

Risks Associated with the Use of Hormonal Substances in Food- Producing Animals

Draft report of the Veterinary Products Committee

May 2005

Contents

Executive Summary	3
Introduction	3
Terms of Reference	3
Overview	3
Conclusions and recommendations	4
1 Introduction	7
1.1 Historical background	7
1.2 Conclusions of the SCVPH 2002 Opinion	9
1.3 Working Group: Terms of Reference	10
1.4 Working Group: Method of working	10
1.5 Overview of biological effects and health end points of concern	11
1.6 Practical use of oestradiol in cattle	14
2 Exposure to hormonally active substances	18
2.1 Analytical techniques: recent data/evaluation of SCVPH Opinion	18
2.2 Bioassays for screening: recent data/evaluation of SCVPH Opinion	19
2.3 Conclusions and recommendations	19
3 Bioavailability of hormonally active substances	19
3.1 Metabolic pathways: recent data/evaluation of SCVPH Opinion	19
3.2 Conclusions and recommendations	20
4 Cancer risks of oestrogenic substances	21
4.1 Breast cancer risk: recent data	21
4.2 Reduced breast cancer risk in future	22
4.3 In utero exposure and breast cancer risks	22
4.4 Oestrogen and the human gut	22
4.5 Conclusions	22
5 Altered gene expression by oestrogenic substances	23
5.1 Recent data/evaluation of SCVPH Opinion	23
5.2 Conclusions and recommendations	23
6 Genotoxic & mutagenic effects of oestrogenic substances	24
6.1 17 β -oestradiol: Recent data/evaluation of SCVPH Opinion	24
6.2 Testosterone & progesterone: Recent data/evaluation of SCVPH Opinion	26
6.3 Zeranol and trenbolone: Recent data/evaluation of SCVPH Opinion	26
6.4 Melengestrol acetate: Recent data/evaluation of SCVPH Opinion	26
6.5 Conclusions and recommendations	27
7 Developmental & reproductive effects of hormonally active substances	28
7.1 Recent data/Evaluation of SCVPH Opinion	28
7.2 Conclusions	31
8 Environmental impact of hormonally active substances	31
8.1 Recent data/Evaluation of SCVPH Opinion	31
8.2 Conclusions and recommendations	33
9 Other considerations	34
9.1 Formal risk assessment of hormonally active substances	34
9.2 Ban on over thirty-month cattle	34
10 Conclusions and recommendations	35
10.1 Current scientific evidence for or against adverse effects	35
10.2 Overall conclusions and recommendations	36
References and Bibliography	39
Glossary	43
Appendix A: Tables of ADIs set for hormonally active substances	47
Appendix B: The 17 EU-funded studies and related publications	48
Appendix C: Metabolism and pharmacokinetics of growth promoting hormones	53
Appendix D: Membership and Expertise of the VPC Working Group on Hormones	59

Executive Summary

Introduction

In April 1999, the European Commission published an Opinion of the Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) on the potential risks to human health from the residues in meat and meat products of hormonally active substances used for growth promotion purposes in cattle, in particular the six hormones, 17 β -oestradiol, testosterone, zeranol, progesterone, trenbolone acetate and melengestrol acetate. The SCVPH concluded that risks associated with the consumption of meat from hormone-treated cattle may be greater than previously thought. The Opinion expressed in the SCVPH report were subsequently assessed by a sub-committee of the UK Veterinary Products Committee (VPC), and the European Safety Working Group of the Committee for Veterinary Medicinal Products (CVMP). The VPC sub-committee and the CVMP Working Group reported their evaluations later that year (1999) and both groups were unable to support the SCVPH's conclusions. The UK Government accepted the view of the VPC, although it continued to fulfil its obligation to enforce the EU ban on the use of all hormonally active substances as growth promoters in food producing animals¹.

In the light of the 1999 evaluations by VPC and CVMP, SCVPH released a review of their Opinion in May 2000; this stated that their original conclusion did not need revising.

In early 1998 the European Commission sponsored 17 research studies to evaluate the health risks posed by eating hormone-treated meat and environmental effects of hormone use. Following completion of these studies, SCVPH released a second Opinion in April 2002 that confirmed the views of the first SCVPH Opinion and concluded that no amendments were justified. In response to this second Opinion, the UK Government asked the VPC to re-examine the scientific evidence for a ban on the use of hormones in food-producing animals, and a sub-committee of the VPC was formed in November 2002 (VPC Working Group on Hormones) to carry out this task. The VPC Working Group findings form the content of this report.

Terms of Reference

To evaluate the latest Opinion of the Scientific Committee on Veterinary measures relating to Public Health (SCVPH) dated April 2002 and advise on its conclusions, and to advise on whether the latest Opinion of the SCVPH, and the research studies on which it is based, addresses the conclusions reached in the report by the VPC Working Group published in October 1999.

Overview

Following a critical evaluation of the scientific reasoning and methods of argument adopted in the key papers and studies cited in the SCVPH 2002 Report, the Working Group were unable to support the conclusion reached by the SCVPH that risks associated with the consumption of meat from hormone-treated cattle may be greater than previously thought. The weight of evidence at present available suggests that likely levels of human exposure to hormonally-active substances in meat from treated animals would not be sufficient to induce any measurable physiological effect². In reaching this conclusion the Group acknowledges there are important gaps in the evidence base that preclude producing definitive risk assessments for 17 β -oestradiol or the other five hormonally active substances.

¹ The use of hormonal growth promoters in food producing animals, including the six hormonal substances covered in the 1999 SCVPH Report, has been banned in the European Community since 1988. Third countries [non-EU countries] are required to guarantee that no animals and no meat coming from animals to which these substances have been administered will be exported to the EU.

² As a worse-case example, it has been estimated that a postmenopausal woman eating a kilogram of meat (kidney) with the highest concentration of oestradiol detected (56 ng/kg) would experience an increased oestrogen level of 0.01% of average endogenous production.

Conclusions and recommendations

1. The Working Group were of the view that human exposure to residues of hormonally-active substances, including growth promoters in meat, could exert biological effects if exposure is at a sufficiently high level. Therefore, the two key issues are:
 - (i) determination of the dose-response for induction of biological effects by the hormonally-active substances in test animals and, ideally, humans in order to identify a Lowest Observable Effect Level (LOEL), and
 - (ii) determination of the level (and range) of the additional human exposure and uptake from eating meat from treated animals.
2. These determinations should be made in adults and in developing (fetal/neonatal) animals and humans to identify the most sensitive index of effect. These effects would be in addition to those occurring naturally due to endogenous hormones.
3. The research so far has provided some, but not all the basic, but essential information outlined above. Without it, no definitive conclusions can be drawn; although the weight of available evidence suggests that likely levels of human exposure to hormonally-active substances in meat from treated animals would not be sufficient to induce any measurable biological effect.
4. Specifically, it is very unlikely that the presence of 17 β -oestradiol and its metabolites in meat from treated animals would significantly increase the risk of adverse effects in consumers. This is due to their low concentrations in comparison with those arising from endogenous production and from other dietary sources. Any increase would be likely to be small in the context of the entire food basket.
5. In reaching these conclusions, the Working Group expressed a number of qualifications and reservations based on the current lack of evidence of a risk to humans. These included:
 - all scientific judgements made by the Working Group were based on the assumption that the consumer is exposed to residues at no greater concentrations than those that would be caused by the “correct” or “recommended” use of the exogenous hormones, be it for growth promotion or other permitted zootechnical uses or therapeutic purposes;
 - the Working Group understand that misuse of hormonally-active substances for growth-promotion was more likely than misuse for oestrus synchronisation or therapeutic uses; and
 - substances with hormonal action may be used in combination, both legally and illegally, while the toxicological and safety factors available (e.g. ADIs) only relate to single substances.
 - the Working Group had to decide what to do in the absence of information or where there was uncertainty of interpretation of information. One Member expressed the view that for the substances under consideration, there was a large element of uncertainty, so the precautionary principle should become the primary consideration. The many uncertainties associated with the current lack of knowledge could be addressed by further research where this was both feasible and affordable. The Working Group was unanimous that all uncertainties must be made clear, especially those that were considered crucial in the risk assessment process.

6. As has been noted in this report, and acknowledged in the SCVPH Opinion, there are important gaps in the evidence base that preclude producing definitive risk assessments for 17 β -oestradiol or the other five hormonally-active substances. Not all data gaps are equally important for the purposes of risk assessment and the Working Group highlighted a number that could improve future risk assessments. As an example, it would be helpful if the CVMP and JECFA could make available data on pharmacokinetics and metabolism of assessed compounds that were supplied in manufacturers' dossiers. This openness and transparency would allow greater public scrutiny of the facts and confidence in the hazard and risk assessments produced.
7. The Working Group felt that none of the basic issues could be addressed without a structured approach. There was a need to establish precisely the:
 - relationships between the potential use of growth-promoters (including over-use) and concentrations of residues in meat;
 - levels of exposure in consumers (i.e. taking account of intake, absorption, bioavailability and metabolism); and
 - dose-response relationships for the effects of the hormonally-active substances (and their metabolites) in experimental animals or in humans.
 - further data on lipoidal oestrogens, possible bioaccumulation and possible synergistic effects of cocktails of hormonal substances would also be desirable
8. The Working Group noted specific needs:
 - To establish in humans the detailed relationship between systemic exposure to specific hormonally-active substances and the amount of meat consumed from treated animals.
 - To establish in experimental animals the relationship between intake of hormonally-active substances, or their metabolites, and target-organ effects (selecting the likely most sensitive target organ depending on the nature of the activity of the compound). This study to be conducted for adults and then fetal and/or neonatal exposure to be considered.
 - To consider lipoidal esters of oestrogen in future studies of the possible passage of oestrogen in implants through cattle to humans. The bioavailability and metabolism of lipoidal esters following ingestion should be investigated to allow the biological significance of the oestrogens to be assessed.
 - To carry out studies to confirm whether the ADI for pre-pubertal boys could be exceeded if they consumed a standard³ 500g portion of meat from an animal that had been treated with a number of hormonal implants. If confirmed this would be of concern.
9. The following need to be established in order to improve future risk assessments:
 - the precise relationship between the potential use of growth-promoters and concentrations of residues in meat
 - levels of exposure in consumers

³ The JECFA veterinary hypothetical diet assumes daily consumption of 300g muscle, 100g liver, 50g kidney and 50g fat.

- dose-response relationships for the effect of hormonally active substances (and their metabolites) in experimental animals and humans
- the bioavailability, metabolism and possible bioaccumulation of lipoidal esters of oestrogen following ingestion of meat from implanted cattle
- the possible synergistic effects of cocktails of hormonal substances
- a validated technique to detect and assign low residual concentrations of oestradiol in the finished edible products to natural sources or implant residues.

1 Introduction

1.1 Historical background

The use of hormonal growth promoters in food-producing animals has been a sensitive issue of debate in the UK and elsewhere for several years. The UK Government states that decisions on policy must be made and underpinned in a transparent fashion on the best available and most robust scientific evidence with any uncertainties clearly identified.

In September 2002, Ministers asked the Veterinary Products Committee to consider the latest European Commission's Scientific Committee on Veterinary measures relating to Public Health (SCVPH) Opinion (April 2002) and other scientific evidence. Our observations and conclusions are set out in this report. The chronological sequence of events that led to the current position and commissioning of this report are as follows:

Hormonally active substances, such as diethylstilboestrol (DES) had been used for growth promotion since the early 1950's. Concerns about a possible risk of cancer from residues of such substances were raised in the early 1970's. The European Community introduced a ban on the use of DES in 1987 and banned the use of all hormonally-active substances as growth promoters in food producing animals in 1988. A similar condition was placed on all countries wishing to export meat from such animals to the European Community.

