JB Morgan and GW Horn, organisers]. Stillwater, OK: Oklahoma State University.

Wiseman H & Duffy R (2001) New advances in understanding of the role of steroids and steroid receptors in disease. *Biochemical Society Transactions* **29**, 205–209.

Xie W, Barwick JL, Simon CM, Pierce AM, Safe S, Blumberg B, Guzelian PS & Evans RM (2000) Reciprocal activation of xenobiotic response genes by nuclear receptors SXR/PXR and CAR. *Genes and Development* **14**, 3014–3023.

Xie W & Evans RM (2001) Orphan nuclear receptors: The exotics of xenobiotics. *Journal of Biological Chemistry* **276**, 37739–37742.

Zacharewski T (1997) *In vitro* bioassays for assessing estrogenic substances. *Environmental Science and Technology* **31**, 613–623.

Zarkawi M, Galbraith H & Hutchinson JSM (1991*a*) Influence of trenbolone acetate, zeranol and oestradiol-17β implantation on growth performance and reproductive function in beef heifers. *Animal Production* **52**, 249–253.

Zarkawi M, Galbraith H & Hutchinson JSM (1991*b*). The action of trenbolone acetate, a synthetic anabolic agent on ovarian function in the guinea pig. *Laboratory Animals* **25**, 117–121.

Zhu LJ, Hardy MP, Inigo IV, Huhtaniemi I, Bardin CW & Moo-Young AJ (2000). Effects of androgen on androgen receptor expression in rat testicular and epididymal cells: a quantitative immunohistochemical study. *Biology of Reproduction* **63**, 368–376.