The United States and Canada objected to this ban to the World Trade Organisation (WTO). In 1997, the WTO Expert Panel found that the ban was not based on science – for example, on a risk assessment or on relevant international standards⁴.

The European Commission appealed against this ruling. In February 1998, the Appellate Body upheld the WTO Expert Panel's view, in that they found that the ban had been imposed without credible evidence to indicate that there were health risks posed by eating hormone-treated meat. The European Commission was given 15 months to remove the ban or produce a risk assessment.

In early 1998, the European Commission sponsored 17 research studies to respond to the findings in the Appellate Body report. These covered toxicological and carcinogenicity aspects, residue analysis, potential abuse and control problems and environmental effects of hormone use.

At the end of 1998, the SCVPH was asked to carry out an assessment of the risk to human health from the use of the six hormonally-active substances, particularly from residues from bovine animals where such substances were administered for growth promotion. There were: 17 β -oestradiol, testosterone, zeranol, progesterone, trenbolone acetate and melengestrol acetate. In April 1999, the SCVPH produced its first Opinion on the subject (SCVPH, 1999).

The SCVPH concluded that the risks from hormone-treated meat were higher than previously thought. It indicated that there was a significant body of evidence suggesting that 17 β -oestradiol should be considered a complete carcinogen. It also concluded, with different levels of evidence, that there were risks to consumers from the other 5 hormones examined.

Overall the SCVPH concluded that no threshold concentrations could be defined for the hormones - this precluded the setting of Acceptable Daily Intakes (ADIs) or Maximum Residue Limits (MRLs). However, they were unable to estimate the level of any risk.

The then Minister of Agriculture, Fisheries and Food, Nick Brown MP, asked the Veterinary Products Committee (VPC) to assess the evidence in the SCVPH Opinion. The VPC set up a sub-

⁴ The WTO Panel report numbers - WT/DS26/R/USA AND WT/DS48R/CAN

committee to do this, which reported in October 1999. The Safety Working Group of the Committee for Veterinary Medicinal Products (CVMP) – the European Commission’s own organisation with responsibility for advising on the safety of veterinary medicines - also examined the SCVPH Opinion.

The VPC sub-committee was unable to support the SCVPH’s conclusion that the risks associated with eating hormone-treated meat might be higher than previously thought. The Working Group also found that they had sufficient concerns about the scientific reasoning in a number of key areas, to throw serious doubt on the conclusions of the SCVPH. However, the Group identified a number of areas where additional expert evidence should be sought to add to the data and help prevent selective scientific conclusions being drawn in the future.

The CVMP also produced a report in 1999 in response to the SCVPH Opinion (EMEA/CVMP/885/99). The CVMP was unconvinced by the SCVPH data and arguments, and concluded that its (the CVMP) previous recommendations with regard to the ADIs and MRLs of the five hormones they had examined were still applicable (17 β -oestradiol, altrenogest, progesterone, flugesterone acetate and norgestomet). The CVMP also noted that its conclusions were practically the same as the FAO/WHO Joint Expert Committee on Contaminants and Food Additives (JECFA, 1999). Tables of ADIs are given in Appendix A.

The UK Government accepted the view of the VPC - that they were unable to support the conclusion of the SCVPH of a higher risk than previously thought from eating hormone-treated meat. The UK has, however, always fulfilled its obligations to enforce the EU ban.

In May 2000, the SCVPH released a review (SCVPH, 2000) of their Opinion having examined the reports of the VPC and CVMP. The SCVPH noted that the two evaluation reports showed a high degree of consensus on the possible risks. It did not seek to answer the questions raised in the reports, but concluded that they did not provide convincing data and arguments that demanded revision of the SCVPH’s previous conclusions. The SCVPH review also said that there were obvious gaps in the present knowledge on the hormones in relation to animal metabolism and residue deposition. They expected that the EU’s research programmes would provide additional data on these topics.

Following the completion of the 17 Studies sponsored by the European Commission (EC), the SCVPH were asked to review their previous Opinions of 1999, 2000, the data from the 17 studies and other recent scientific literature from any source. In April 2002, the SCVPH released another Opinion (SCVPH, 2002). This confirmed the views in the previous SCVPH Opinion and concluded that no amendments to these were justified. The SCVPH’s general and overall conclusions are given in the following section.

In September 2003, the European Parliament and Council of Ministers passed Directive 2003/74/EC⁵. This Directive puts further restrictions on the use veterinary medicinal products containing oestradiol or its ester-like derivatives. Originally, the intention was for all uses of 17 β -oestradiol to be banned and restrictions tightened on other hormones. The UK and other Member States expressed concerns about the loss of valuable therapeutic products.

This Directive now requires that oestradiol and its derivatives may not be used for oestrus induction/synchronisation in cattle, horses, sheep or goats after October 2006. These substances may still be authorised for the treatment of foetus maceration or mummification and the treatment of pyometra in cattle, or oestrus induction in cattle, horses, sheep or goats. However, the Directive requires the European Commission to present a report by October 2005 on the possible alternatives to oestradiol for these therapeutic uses.

⁵ Available at: http://europa.eu.int/eur-lex/pri/en/oj/dat/2003/l_262/l_26220031014en00170021.pdf

1.2 Conclusions of the SCVPH 2002 Opinion

“The review of the 17 studies launched by the European Commission and a recent scientific literature allows the following conclusions:

- Ultra-sensitive methods to detect residues of hormones in animal tissues have become available, but need further validation.
- Studies on the metabolism of 17β -oestradiol in bovine species indicate the formation of lipoidal esters, disposed particularly in body fat. These lipoidal esters show a high oral bioavailability in rodent experiments. Thus, the consequence of their consumption needs to be considered in a risk assessment.
- Experiments with heifers, one of the major target animal groups for the use of hormones, indicated a dose-dependent increase in residue levels of all hormones, particularly at the implantation sites. Misplaced implants and repeated implanting, which seem to occur frequently, represent a considerable risk that highly contaminated meats could enter the food chain.
- There is also a dose dependent increase in residue levels following the oral administration of melengestrol acetate at doses exceeding approved levels, with a corresponding increased risk that contaminated meats could enter the food chain.
- Convincing data have been published confirming the mutagenic and genotoxic potential of 17β -oestradiol as a consequence of metabolic activation to reactive quinones. *In vitro* experiments indicated that oestrogenic compounds *might* alter the expression of an array of genes. Considering that endogenous oestrogens also exert these effects, the data highlight the diverse biological effects of this class of hormones.
- No new data regarding testosterone and progesterone relevant to bovine meat or meat products are available. However, it should be emphasized that these natural hormones are used, only in combination with 17β -oestradiol or other oestrogenic compounds in commercial preparations.
- Experiments with zeranol and trenbolone suggested a more complex oxidative metabolism than previously assumed. These data need further clarification as they might influence a risk assessment related to tissue residues of these compounds.
- Zeranol and trenbolone have been tested for their mutagenic and genotoxic potential in various systems with different endpoints. Both compounds exhibited only very weak effects.
- Data on the genotoxicity of melengestrol acetate indicate only weak effects. However, pro-apoptotic effects were noted in some cell-based assays, which were attributed to the impurities in commercial formulation. Further experiments should clarify the toxicological significance of these impurities.
- Model experiments with rabbits treated with zeranol, trenbolone or melengestrol acetate, mirroring their use in bovines, were designed to study the consequences of pre- and perinatal exposure to exogenous hormones. All compounds crossed the placental barrier easily and influenced to varying degrees the development of the foetus, at the doses used in the experiments.
- Epidemiological studies with opposite-sexed twins, suggest that the exposure of the female co-twin *in utero* to hormones results in an increased birth weight and consequently an increased adult breast cancer risk
- Several studies were devoted to the potential impact of the extensive use of hormones on the environment. Convincing data were presented indicating the high stability of trenbolone and melengestrol acetate in the environment, whereas preliminary data were provided on the potential detrimental effects of hormonal compounds in surface water.

In conclusion, after re-appraisal of the data from the 17 studies and recent scientific literature, the SCVPH confirms the validity of its previous Opinions (in 1999 and 2000) on the Assessment of Potential Risks to Human Health from Hormone Residues in Bovine Meat and Meat Products, and that no amendments to those opinions are justified.” (SCVPH, 2002)

1.3 Working Group: Terms of Reference

The VPC has again been asked to examine the scientific evidence for a ban on the use of hormones in food-producing animals and to advise on whether therapeutic uses pose any risk to consumers. The advice from the VPC will be used to inform the UK position when the review of 17 β -oestradiol products takes place. The Working Group Terms of Reference are:

- to evaluate the latest Opinion of the Scientific Committee on Veterinary measures relating to Public Health (SCVPH) dated April 2002 and advise on its conclusions and
- to advise on whether the latest Opinion of the SCVPH, and the research studies on which it is based, addresses the conclusions reached in the report by the VPC Working Group published in October 1999.

1.4 Working Group: Method of working

In November 2002 the VPC, in conjunction with the VMD (which acted as Secretariat), set up a small Working Group consisting of a number of VPC members and a number of non-VPC scientific experts. The composition of the Working Group was intended to cover the areas of specialism needed and the membership and their expertise and experience are to be found in Appendix E.

The Working Group met on five occasions, the first of which was to clarify the remit of the exercise and to ascertain whether the Working Group covered the areas of expertise required. Two conclusions arose at this first meeting. Firstly, it was agreed that the 17 EU-funded reports and any ensuing publications on which the SCVPH had in part relied for their 2002 Opinion would need to be obtained and, secondly, because the SCVPH had addressed potential environmental impacts of hormonally active substances used in food production, we would also need to do so, although, strictly, it was beyond the remit of the SCVPH. The Working Group consulted an environmental expert.

In subsequent Working Group meetings, a report structure was agreed and tasks allocated to members. It was also agreed that, in addition to the SCVPH Opinion and the 17 EU-funded studies, other recent relevant scientific publications would be sought, evaluated and included, based on the knowledge and expertise of Working Group members. One particular point to note was the effort required for the VMD Secretariat to obtain the 17 EU-funded reports used by the SCVPH. The Working Group appreciated the persistence of the Secretariat that eventually enabled them to be obtained from the Commission. Full details of the 17 studies are given in Appendix B; individual studies are referred to in this report as studies 1 to 17. In some cases, only the study report submitted to the EC was available. In other cases, peer-reviewed publications were available, based on results within some of the study reports. Some of the study reports have not, as yet, been published in journals and thus have not been subject to the normal peer-review process.

The Contract reports from the 17 studies and their associated papers were scrutinized and evaluated by appropriate members of the Working Group. The Working Group noted that the 17 Studies were of limited value in the selection of topics that need to be covered. There was also a degree of repetition. New information is available in some areas, but there are still gaps that have been highlighted within earlier sections. Other gaps will be noted in later sections of this report.

The Working Group also agreed that, apart from a scientific evaluation of existing data, it was important to highlight significant gaps in knowledge and areas where uncertainty existed. This was felt to be particularly relevant as the Working Group was aware that it had no mandate to make, or even propose, on policy in this area; thus, it was crucial for the report to express uncertainties where they existed in the science base which would inform policy makers.

Another issue of concern within the Working Group was to ensure that the reader understood the various uses to which hormonal substances had been, or could be, used in bovine meat production and ensuing meat products. Their use as growth promoters may be regarded as zootechnical. This was the original use for which the EU ban was intended. Oestrus induction in cattle and horses is also a zootechnical use whereas other veterinary uses such as the treatment of pyometra are regarded as therapeutic.

Although the toxicological evidence of the substances under discussion here is based on studies on specific laboratory animals, the actual risk assessment to humans will be dependent on the dose used, the time of administration relative to slaughter, the pharmacological formulation and route of application to these meat-producing animals, and information on human consumption; questions of whether risks, however minimal, should be permitted - depending on whether the use is zootechnical, hence commercial or therapeutic, and so to the benefit of the health and welfare of the animal - are beyond the mandate of the Working Group.

The Working Group noted that illegal or improper use of growth-promoting substances, in the form of implants and/or in feed might present an added exposure to humans who consumed meat or meat products from animals so treated. However, this would be no different from the illegal or inappropriate use of any veterinary products and, as such, is beyond the remit of the Working Group. However, it is something that does need consideration.

1.5 Overview of biological effects and health end points of concern

Hormones are vital in normal development, maturation and physiological functioning of many vital organs and processes in the body. However, like any other chemicals of natural or synthetic origin, hormones may be toxic to living organisms under certain circumstances. The toxicity may be due to an excess of its normal ('physiological') action. This may be the result of excessive exposure to the substance, for example following absorption of a large dose, or because the physicochemical nature of the substance gives it greater (more 'potent') or more prolonged activity of the same type, or because the hormonal action (endocrine effect) occurs at an abnormal time during development or adult life, or is an action on an organism of the inappropriate sex. Hormones, like other chemicals, may also exert direct toxic actions not related to their endocrine ('physiological') effects.

Hormones are very 'active' substances, where small doses may have profound effects. However, this does not mean that they do not follow typical dose-response relationships, or that all their actions are necessarily permanent in the current or future generations. The relationship between the dose applied to an organism and the effect produced may be linear or it may follow a more complex relationship because it will depend on the extent of absorption of the substance, how it is transported, metabolised and excreted from the body ('pharmacokinetics') and its access across internal barriers to its sites of action. The severity and duration of hormone-induced toxicity will therefore be related to the local concentration at the target site in the body, at least above any possible minimum threshold for an effect and below the level at which the mechanism causing the effect becomes saturated and the action reaches its maximum. Many toxic actions are reversible once exposure ceases because normal physiological processes and repair mechanisms return the affected cells and organism to normality. However, irreversible damage may result if there is extensive damage to a tissue.

1.5.1. Hormones in context

Endogenously produced sex steroids exert a wide range of effects on the body with most tissues/organs affected to a greater or lesser degree (not just the reproductive organs). These effects

vary according to age and gender. Therefore, exposure to exogenous steroidogenically active compounds at certain levels has the potential to affect many organs.

Overwhelming evidence suggests that sex steroids exert effects that are dose-dependent and that a threshold dose exists, below which, no biological effect will occur. This threshold may vary according to age, gender and tissue/organ.

Production, bioavailability/metabolism and action of endogenous sex steroids is closely controlled. Exogenous exposure to synthetic sex steroids may therefore be compensated for such that no biological effect ensues.

During development, sex steroids play an *organisational* role that involves programming of various tissues/organs in a gender-specific way. These effects are largely irreversible (e.g. sexual differentiation of the external genitalia).

Differences in the level of exposure to sex steroids during development between individuals can occur naturally (e.g. higher exposure in twin pregnancies) and this may alter predisposition to future disease such as breast or testicular cancer.

Importantly, for the six hormonal substances considered, effects of concern would have thresholds, although they may be difficult to define. As noted above, because of 'feedback' systems within the body, the introduction into the body of an exogenous source of a sex steroid hormone may be compensated for, so that no biological effect is produced. However, this may not apply to the fetus, postmenopausal women or pre-pubertal children – thus making these groups potentially more vulnerable.

The major areas of concern relating to health effects of hormonal substances in bovine meat and meat products relate to **cancer, mutagenicity and reproductive effects**, in particular **endocrine disruption**. Generally, cancer and mutagenicity are well described, reasonably well understood by most readers and need little general description, though some background considerations are included in Sections 4 and 6 respectively. However, endocrine disruption has become, in recent years, an area where there has been concern about potential harmful outcomes for a wide range of chemicals hitherto unsuspected of causing such effects. Many of these are less well described and the Working Group felt it would be helpful to include a scene-setting description of endocrine disruption as a background against which to describe the most recent studies.

1.5.2 Endocrine disruption

Hormones such as androgens and oestrogens play important roles in the day-to-day function of the body. Effects are not restricted to the reproductive system but are pervasive, affecting most tissues in the body, including the brain, bone, muscle, liver, fat, cardiovascular and immune systems (WHO 2002). During development, androgens and oestrogens also play important organising effects in which the function of certain tissues may be permanently altered. The role of androgens in masculinising the male (reproductive system, genitalia, brain and rest of the body) during fetal life is the most dramatic example of this. It is well established that when there is inappropriate production or inhibition of normal androgen/oestrogen production/action, whether in fetal, childhood or adult life, then important health disorders are likely to occur (WHO 2002).

Against this background, it is understandable that there has been widespread concern about the potential health consequences that might result from human exposure to environmental chemicals that possess intrinsic oestrogenic, androgenic or anti-androgenic activity. In addition to xenobiotics, such effects are also attributed to phytoestrogens⁶. These chemicals have been loosely termed

⁶ Phytoestrogens are a group of chemicals produced naturally by certain edible plants. The commonest are the isoflavones (e.g. genistein, found in soybeans and legumes), coumestans (found in young sprouting legumes), lignans (found in linseed, many cereals, fruits and vegetables) and prenylated flavonoids (found in hops and

'endocrine disruptors', based on their potential to alter normal hormone action (Crisp *et al.*, 1988; Bolt *et al.*, 2001).

Whether or not this potential is realised in the body of the recipient is dependent on many factors, one of which is the potency of the chemical in question i.e. what dose of compound will exert a detectable effect? The oestrogenic potency of any ingested compound is determined by a combination of factors. These include:

- absorption, metabolism, entero-hepatic recirculation,
- binding to plasma proteins such as sex hormone-binding globulin (SHBG)⁷, and
- affinity for binding to either oestrogen receptor- α (ER α) or oestrogen receptor- β (ER β).

Other factors such as rates of breakdown of the compound, and local availability in particular tissues may also be important. It is not easy to predict from simple measurements of any one of these parameters what the overall oestrogenic potency of a compound will be in the whole animal. Most studies on the oestrogenic potency of compounds to which there is human exposure, have utilised *in vitro* cell transfection systems that assess the ability and affinity of the compound to compete for binding to oestrogen receptors, ER α or ER β . Compounds that have a high affinity for these receptors are likely to be potent oestrogens and may, therefore, exert biological effects on oestrogen target tissues. However, as other factors mentioned above can influence potency, studies involving *in vivo* administration of such compounds provide the most accurate guide as to whether or not they may have target tissue effects. The immature rat uterotrophic assay is the most widely used endpoint of oestrogen bioactivity *in vivo*. Though this provides perhaps the most useable measure of oestrogenic potency *in vivo*, activity in this assay does not necessarily mean that there would be similar biological activity at other oestrogen target sites, such as in the breast or in bone (Jordan 1998).

Most endocrine disruptors have only very weak intrinsic hormonal activity when compared with the natural (endogenous) hormones (testosterone, oestradiol) that are made within the body (WHO, 2002). It is also commonly forgotten that all hormonal systems in the body are tightly controlled and this usually involves a feedback 'balancing system' or systems that constantly check the levels of action of the hormone in question and adjusts its concentration up or down accordingly (WHO, 2002). Therefore, in theory, exposure to exogenous endocrine disruptors at a concentration sufficient to cause an effect should be compensated for by altered production of the endogenous hormone in question.

beer). Human dietary exposure can therefore be substantial, although very variable depending on the composition of the diet. A recent in-depth review of the evidence for both adverse and beneficial effects of dietary phytoestrogens concluded that much of the available data is equivocal and fails to distinguish between effects of the compounds themselves and effects of other dietary constituents (COT 2003). However, it was also emphasised that the nature and extent of any *potential* effects of phytoestrogens in the diet will depend critically on both the level of exposure and the age at exposure.

⁷ In humans, sex steroids do not circulate in the bloodstream in a readily bioavailable form. The majority (>95%) is bound to SHBG or other plasma proteins, such as albumin. An equilibrium exists between the amount of protein-bound and free sex steroid in plasma (Hammond 2002). Although endogenously produced sex steroids bind to SHBG, many synthetic steroidal compounds do not e.g. the potent oestrogens, diethylstilboestrol (DES) and ethinyl oestradiol. The possibility that ingested compounds may bind to SHBG and displace already bound endogenous sex steroid, thus making the latter bioavailable, must also be considered. Theoretically, a compound with minimal or no intrinsic oestrogenicity itself could induce oestrogenic effects if it was able to bind with higher affinity than oestradiol to SHBG and thus displace it.

An important exception to this principle is exposure in fetal/perinatal life when hormones are exerting organisational effects and feedback systems may not be operative (WHO, 2002). Also in postmenopausal women, no ovarian oestrogen synthesis occurs and the residual oestrogen production, which occurs predominantly in sub-cutaneous fat, is not subject to significant feedback control. Thus in these circumstances, ingestion of exogenous hormone will lead to additive increments of exposure.

Some compounds may possess no intrinsic hormonal or anti-hormonal activity, yet still be capable of exerting hormonal effects. This can occur if the compound affects the production, bioavailability or metabolism of endogenous (potent) hormones. Such compounds potentially pose a more serious health threat, as alterations in endogenous hormones will cause clinical disorders; there are a growing number of examples of such compounds (Sharpe & Franks 2002). Evaluation of potential effects of residues of hormone growth promoters in meat ingested by humans therefore needs to be considered against the background outlined above. In contrast to most endocrine disruptors, several of the growth promoters are intrinsically potent hormones (e.g. oestradiol). This means that effects are more likely if there is significant human exposure. However, as human exposure is most likely to residues of the growth-promoting agent present in muscle/fat, which will normally be at very low concentrations (pg to μg /kg/day). This may rule out possible effects (Henricks *et al.*, 2001; Lange *et al.*, 2001; Maune *et al.*, 2001). Moreover, as human exposure will be via food, the absorption and metabolism of the compound in the gut becomes very important. Oral absorption of oestradiol is good, however the quantity reaching the systemic circulation is greatly reduced by extensive first-pass metabolism in the intestines and liver, and oestradiol is generally considered to be inactive when administered orally (see Appendix C and JECFA, 2000).

1.6 Practical use of oestradiol in cattle

This section focuses on cattle, as they are the main food producing species in which oestradiol products are used for therapy or growth promotion. In order to put the contribution to the food chain from therapeutic and zootechnical use of oestradiol in context the endogenous production of oestrogens arising at various stages of the reproductive cycle should be considered.

The reproductive cycle of the cow

The reproductive cycle of the 'average' dairy cow, calving approximately once a year, involves 4 to 5 oestrous cycles followed by pregnancy; on average she spends approximately 75% of the year being pregnant and produces milk for all but the last 40-60 days of pregnancy.

Endogenous production of oestradiol and oestrogens varies throughout the reproduction cycle. In the 'cycling' cow there are 2 or 3 small peaks of oestradiol during the 21-day oestrous cycle, which accompany waves of follicular development, and one major peak at oestrus. Reported concentrations of oestradiol in plasma and milk vary according to the assay method used but are typically 4-5 times higher during oestrus than in the remainder of the cycle. During pregnancy oestradiol concentrations in plasma and milk rise dramatically and are typically 10 fold higher than in the cycling animal. Therefore most milk comes from pregnant animals and thus contains higher concentrations of natural endogenous oestrogens.

In addition, a proportion of cows/heifers entering the food chain are pregnant. Meat from these individuals can also contain higher levels of oestrogen produced by the foeto-placental unit. When the predicted removal of the ban on the inclusion of meat from cattle over 30 months into the food chain occurs, approximately 25% of cull cows entering the food chain are likely to be pregnant (Singleton and Dobson, 1995). Meat from these animals will add significantly to the oestrogen concentrations currently entering the food chain from this source. However it should be noted that removal of the restriction would only return the oestrogen load to pre-ban levels.

Therapeutic use of oestradiol in cattle

The uses and indications for oestradiol salts have been recognised for some time and are clearly defined. Oestradiol benzoate is authorised for the treatment of pyometra and endometritis in cattle. Therapy may also be beneficial to enhance oestrous behaviour in suboestrous or anoestrous animals in the induction of lactation as well as in the dilation of the cervix in cases of abortion. Various oestradiol salts are also luteolytic and are incorporated into oestrous synchronisation devices (PRIDs). Equally the administration of 1mg of oestradiol by injection in conjunction with the intravaginal progesterone-releasing device (Cidr) increases synchrony and may enhance the expression of oestrous behaviour.

Intra uterine infections after calving

Post-partum endometritis occurs mainly in dairy cows. Various reports estimate that prevalence in these animals ranges between 3 and 8 per cent. This condition occurs during early lactation when discharges need to be eliminated to aid hygienic conditions for milk production.

A proportion of the cases occur in cows that have already experienced a post calving ovulation and have functioning corpora lutea. A proportion of these will respond to a luteolytic injection by producing endogenous oestrogens and resolving the condition by “self-cure”. However a significant proportion simultaneously experience post-partum anoestrus due to ovarian inactivity. This subgroup is not suitable for luteolytic therapy. For this group there are two alternative strategies. The first uses an intramuscular injection of oestradiol benzoate to mimic the effects of normal ovarian follicular cyclicity. The result is relaxation of the cervix, improved muscle tone and increased supply of leucocytes to the uterus. These induced changes result in evacuation of the uterine contents and elimination of infection. Following the injection, the blood concentration of oestradiol does not rise above the normal physiological range. Indeed, as the remnants of the foeto-placental unit are a source of oestrogen, early evacuation may result in a more rapid fall in milk oestrogen concentrations in these cows. The second accepted therapy involves intra-uterine infusion(s) of an antimicrobial (often an antibiotic) solution. This approach aims to reduce the intra-uterine infection and thus promote a return to normal ovarian cyclicity.

An overall ban on the therapeutic use of oestradiol and/ or its esters would prevent the former therapy and greater use of antibiotics would be necessary. As these cows are producing milk that may enter the food chain minimal use of antibiotics is required. Also a proportion of these infections may not respond to antibiotic therapy. Therefore, the result of a ban would be an increased risk of antibiotic resistance and reduced standard of welfare for a proportion of cows.

Veterinary medicinal products containing oestradiol

There are currently four veterinary medicinal products containing oestradiol available in the UK outlined in Table 1.6.1. These products are formulated to release their oestradiol content in one burst of short duration and are therefore not suitable for growth promotion. One of these products is licensed exclusively for use in the bitch whilst the other three are licensed for zootechnical and therapeutic uses in the cow. Crestar devices are used purely for oestrous synchronisation in dairy and beef heifers and beef cows, and are not used in lactating dairy cows. PRIDs are used both for treatment of suboestrous and anoestrous as well as for oestrous synchronisation in both beef and dairy animals.

Table 1.6.1 Currently available oestradiol containing veterinary medicinal products

Product	Active ingredient	Conc ⁿ (mg/ml)	Route of administration	Indications	Dose	Withdrawal
Mesalin	Oestradiol Benzoate	0.2	Subcutaneous or Intramuscular	Mesalliance in the bitch	0.01mg/kg 3 and 5 days post	N/A

					mating	
Oestradiol Benzoate	Oestradiol Benzoate	5	Subcutaneous (bitch) Intramuscular (cow)	Mesalliance in the bitch Pyometra and endometritis in the cow	0.3mg/kg 1-4days post mating 3mg/500kg	N/A Milk - 0d Meat - 15d
Crestar	Oestradiol valerate	2.5	Intramuscular	Oestrous synchronisation in combination with implant	5mg	Not for use in milking cows Meat -14d post implant removal (23-24d)
PRID	Oestradiol Benzoate	N/A	Intravaginal	Oestrous synchronisation and stimulation of ovarian activity in anovulatory and suboestrous cows	10mg	Milk - 0d Meat - 24hrs

Food safety considerations following application of oestrus control products

Crestar is not licensed for use in lactating dairy cattle, and despite the longer half-life of oestradiol valerate, the withdrawal period should ensure that no residues should reach the food chain via meat. In contrast PRID and oestradiol benzoate are licensed for use in both dairy and beef animals. However, as long as withdrawal periods are observed there are no residue implications associated with these products. More specifically the only area of concern would be the intramuscular injection site where significant residues may be present if the withdrawal periods were not observed. (It is worth noting that therapeutic doses of oestradiol result in pg concentrations that have a half-life of 8 hours and do not exceed normal endogenous blood concentrations).

The rationale for continued use is that, used for therapeutic or zootechnical reasons, these products do not cause the concentration of plasma or milk oestradiol to rise outside the physiological range. The use of oestradiol benzoate will cause an elevation in plasma and milk oestradiol concentrations, however these elevated concentrations are still well below those of naturally circulating oestradiol in pregnant animals.

As an illustration, if one considers the total number of treatments with oestradiol benzoate and Prid in 2002 and assumes that they were all delivered to lactating dairy cattle, and take the worst case scenario that this use elevates oestradiol concentrations to the equivalent of a pregnant animal for 2 days. In 2002 there were approximately 94,500 treatments sold (Oestradiol benzoate 29,870 doses; Prid 64,448 doses) - assuming these were all used in the 2.25 million dairy cows in the UK, this equates to 0.042 doses per animal. The worst case impact of this use could therefore be said to be the equivalent of extending pregnancy by 2 hours per cow in the national herd. To put this into context a 1.2% increase in the proportion of dairy cows in calf to continental beef bulls would result in a similar increase in the duration of pregnancy (by virtue of the longer gestation period of these breeds).

The last 30 years has seen an increase in the use of continental sires from virtually zero to approx 30% of dairy cows services; this management change alone has resulted in an additional 5.25

million days of pregnancy or 2.33 extra days per cow – equivalent to some 2.65 million oestradiol treatments per year.

Growth promotion – multiple implantations into cattle

Implanting hormonal growth promoters is currently widespread in the beef cattle industry of many non-EU countries for the better performance in growth and improvement of feed efficiency. In 1999, more than 96% of all US cattle in feedlots were implanted at least once (NAHMS, 2000 cited by Scheffler *et al.*, 2003). These hormonal implants may enhance growth during suckling, growing and finishing stages of production (Mader, 1997; Platter *et al.*, 2003). Implant residues entering the food chain as a result of the implants administered during the suckling and growth phases of production will be lower than those arising from implants during the finishing stages. The weight gains are significant. A combination of trenbolone acetate and oestradiol improved average daily gain and feed efficiency during finishing stages by up to 20% and 13.5% respectively (Duckett and Andrae, 2000). The magnitude of the response to these anabolic implants in the performance of beef cattle varies depending on the type of implants, the quantity of growth promoter, duration of exposure, age of animals and combination of implants. Improved performance in steers originating from the dairy herd has also been noted.

In general, anabolic implants have minimal or negative effects on meat quality including lower marbling, high shear force and advanced carcass maturity resulting in lower quality grades. Repeated (five sequential implants) implanting has been claimed to have detrimental effects detectable by consumer taste panel scores Platter *et al.* (2001). However consumers failed to detect these differences in meat after 7 and 14 days aging when more moderate (two sequential implants) implant regimes were used (Barham *et al.*, 2003). Therefore there is no organoleptic characteristic by which consumers can be expected to detect meat originating from implanted animals. To date there is no validated technique to detect and assign the low residual concentrations of oestradiol in the finished edible products to natural sources or to implant residue. This is an area where research is urgently needed.

These implants are no longer allowed in the European Union (EU), which also prohibits the importation of beef and its products derived from hormone-treated cattle.

Alternatives to oestradiol-containing products

In the absence of oestradiol-containing products, alternatives would need to be employed. For oestrous synchronisation regimes prostaglandin or the progesterone-releasing device (Cidr) could be employed. Alternatives for the treatment of pyometra and endometritis could include the use of prostaglandins for a combination of their direct oestrogenic and luteolytic effects. However, it would not be possible to substitute for the current 'off label' use for enhancement of oestrous behaviour.

Zootechnical versus therapeutic use

The Working Group were of the view that the growth-promotion activity should be seen separately from the other zootechnical uses and the therapeutic uses of 17β -oestradiol and other hormonally active substances. One strongly expressed view was that, if the current EU position on the ban of 17β -oestradiol for growth-promotion purposes were to be maintained and extended, it would be most unfortunate to lose its use for other zootechnical or therapeutic purposes. There was also agreement that the therapeutic uses of 17β -oestradiol were more important than other zootechnical uses. It was noted that many of the alternatives to 17β -oestradiol would also result in a comparable rise in endogenous oestradiol. As an example, the use of prostaglandins, if used as an alternative, would raise endogenous oestradiol concentrations, so having a similar outcome to the administration of 17β -oestradiol in the first place. For this reason alone, it seemed sensible to continue with the use of 17β -oestradiol. It is well established that prostaglandins can exert both respiratory and reproductive effects following accidental exposure; for this reason it was felt that the operator risks associated with the use of prostaglandin products should not be overlooked.

Implications of removal of oestradiol-containing products

Finally it is important to consider the implications of removal of the use of oestradiol containing products in food-producing species. Some of the possible implications of the removal of oestradiol products are:

- there is likely to be an increase in the use of prostaglandins, which have health and safety implications for the operator as well as increasing endogenous oestrogens.
- an increase in the use of antibiotics for the treatment of endometritis.
- the development of microbial resistance due to increased use of antibiotics
- welfare implications through sub-optimal treatment of affected cattle.
- ‘off label’ use of oestradiol-containing products licensed for use in companion animals is likely to occur.
- unregulated use of oestradiol formulated on an *ad hoc* basis from chemical suppliers may occur.

2 Exposure to hormonally active substances

2.1 Analytical techniques: recent data/evaluation of SCVPH Opinion

The SCVPH report discussed four of the 17 EC-sponsored studies which concerned analytical techniques for the detection of trace hormones in meat (Studies 1,6,7 & 8), and one study which developed screening bioassays for known oestrogenic and androgenic compounds in yeast, trout hepatocytes and human endometrial cancer cells (Study 9). Study 1 (*Presence of oestrogen in meat*) would have been of particular relevance, but the Working Group were informed that no publication would be forthcoming⁸.

Studies 6 and 7 are both entitled *Analysis of 500 samples for the presence of growth promoters* and would appear to represent key research involving new methods for the detection of trace hormones in meat, based on GC/MS. However, the report of Study 6 comprises only a one-page abstract of a lecture by Professor RW Stephaney, the text of which includes a number of anecdotes but no new study data. Study 7 is supported by two publications (Marchand *et al.*, 2000, Le Bizec *et al.*, 2000). The derivation of new laboratory methodology is adequately described. But other than the description of steroids in four samples of residue-positive meat and liver, there are no data on samples that reflect the concentrations of the compounds under consideration in a representative set of samples.

Study 8 is entitled *Comparison of assay method* and involves development of an assay method based on HPLC. However, only a small number of random samples were analysed. This study appears to have been conducted by Joachim Liehr’s research group in Houston, and is supported by five publications largely related to his interest in the genotoxicity of oestrogens.

On the basis of the results from these four studies, the SCVPH report concludes, appropriately, that ‘the low number of samples does not allow a qualified validation of typical characteristics such as sensitivity, specificity, accuracy and reproducibility’.

⁸ confirmed in a letter from Dr Belingieri 17/7/2003.

2.2 Bioassays for screening: recent data/evaluation of SCVPH Opinion

The SCVPH 2002 Opinion discussed one study that developed a screening bioassay to detect known oestrogenic and androgenic compounds in yeast, trout hepatocytes and human endometrial cancer (Ishikawa) cells (Study 9; Guevel and Pakdel, 2001). The study revealed a highly variable sensitivity between the tests for oestradiol, and a variable differential response in *in vitro* potency tests that may in part be explained by the metabolism of some of the compounds by trout hepatocyte and Ishikawa cells. No data were derived by application of these techniques to meat; if they were to be so applied, exhaustive chromatography to isolate individual steroids would be required in order for the tests to provide useful data. The SCVPH report concludes that, in view of their lack of specificity and sensitivity, the assays performed in recombinant yeast and trout hepatocytes are not justified. The SCVPH Opinion of 2002 on the unsuitability of the yeast assay is fair. The Working Group noted that this is a profoundly different conclusion from the SCVPH Opinion of 1999, when it was the availability of this highly sensitive (as it was then regarded) new bioassay that led them to consider that previous data on low oestrogen concentrations might be flawed.

2.3 Conclusions and recommendations

The Working Group concluded that a number of new analytical methods have been developed that might helpfully be applied to the analysis of residues in the meat of cattle, but no substantial data have been presented from their application, nor have they been fully evaluated. These new techniques should be applied to meat in sufficient sample sets to provide reliable estimates of the relevant residues in untreated and implanted animals in the form that they enter the human food chain.

The suitability of three complementary bioassays for screening tissues for oestrogenic and androgenic compounds has not been demonstrated. Unless rigorous chromatographic separation techniques are developed, these bioassays should not be used for assessing residues in meat.

3 Bioavailability of hormonally active substances

3.1 Metabolic pathways: recent data/evaluation of SCVPH Opinion

The SCVPH 2002 Opinion discussed two EU-funded studies relating to the bioavailability of hormonally active substances. **Study 3a** (Maume *et al.*, 2001) involved the development of a new assay procedure for quantification of oestradiol levels in edible tissues and subsequent measurements of oestradiol levels in tissue samples from cattle following oestrogen implantation. The new assay included the analysis of lipoidal esters of oestradiol. Validation of the analysis of free oestrogens was complete but was only partial for the analysis of lipoidal esters. Nonetheless, the conclusion that lipoidal esters account for approximately 50% of the total oestradiol concentration in control or single-implanted steers appears sufficiently sound, as is the conclusion that this fraction should be taken into account when assessing the overall intake of oestrogens from treated cattle. **Study 3b** (Hoogenboom *et al.*, 2000, 2001) investigated the metabolism of 17 β -oestradiol by bovine hepatocytes and human intestinal and breast cells and tested their oestrogenic properties in the rat uterotrophic bioassay. These studies showed that 17 α -oestradiol as well as lipoidal esters of 17 β -oestradiol may be formed *in vivo* in animals implanted with 17 β -oestradiol as a growth promoter. 17 α -oestradiol had only about 10% of the *in vivo* oestrogenic potency of 17 β -oestradiol, whereas the lipoidal oestrogens had 10-fold higher potency than 17 β -oestradiol when tested *in vivo* in the rat uterotrophic assay.

Based on these studies the SCVPH 2002 Opinion concluded that metabolism of 17 β -oestradiol in bovine species results in the formation of lipoidal esters, and that these esters are largely disposed in body fat and may contribute significantly to an additional oestrogen exposure via meats. Lipoidal oestrogens may have higher potency in the breast due to their postulated transport via the lymphatic circulation and might potentially bioaccumulate in edible fat or meat. However, their oral bioavailability in humans following dietary exposure via contaminated meat products is unknown.

These studies on 17 β -oestradiol metabolism and evaluation of oestrogenic potency *in vivo* appear to have been well conducted. The demonstration (Study 3b, Hoogenboom, 2000) that certain residues may have potency in the uterotrophic assay is suggestive of bioavailability *in vivo* at this particular oestrogen target site. But it remains unclear whether similar actions would occur at other sites and whether any biological or 'adverse' effect would result. Since the 1999 SCVPH Opinion, more recent data (Study 3a; Maume *et al.*, 2001) have shown that in steers implanted with one (normal practice) or with two or four implants inserted simultaneously (misuse), dose-dependent increases in concentrations of lipoidal oestrogens are found in fat, ranging from 30-40 ng/kg (one implant) up to 100-140 ng/kg (four implants). Similar or slightly higher concentrations of 17 β -oestradiol were detected and much lower concentrations of 17 α -oestradiol. In muscle concentrations of all three compounds were generally <100 ng/kg, whereas relatively high concentrations of 17 α -oestradiol were detected in liver and kidney samples (200-800 ng/kg). Based on the rat uterotrophic studies reported in Study 3b, no significant effects were detected *in vivo* for any of these three oestrogens at doses of 25 nmol/kg/day (~7000 ng/kg) over a 6-day period.

Assuming similar absorption and metabolism profiles in the human and rat, these findings would suggest that consumption of meat/fat from 17 β -oestradiol-implanted cattle is unlikely to provide biologically significant oestrogenic exposure, even if unusually large amounts, from animals bearing 4x the recommended number of implants, were eaten regularly. However, this conclusion makes numerous assumptions relating to absorption, metabolism and bioavailability and takes no account of the (theoretical) possibility that lipoidal oestrogens might bioaccumulate over time in fatty tissue, such as in the breast. The Maume *et al* (2001) paper also considered the levels of oestrogens in animals with multiple implants in relation to the maximum human daily dietary intakes. For adults their estimates indicate an intake of < 5% of ADI from a standard 500g meat intake, but for prepubertal boys the ADI might be exceeded.

The SCVPH conclusion on the formation of lipoidal esters based on *in vitro* oestrogenic activity expressed in T47 D breast cancer cells (Study 3b, Hoogenboom *et al.*, 2001) is reasonable, although it is not clear to what extent hydrolysis of lipoidal esters occurred before binding to ER in T47 D cells, and thus did not reflect a direct effect of the esters themselves. The degree to which any such hydrolysis would occur in humans is unknown.

3.2 Conclusions and recommendations

Theoretically, if considerable amounts of 17 β -oestradiol or lipoidal oestrogens are present as residues or contaminants in hormone-treated meat samples, they could exert significant effects on important oestrogen target tissues such as the breast. However, from the information available it appears that such exposures are unlikely to occur, even in situations in which misuse (i.e. over-implantation) of implants has taken place. This conclusion should be re-assessed when, and if, new data become available to show that bioaccumulation of lipoidal oestrogens in fatty tissue occurs *in vivo* after oral administration. The data from Maume *et al* (2001) in relation to prepubertal boys should be confirmed by others and if confirmed may be a cause for concern in this group.

4 Cancer risks of oestrogenic substances

4.1 Breast cancer risk: recent data

Over the last few years there have been a number of publications that have had a substantial impact on our thinking on the effects of endogenous and exogenous oestrogens on the incidence of breast cancer. The data are directly relevant only to postmenopausal women⁹.

The Endogenous Hormones Breast Cancer Collaborative Group (2002) collated and analysed data from the nine published studies on the relationship between the plasma concentration of steroid hormones in postmenopausal women and the risk of subsequent development of breast cancer. Statistically significant relationships were found for several hormones. The strongest associations were for 17 β -oestradiol (stronger still when only the protein-free fraction was considered) and testosterone. The relationship with testosterone was markedly weakened after adjustment for 17 β -oestradiol; this is consistent with the relationship being determined by conversion of testosterone to 17 β -oestradiol by the action of the aromatase enzyme. The Collaborative Group estimated that the relative risk for breast cancer was 1.25 for each doubling of plasma 17 β -oestradiol concentrations. It is, however, near certain that this underestimates the true risk¹⁰, since only a single blood sample from each subject was analysed in each of the studies. Additionally, the studies used a wide variety of analytical techniques, some of which were inaccurate and/or inappropriate for use in postmenopausal women.

Recent publications from two very large studies have confirmed that use of hormone replacement therapy (HRT) by postmenopausal women for several years significantly increases their risk of breast cancer (Beral, 2003; Chlebowski *et al*, 2003). The Women's Health Initiative (WHI) conducted a randomised, placebo-controlled trial of combination HRT (oestrogen plus progestin) versus no HRT in 16,608 North American women and found that breast cancer incidence was increased with a hazard ratio of 1.24 (Chlebowski *et al*, 2003). Notably, this study also reported that the breast cancers presented at a significantly later stage in the HRT users. The Million Women Study (MWS) recorded HRT usage in around one million postmenopausal women in UK (Beral, 2003). Consistent with the WHI study, a higher incidence of breast cancer was found in combination HRT users. Importantly, in the context of the possible impact of ingested exogenous oestrogenic residues, MWS also reported a higher incidence in women taking oestrogen-only HRT (relative risk of 1.30 versus never users). These two studies confirm that incidence of breast cancer in postmenopausal women is enhanced by the regular ingestion of oestrogens, mainly in the form of oral conjugated equine oestrogens, in quantities sufficient to reduce menopausal symptoms and preserve bone integrity. However, it is not possible from these studies to ascertain a concentration of 17 β -oestradiol that does not enhance the risk of breast cancer (i.e. a NOAEL cannot be established).

Yen *et al* (1975) have described the effects of ingested oestradiol on plasma oestradiol, oestrone and gonadotrophin concentrations, in postmenopausal women. Their data showed that 2 mg micronized oestradiol led to a maximum plasma concentration of 110 pg/ml 5 hours after ingestion and this was a 437% increase i.e. from a starting level of 20 pg/ml, falling to baseline by 24 hours. Thus the increment in oestradiol from ingesting 2 mg was 90 pg/ml = 320 pmol/L. Over 24 hours the mean increment would be no more than 150 pmol/L.

⁹ In postmenopausal women, no ovarian oestrogen synthesis occurs and the residual oestrogen production occurs predominantly in sub-cutaneous fat and is not subject to significant feedback control; in these circumstances, ingestion of exogenous hormone leads to additive increments of exposure.

¹⁰ It has been estimated by the evaluation of multiple samples that the true relative-risk from plasma oestradiol is double that estimated by single-sample studies (Hankinson *et al*, 1995). Thus the relative risk of breast cancer from a doubling of oestradiol concentrations is likely to be approximately 1.50.

The highest concentration of oestradiol detected in meat was 56 ng/kg in kidney (Arnold 1999). For a postmenopausal woman eating a kilogram of such kidney from a treated animal, the theoretical increment would therefore be 0.004 pmol/L (based on the finding that the 97.5th percentile of consumers eat 40g of kidney per day). This increment is approximately 3 orders of magnitude below the most sensitive assays available and below any concerns related to breast cancer risk. Assuming that all of the oestradiol in meat is bioavailable and unaffected by food preparation, this would be expected to lead to mean concentrations increasing from approximately 40 pmol/L to 40.004 pmol/L, an increase of only 0.01%.

4.2 Reduced breast cancer risk in future

At present in the UK, there are over 100,000 women receiving tamoxifen for treatment of breast cancer, of whom about 75% are postmenopausal. Modern aromatase inhibitors (e.g. anastrozole, letrozole, exemestane) have shown themselves to be superior in efficacy to tamoxifen (Smith & Dowsett, 2003), and it is widely expected that in the next few years this population will instead be treated with aromatase inhibitors. The efficacy of these compounds is determined by their suppression of plasma oestradiol concentrations from 25 pmol/l to below the detection limit of available assays (<3 pmol/l). However, their efficacy could be compromised by the ingestion of oestradiol in doses that achieved increments of plasma oestradiol in single figures of pmol/l.

4.3 In utero exposure and breast cancer risks

Evidence to support a role of intra-uterine factors such as 17 β -oestradiol concentrations and development of breast cancer in the female are well established (Braun *et al*, 1995; Swerdlow *et al* 1997). One of the approaches used to explore the potential involvement of hormones in affecting predisposition to cancer is to compare twin versus singleton pregnancies, as oestrogen concentrations in twin pregnancies are invariably higher than in singleton pregnancies (Kappel *et al.*, 1985). The SCVPH report discussed one EC-commissioned study based on the Swedish Twin Registry that sought to evaluate whether risk of breast cancer was higher in twins (Study 13; Kaijser *et al.*, 2001). This study is critically evaluated in Section 7.

4.4 Oestrogen and the human gut

Ingestion of meat from animals treated with hormonally-active substances is likely to result in highest levels of exposure in the gut. Therefore, potential effects of oestrogenic and/or androgenic compounds on the gastrointestinal tract need to be considered. There are clear gender-related differences in gastric acid production (40% higher in males; Feldman *et al*, 1983) and in the incidence of gastroduodenal ulcers (higher in men; Hawkey *et al*, 2002), Crohn's disease (higher in females; Chang & Heitkemper 2002) and colorectal cancer (higher in males than in premenopausal women; DeCosse *et al*, 1993).

There is reasonably convincing evidence that the gender difference in gastric acid production and colorectal cancer stems from differences in oestrogen production/action in males versus females, as oestrogen treatment reduces gastric acid production (Campbell-Thompson *et al.*, 2001), and oestrogen exposure, whether endogenous or via hormone-replacement therapy, is protective against colorectal cancer (DeCosse *et al*, 1993). The precise involvement of oestrogens in progression of Crohn's disease is less clear (Cosnes *et al*, 1999). Based on these observations, it would be concluded that any additional exposure of the gut to oestrogenic compounds present in meat from growth-promoted animals would have a health-beneficial, rather than -detrimental, effect.

4.5 Conclusions

The Working Group noted that recent studies have confirmed hormone replacement therapy increases the risk of breast cancer in postmenopausal women. However, it also noted that the

maximum increase in oestradiol levels which might occur following consumption of oestradiol-treated meat by a postmenopausal woman is most likely to be below any concerns related to cancer risk. Oestrogen-therapy appears to be protective against colorectal cancer, and therefore, arguably, any additional exposure following ingestion of oestradiol-treated meat would, if anything, have a health-beneficial effect for this commonly occurring cancer.

5 Altered gene expression by oestrogenic substances

5.1 Recent data/evaluation of SCVPH Opinion

The SCVPH report discussed one study that measured changes in gene expression of a number of hormone sensitive genes in a breast cancer (MCF7) cell line (Study 17; Leffers *et al.*, 2001). The study found the expression of the different hormone responsive genes varied for the different oestrogens (zearanol and five related compounds, 17 β -oestradiol and three other oestrogenic substances). The SCVPH's only comment on this study was to note the down-regulation of GST μ 3, a Phase II enzyme that is involved in protection against DNA damage by free oxygen radicals.

The results showed that zearanol was of similar potency to 17 β -oestradiol in this test system, although there were gene-specific differences in the levels of expression following treatment with the oestrogenic substances. Zearanol was much more potent than the naturally occurring fungal contaminant, zearalenone. It was also noted that zearanol as a mycotoxin may arise from fungal growth in cereals. Although interconversion of these substances can occur, this occurs at a low rate, suggesting zearanol may pose a greater hazard than the widely occurring zearalenone. Zearanol was the most potent inhibitor of the expression of MRG1/p35srj which is involved with a nuclear transcription activation factor.

A number of authors have demonstrated that zearanol shows none or only limited binding to cellular binding proteins in contrast to 17 β -oestradiol. This indicates that zearanol may be more potent in hormonal activity than 17 β -oestradiol, due to higher bioavailability (Ben-Rafael *et al* 1986, Mastri *et al* 1985, Shrimanker *et al* 1985, Nagel *et al* 1998). Currently, its bioavailability by the oral route following consumption of meat products containing zearanol is not known. However, the higher oestrogenic potential seen in this *in vitro* test system is not consistent with reports of lower oestrogenic potential in a variety of *in vivo* assays e.g. vaginal cornification, uterotrophic assay, depression of serum gonadotrophin concentrations in castrated monkeys (reviewed in Lindsay, 1985). Furthermore zearalenone and zearanol were shown to have similar physiological effects in a variety of *in vivo* assays. This suggests that this gene expression test system may not be a good indicator of *in vivo* oestrogenic potency.

Leffers *et al.*, (2001) also showed 17 β -oestradiol down-regulated GST μ 3 at extremely low concentrations and suggested that this response might be a result of the altered redox status within the cell, rather than due to regulation by the oestrogen receptor. Together with the down-regulation of other phase II genes, they suggested that this could reduce protection against DNA damage and that changes in the relative balance of gene expression of Phase I and Phase II metabolism may be important in the proposed production of genotoxic catechol metabolites of 17 β -oestradiol.

5.2 Conclusions and recommendations

The evidence that oestradiol gives rise to genotoxic metabolites is considered further in Section 6. The low binding activity of zearanol and its ability to alter gene expression of important hormone responsive genes makes it important to determine the bioavailability and biological significance of zearanol residues in meat. Initially this would require studies of serum concentrations following consumption of meat from zearanol-treated animals.

6 Genotoxic & mutagenic effects of oestrogenic substances

In its 1999 Opinion, the SCVPH concluded there was sufficient evidence that 17 β -oestradiol was genotoxic. This Opinion was based on positive responses in a variety of *in vitro* indicator assays. The VPC sub-group report (1999) pointed out that standard mutagenicity tests on 17 β -oestradiol (bacterial mutation, mammalian gene mutation, *in vitro* micronuclei, the bone marrow micronuclei test and germ cell cytogenetics assay) were all negative. Furthermore, the studies on which the SCVPH based their Opinion were all non-standard studies (methotrexate resistance, microsatellite formation), or were unconvincing due to the absence of a dose-response. The SCVPH concluded, however, that there was evidence for induction of oxidative damage, DNA adducts and aneuploidy.

6.1 17 β -oestradiol: Recent data/evaluation of SCVPH Opinion

The 2002 SCVPH Opinion states there is now conclusive evidence that 17 β -oestradiol is genotoxic since it induces mutations in mammalian cells, oestradiol metabolites induce mutations in mouse skin *in vivo* and catechol oestrogen quinones form DNA adducts in cultured cells and mouse skin. This SCVPH Opinion is based on a study commissioned by the EC (Study 3; Hoogenboom *et al.*, 2000, 2001) and other published papers. The study by Hoogenboom and colleagues showed that 17 β -oestradiol and several of its metabolites were negative in a series of apparently well-conducted bacterial mutagenicity assays using a variety of strains and metabolic activation conditions employed in order to improve the potential sensitivity of the test. Furthermore, they also reported negative responses in the Comet assay using human intestinal cells (CaCo-2). The other published papers considered by the SCVPH are discussed below.

A number of recent papers strengthen the evidence that 17 β -oestradiol can be activated to produce genotoxic metabolites by its conversion into catechol oestrogens which may be oxidized to form semiquinones and quinones (e.g. Lavigne *et al.*, 2001; Cavalieri *et al.*, 2000; Liehr, 2001). These quinones can form DNA adducts leading to depurination. The metabolites may also generate potentially mutagenic oxygen radicals by redox cycling. Inactivation via O-methylation, or glucuronidation or sulphation also occurs.

The mutagenic potential of oligodeoxyribonucleotides adducted with hydroxyoestrogen moieties was studied (Terashima *et al.*, 2001). A series of synthetic oligonucleotides were produced, each containing a modified nucleotide. These were used to create vectors, which were then used to transfect COS-7 monkey kidney cells. They were shown to induce G to T transversions in this model system. This study demonstrates that oestrogen metabolite adducts introduced into naked DNA are pre-mutagenic. The system bypasses normal cellular controls (activation/inactivation pathways and DNA repair) of the intact animal.

A metabolite of 17 β -oestradiol, 2-methoxyestradiol (2-MeE2), was claimed to induce transformation in the SHE assay, chromosome aberrations and mutations at the HPRT and Na⁺/K⁺ ATPase loci (Tsutsui *et al.*, 2000a). The study is poorly reported. 2-MeE2 induced mutations at only one of the doses tested, which was a mid-point dose in the case of the Na⁺/K⁺ ATPase assay. Furthermore, it is not clear whether cytotoxicity has been assessed appropriately and the assays lacked statistical power due to the low control frequency and low numbers of cells analysed. At best the evidence is marginal. The induction of chromosome aberrations is even less scientifically convincing. Concurrent cytotoxicity data are not given, but assuming the concentrations are similar to those measured in the mutation assay, then an increase in aberrations was only seen at toxicity concentrations in excess of the internationally acceptable limits. Nearly all the damage was due to “chromosome pulverization”, an effect attributed by the authors to asynchronous division within multinucleate cells, and not therefore due to clastogenicity. Aneuploidy and polyploidy were also induced. While there is some evidence that the metabolite was able to induce cell transformation –

the significance of this finding is less clear as the SHE assay detects both genotoxic and non-genotoxic substances. A wider range of metabolites was tested in the same systems (Tsutsui *et al*, 2000b). Despite significant methodological and reporting inadequacies, there does appear to be some evidence that some of the metabolites can induce chromosome aberrations, mutations and cell transformation in SHE cells. 4-hydroxyestrone and 2-methoxyestrone, but not 17 β -oestradiol, oestrone, 2-hydroxyoestradiol or 4-hydroxyoestradiol induced mutations at the HPRT locus. Estrone, 4 hydroxyestrone and 4-hydroxyoestradiol, but not oestradiol or the other hydroxyl metabolites tested, gave some evidence of weak induction of chromosome aberrations.

The evidence that 17 β -oestradiol is a point mutagen is derived from the publication of Kong *et al* (2000), based on the induction of mutations at the HPRT loci in V79 cells. However, this study is not acceptable by generally recognized standards of quality or accuracy. In the study report there is insufficient information to evaluate whether an adequate number of cells was treated, there is no dose-response (significant increases were seen in cultures treated with 10⁻¹¹, 10⁻¹⁰, 10⁻⁷ and 10⁻⁶ M 17 β -oestradiol but not with 10⁻⁸ and 10⁻⁹M) and there is no evidence that the protocol ensured the independence of the individual mutant colonies picked for assessing the mutation spectra. The mutation induction data appears to be based on separate experiments, combined into a single results table, making it impossible to determine the data obtained in each separate experiment and thus to see how the reported increases relate to control (or spontaneous) values. Some of the DNA base changes in the “induced” mutants are incorrectly assigned. A key observation is the occurrence of two “hotspots” of mutation. These specific changes are rarely found and furthermore may have arisen due to failure to ensure the independence of mutants selected¹¹. Certainly, the postulated mechanism of action of oestradiol due to free-radical induced DNA damage would not be predicted to produce such a unique profile of DNA base changes. Our current understanding of spontaneous mutation indicates that a major fraction of mutations originate from oxidative damage. Thus, if the proposed genotoxicity of oestradiol is due to oxidative damage, then one might predict a mutant profile similar to those produced spontaneously. Therefore, this study cannot be considered sufficient evidence of mutation potential and the claim that 17 β oestradiol does not act via a receptor because mutation is not reduced in the presence of an anti-oestrogen is also not substantiated.

The study of Chakravarti *et al* (2001) is cited as evidence that oestradiol-3,4-quinone induces mutations in mouse skin *in vivo*. Dorsal skin of SENCAR mice was treated with this metabolite and the mice were sacrificed after one hour to measure DNA adducts and at intervals thereafter for measuring mutations in the H-ras gene. The dose used was 200nmol oestradiol-3,4-quinone; the treated surface area was not defined. The mutations induced were sequenced. N3-adenine adducts (rapidly depurinating) and N7 guanine adducts (slowly depurinating) were seen. It appears that those arising at N3, but not at N7 guanine, gave rise to mutations. Whilst this study provides evidence that a metabolite of 17 β -oestradiol can give rise to a genotoxic effect *in vivo*, the mutation frequencies obtained (2.2 x 10⁻⁵ mutations per base pair) are extremely high and there is no concurrent measure of toxicity. The relevance of this dose level to levels to which humans are exposed from eating meat from treated animals would require further investigation.

In a study not considered in the SCVPH Opinion, Yared and colleagues reported on the genotoxic effects of oestrogens in breast cells using the micronucleus and Comet assays (Yared *et al.*, 2002). 17 β -oestradiol, oestrone and oestriol were tested for their ability to induce micronuclei in an assay using cytochalasin B and DNA damage detected by the Comet assay in a human mammary cell line (MCF-7) and primary human mammary epithelial cells, both of which have the oestrogen receptor. Oestradiol induced an increase of micronuclei at 10⁻⁹M. Higher concentrations also showed an increase above controls, but in an inverse dose-response. Oestrone induced a dose-related response in micronuclei. No increase was observed for oestriol. A dose-related induction of proliferation was also observed for all compounds. Positive responses in the Comet assay were seen for β -oestradiol and oestrone and to a lesser extent for oestriol in both cell types.

¹¹ Mammalian Gene Mutation Database, available at: <http://lisntweb.swan.ac.uk/cmgt/index.htm>.

6.2 Testosterone & progesterone: Recent data/evaluation of SCVPH Opinion

Testosterone had previously been reported to be negative in the L5178Y gene mutation assay and in *in vivo* somatic and germ cell assays for chromosome aberrations (Richold, 1988). No data were available on progesterone. The SCVPH considered further the JECFA/WHO evaluation of these hormones (WHO Food Additives Series 43; WHO, 2000) and considered there is no evidence that progesterone or testosterone has genotoxic potential. No other publications have been published to add to this.

6.3 Zeranol and trenbolone: Recent data/evaluation of SCVPH Opinion

The 1999 SCVPH Opinion concluded that trenbolone was not genotoxic on the weight of evidence from numerous studies. Isolated positive responses were reported for micronucleus induction and cell transformation in SHE cells but not in C3H10T1/2 cells. There were, however, no standard assay results available on zeranol. On the basis of further work commissioned by the EC (Study 2, Metzler & Pfeiffer, 2001) the SCVPH 2002 Opinion concluded that these substances exhibit only very weak effects.

The mutagenicity of these substances was also investigated in Study 2 (Metzler and Pfeiffer, 2001). β -trenbolone was negative in a cell mutation assay (V79/hprt) and at the lacI loci in *E. coli*. Marginal positive results were claimed for micronuclei induction in V79 cells and for DNA adducts in hepatocytes. Zeranol did not induce DNA adducts in rat hepatocytes, mutations at the lacI locus in *E. coli* or mutations in mammalian cells (V79/hprt). A borderline response was seen for induction of micronuclei *in vitro*. The positive micronucleus responses for both compounds were only obtained at near-cytotoxic concentrations. The authors conclude that further work is required to evaluate the genotoxicity of these substances and their metabolites, and that non-standard systems may be required to detect weak effects.

The Working Group considered there to be methodological flaws with Metzler and Pfeiffer's gene mutation study – insufficient cells were treated and maintained through the expression period and assessed for mutations; a single dose only was assessed and the cytotoxicity values are not presented. Similarly the micronucleus results were obtained at a single concentration only and the measures of cytotoxicity were not presented, although it would appear that near cytotoxic concentrations were used. The method did not use the cytocholasin B method and its sensitivity was consequently affected by the inhibition of cell proliferation by trenbolone.

A further study considered by SCVPH involved the interaction of hormonal substances and their metabolites with sex hormone-binding globulin (SHBG) or the analogous sex hormone-binding protein (SBP) from trout plasma (Study 10). Zeranol and its metabolites were included in this study, and were found to have low binding affinity to these proteins; This would result in high bioavailability when present in plasma, but also fairly rapid metabolism. This unpublished study is discussed further in Section 6.4.

6.4 Melengestrol acetate: Recent data/evaluation of SCVPH Opinion

At the time of the SCVPH 1999 Opinion the information available on melengestrol acetate (MGA) was sparse. In its 2002 Opinion, the SCVPH considered the recent JECFA evaluation (WHO Food Additives Series 45, WHO 2000), but noted that most of the references were to unpublished reports. An EC funded study addressed this issue (Study 4, Metzler and Pfeiffer, 2001) and showed that MGA was negative in a cell mutation assay (V79/hprt), in a micronucleus test in V79 cells and in a

gene mutation assay for LacI mutations in *E. coli* (Metzler and Pfeiffer, 2001). SCVPH (2002) concluded from this study that MGA showed only weak effects. However, the Working Group considers the published study provides insufficient information for evaluation and thus no conclusion can be made on the mutagenicity of MGA.

6.5 Conclusions and recommendations

17 β -oestradiol

Most of the “new” information referred to in the SCVPH report has been generated using non-standard methodologies that produce information of questionable relevance to effects that may occur in the intact animal. A number of the studies discussed in the report are of poor quality. However, there is now additional evidence that metabolites of 17 β -oestradiol can form DNA adducts *in vitro* (Yagi *et al.* 2001; Cavalieri *et al.* 2000) and *in vivo* (Cavalieri *et al.* 2000). While the catechol metabolites of 17 β -oestradiol induce DNA adducts in SHE cells, 17 β -oestradiol itself does not do so (Yagi *et al.* 2001). There is some evidence for the induction by oestradiol of DNA damage (single strand breaks) and micronuclei formation in cells with the oestrogen receptor (Yared *et al.* 2002). It is not known whether the micronuclei are due to clastogenicity or to aneuploidy.

Evidence for the induction of mutations by 17 β -oestradiol itself has only been obtained in non-standard assays, including those without normal cellular controls (e.g. Terashima *et al.* 2001). There is still no evidence that 17 β -oestradiol itself is a gene mutagen. The key study of Kong *et al.* (2000), purporting to show that 17 β -oestradiol is a gene mutagen, suffers methodological and interpretation flaws. The mutagenicity seen in the *in vivo* study involving skin painting (Chakravarti *et al.*, 2001) may have been associated with extreme doses. There is, however, some evidence for a clastogenic potential for 17 β -oestradiol (reviewed by Cavalieri *et al.* 2000), although some studies have failed to differentiate between aneuploidy and structural damage. There is further evidence that oestradiol is an aneugen and an inducer of other genotoxic effects (e.g. DNA amplification, microsatellite formation). The significance of these latter endpoints for hazard and risk evaluation is still not clear.

Overall, the weight of evidence from many genotoxicity studies, both standard and non-standard, indicates there may be a genotoxic potential for metabolites of 17 β -oestradiol, but this direct evidence is by no means substantial. However, there is further indirect evidence for genotoxicity. A plausible hypothesis has been advanced (Santer, 2003; Cavalieri *et al.* 2000; Liehr, 2001) that 17 β -oestradiol is carcinogenic in humans and animal models by a combination of effects on cell proliferation and by genotoxicity. The hypothesis is primarily based on reasonable evidence that 17 β -oestradiol is not carcinogenic solely due to epigenetic phenomena such as induction of cell proliferation.

Although there is evidence that oestrogen metabolites may be directly genotoxic *in vitro*, *in vivo* their formation is affected by opposing activation and inactivation metabolic pathways, the presence of anti-oxidants and DNA repair capacity and thus it is likely this genotoxicity will have a thresholded response. The importance of anti-oxidant defence systems is demonstrated by the reduction in transformation and formation of DNA adducts by oestrogen metabolites in the presence of ascorbic acid (Yagi *et al.* 2001).

To date, there are no standard tests conducted *in vivo*, even on 17 β -oestradiol metabolites, which indicate a mutagenic potential for 17 β -oestradiol *in vivo*. Since both DNA repair pathways, antioxidant defence and Phase II inactivation pathways can be overwhelmed at high doses, it is necessary to obtain evidence of genotoxicity in well-conducted assays, employing realistic dose levels.

It is important to determine whether the 17 β -oestradiol metabolites can be produced *in vivo* in sufficient quantities to result in genotoxicity. Thus it is recommended well conducted *in vivo* studies are performed to determine whether 17 β -oestradiol is able to induce genotoxic damage *in vivo* under realistic exposure conditions

Testosterone and progesterone

On the basis of the limited information available to the Working Group, there is not further evidence of genotoxicity of these substances and no recommendation for further work.

Zeranol and trenbolone

The Working Group concluded that there are insufficient data to indicate zeranol or trenbolone are genotoxic. This conclusion is the same as that reached in the (previous) 1999 SCVPH Opinion. Further studies would be required to evaluate this fully.

Melengestrol acetate

The Working Group considered there to be insufficient data available to evaluate the genotoxicity of MGA.

7 Developmental & reproductive effects of hormonally active substances

The potential for chemicals with intrinsic endocrine activity to perturb development and function of the reproductive system, especially in the male, has been a driving force for concern about the issue of environmental endocrine-active chemicals. Results of studies in experimental laboratory animals have been equivocal, and there is as yet no data to show that such effects occur in humans due to any environmental chemical exposure.

7.1 Recent data/Evaluation of SCVPH Opinion

The SCVPH 2002 report considered three EU-funded studies that addressed reproductive and developmental sequelae of exposure to hormonally active compounds. One was an animal study, involving gestational and lactational exposure of rabbits to zeranol, trenbolone acetate (TBA) and MGA (Study 11; Lange *et al.*, 2002). The other two were human studies: a retrospective case-control follow-up of young men and women suspected of having been exposed to meat from hormone-treated animals when they were children in 1977 (Study 12; Chiumello *et al.*, 2001) and a study based on data from the Swedish Twin Registry, looking at breast cancer risks in twins (Study 13; Kaijser *et al.*, 2001).

Study 11: Studies to directly assess the effects of zeranol, TBA and MGA on the development of the testis and reproductive system in rabbits were investigated. These studies involved gestational and lactational exposure to these compounds at moderate or high doses, ranging from 0.25 mg/kg/month by implant for zeranol to 0.5 mg/kg/day for MGA. Exposure to TBA or MGA was also investigated during adulthood. The SCVPH quote the authors conclusions as indicating “that prenatal exposure to low doses of MGA, TBA or zeranol may affect the function of the reproductive tract in rabbits, although the effects are not as severe as those observed after exposure to the high doses. The effects are most pronounced if the exposure occurred early in life. All three compounds readily cross the placental barrier and accumulate to a variable degree in fetal tissues. The effects of zeranol and TBA are more severe than the effects of MGA in animals exposed during development, however, MGA has marked effects on spermatogenesis when administered in adults”. As only a superficial description of the results of this study were given in the SCVPH 2002

Opinion, the Working Group found it difficult to draw any conclusions with confidence. The only mention of a *significant* change was an increase in concentrations of oestrone after exposure to either MGA or to zeranol during early adolescence, a change unlikely to be of biological significance.

The final report on Study 11 provides a more conclusive view on this study. Pilot studies used relatively high doses of the test compounds and this led to various problems that resulted in use of lower levels of exposure for the main (reported) study. In the pilot study, treatment with zeranol (dose unspecified, but an implant that delivered >0.25mg/kg/month) resulted in major reproductive abnormalities, including cryptorchidism and gross suppression of spermatogenesis. However, no details of this pilot study are given (other than a description of testicular histology) and the use of a lower dose for the main study suggests that it was considered that the pilot study was compromised in some way. Similarly, prenatal exposure to TBA in the pilot studies was confounded by major perinatal mortality of the offspring and only one dam gave birth to offspring that survived after exposure to the lowest dose of TBA (0.5 mg/kg/week).

In the main study, only minimal effects were observed in animals exposed to doses of the three test compounds during different life phases (for zeranol, a monthly subcutaneous implant of 0.25 mg/kg to the dam; for TBA a weekly subcutaneous injection of 0.5 mg/kg; for MGA 0.5 mg/kg orally daily). No consistent significant treatment-related effects were observed, though four cases of unilateral cryptorchidism were observed, two after adolescent exposure to TBA and one after adolescent exposure to zeranol; one animal with unilateral cryptorchidism was observed after gestational exposure to TBA. No other gross changes of the reproductive system were observed. Abnormal spermatogenesis, as evaluated by a non-standard, but published, method, was evident in the cryptorchid testes as expected. But there was no evidence for abnormal changes in scrotal testes (though this is based on deductions from the limited tabulated data provided), with the possible exception of animals exposed in adulthood to MGA. The latter (small) effect most likely occurs as a consequence of the progestational activity of MGA. Even assuming that MGA does have effects on spermatogenesis when administered to adult rabbits, the dose used (0.5 mg/kg/day) is presumed to have no relevance to humans exposed via residues in meat, unless there is ingestion of part of an implant.

Based on cellular morphology, the report refers to the abnormal persistence of “single” ‘fetal-like’ germ cells in the testes of treated animals, although it is not specified in which treatment groups these cells were noted. Such cells are of interest because testicular (germ cell) cancer in humans probably arises from transformed fetal germ cells that have persisted in the testis since fetal life. However, the study was unable to confirm the possible fetal nature of these cells using a battery of specific markers as none of the available antibodies worked on rabbit tissues. Evidence for effects of TBA and zeranol on gonocyte development in the fetal testis was provided by increased numbers of these cells being immunopositive for PG-2, but as the role of this antigen is unknown, it is not possible to evaluate the significance of this observation (which was based on only two animals per group).

Sporadic changes in reproductive hormone concentrations were reported at certain ages in certain treatment groups, but no consistent, easily interpretable pattern was observed; no evidence of major dysfunction of the testis or of the hypothalamic-pituitary-testicular axis emerged from this study. Similarly, no evidence for any change in semen quality was found in any of the treatment groups. From limited studies on maternal and fetal samples from control and treated animals, it was shown that residues of MGA and zeranol and metabolites of TBA were clearly identifiable in various tissues of relevant treated animals, but were not detectable in controls.

This study experienced confounding problems due to ‘side-effects’ of the administered compounds during pregnancy. This is not unusual as pregnancy is susceptible to hormonal disruption, as it is a hormone-dependent process (Witorsch 2002). This may indicate that the doses of the test compounds being used were too high, although this was not a specified conclusion of the report.

The use of generally lower doses for the main study largely avoided these confounding problems and provided only sketchy evidence for any significant impact on reproductive development and function as a result of *in utero* or postnatal exposures to MGA, TBA or zeranol. Perhaps the only lingering concern was the sporadic occurrence of cryptorchidism, which was confined to treated animals, though this was restricted to animals exposed during adolescence; this may either indicate that the cryptorchidism was treatment-unrelated (i.e. the problem was present prior to initiation of treatment, as cryptorchidism is not uncommon in rabbits) or that the final stage of inguinal testicular descent had been compromised. The latter is well established to be an androgen-dependent process, but the *very limited* data available for testosterone concentrations show no indications of suppression.

Other than the occurrence of cryptorchidism, none of the other findings in treated animals were suggestive of consistent, abnormal changes in development or function of the male reproductive system. Moreover, they occurred in animals in which exposure to the growth promoting hormones was far in excess of that likely to occur in humans as the result of ingestion of meat/fat from growth promoter-treated cattle. This provides reassurance that adverse effects on the developing human male reproductive system are unlikely to occur.

Studies 12 and 13: The SCVPH 2002 Opinion concluded that *in utero* or pre- and peripubertal exposure to hormones (including animal evidence on synthetic products) may affect pubertal development and that epidemiological studies with opposite sexed twins indicated prenatal exposure to hormones may be linked to adult cancer risk. These conclusions derived from two EC-funded studies (Studies 12, 13).

Study 12 is extremely difficult to interpret and has several shortcomings. It followed an outbreak of gynaecomastia in school children in Italy in 1977, when it was *presumed* that accidental exposure to an oestrogenic compound of some sort was involved. The source and nature of the compound were never identified. In this situation the presumed exposure mimics what would happen normally during natural puberty when endogenous oestrogen concentrations would rise and stimulate breast development (in the female). If such exposure were continued over a period of time, effects on final height and other parameters might occur that could have significant impact for the individual. Remarkably, in the follow-up study height was not measured (or is not listed on the questionnaire or in the final report). Instead the focus was solely on reproductive issues and only minor changes were found. The most significant finding was an increased incidence of small (atrophic) testes in men who had attended the affected school in 1977. However, even this finding is suspect. First, it is well established that individuals who believe they may have a reproductive problem are more likely to volunteer/participate in studies that involve clinical examinations and blood tests related to reproduction (they get a free check-up); evidence of this was apparent from the report. Second, it is completely unknown whether or not the boys with atrophic testes were 'exposed' to the contaminated meat. Third, as this contamination was not proven, nor the nature of any hormonal contaminant identified, it is not possible to draw conclusions from this study.

Even if it accepted that the children in question had been exposed to an oestrogen such as DES, used for meat growth promotion, there is no evidence to suggest that such outbreaks are other than very isolated and rare phenomena. This suggests that exposure of children to oestrogenic compounds in meat is not sufficient to induce precocious breast development to the point where it is clinically significant. The most frequent occurrence of precocious puberty in girls arises in individuals who have been adopted at an early age from a developing country and then reared in a Western country (Tuvemo and Proos, 1993; Virdis *et al* 1998). What underlies the extraordinarily high incidence (20-25%) of precocious puberty in such individuals is still unclear but may involve precocious activation of the hypothalamic-pituitary axis.

Study 13 (Kaijser *et al* 2001) was based on the Swedish Twin registry and sought to evaluate whether subsequent risk of breast cancer was higher in twins. This study showed that with increasing female birthweight the risk of developing breast cancer in pre- or postmenopausal life

was increased step-wise; Though comparison of twins versus singletons can reveal a relationship between oestrogen concentrations in pregnancy and the risk of reproductive cancer in the offspring (Braun *et al* 1995; Swerdlow *et al* 1997), there are several difficulties in making such associations. First it is unclear what the relationship is between oestrogen concentrations measured in blood of the pregnant mother and those in the fetus, in particular the concentrations in oestrogen target tissues. Second, in twin pregnancies there is normally lower birthweight and this and other factors that affect growth of the fetus *in utero* can be significant risk factors for development of reproductive cancers in both sexes. This is illustrated in Study 13 in which risk of breast cancer in a female twin was considerably increased when there was a male twin present, and this appeared to be related partly to an increase in birthweight of the female twin. The latter effect might be related to increased androgen exposure from the male fetus, as the female fetus makes negligible amounts of sex steroids.

7.2 Conclusions

While it is reasonable to conclude that the hormone environment *in utero* is a factor in determining subsequent risks of some reproductive cancers, this is a complex area to interpret. It is certainly not straightforward to conclude on the basis of these findings that pre-natal exposure of the fetus to exogenous hormones, in particular hormones used as growth promoters in livestock, will be capable of inducing comparable effects. Issues such as potency, bioavailability, pharmacokinetics, transfer to the fetus, all have to be taken into account.

By reference to offspring from women who were treated with extremely high doses (>0.1mg/kg/day) of the potent oestrogen diethylstilboestrol during pregnancy, only a very modest increase in testicular cancer risk occurred in the male offspring (Toppari *et al* 1996) and only rare cases of vaginal cancer occurred in the female offspring (Noller 1983). It would therefore seem unlikely that exposure to the less potent growth promoting compounds, at what would be very much lower concentrations (Henricks *et al* 2001; Lange *et al* 2001; Maume *et al* 2001), would pose a significant risk with regard to the development of reproductive cancers. Moreover, experimental studies in rodents that involve administration of test compounds to pregnant animals and consequent exposure of the fetus may be poor models for the human, because of major differences in endogenous hormone concentrations, timing/duration of fetal development etc (Witorsch 2002). Again, the dramatic changes in diet, BMI, later age at first pregnancy, rates of smoking etc in women in Western countries over the past 50 years have established effects on fetal growth and development (Sharpe & Franks 2002). Against this changing background, discerning potential contributory effects from low-level exposure to growth-promoting hormones or their metabolites is probably an impossible task.

8 Environmental impact of hormonally active substances

8.1 Recent data/Evaluation of SCVPH Opinion

Although the SCVPH Opinion (2002) concentrates on risks to human health, Section 6 (p 21) and Annex 1 (pp. 24-27) also consider environmental effects. Three studies are mentioned by the SCVPH in relation to environmental effects:

Study 14: *Endocrine disrupting activity of anabolic steroids used in cattle*. The paper by Schiffer *et al.* (2001) contains results from this study that are of relevance to environmental risk assessment.

Study 15: *Screening water samples for estrogenic and androgenic anabolic chemicals*. The results from this study have not yet been fully published, but a discussion is published in a brief paper by Jégou *et al.* (2001).

Study 16: *Endocrine disrupting effects of cattle farm effluent on environmental sentinel species*. The results from this study have been published in brief form in a review by Orlando and Guillette (2001).

The Working Group evaluated the SCVPH's conclusions and compared these with the published evidence from the three cited studies. Additional information was not sought, and it is possible that further publications have emerged from the three SCVPH studies.

Section 6 of the SCVPH opinion simply states that previous SCVPH opinions have not addressed environmental concerns, but that relevant results from the 17 Studies are presented in Annex 1. This section also draws attention to the existence of a report by the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE 1999). This report is an overview of the evidence for endocrine disruption, particularly in wildlife. It does not specifically address risks from hormone residues in beef, but does briefly consider human and wildlife studies that examine the effects of oestradiol and 17 α ethinyloestradiol. The main focus for wildlife studies is on chlorinated organic compounds (e.g., PCBs and DDT) and TBT, rather than hormones. Several recommendations are made to improve the predictive and monitoring tools for detecting endocrine disrupting chemicals that might occur in the environment. However, the direct relevance of this CSTEE report to the environmental risk assessment of hormones used in beef is rather limited

The SCVPH Opinion Annex 1 reviews the three studies (Studies 14, 15 and 16) with relevance for the environment, and draws five principal conclusions:

- Aquatic animals are most sensitive to endocrine disruptors due to a greater potential for tissue accumulation.
- The environmental impact of anabolic steroids is potentially great.
- Further studies to determine the biological and chemical stability of such steroids in soil and water are warranted.
- Little information is available on the endocrine disrupting potential of the metabolites of MGA.
- Surface water downstream from a cattle feedlot was contaminated with oestrogenic and androgenic compounds, but the identity of these could not be established. Fish morphology near to cattle feedlots showed signs of endocrine disruption but, once again, the specific cause of this could not be identified.

The Working Group considered these five conclusions in turn, as follows:

(i) *Aquatic animals are most sensitive to endocrine disruptors due to a greater potential for tissue accumulation.* Two issues are confused here: the inherent sensitivity of an organism to a toxicant and the extent to which organisms in different environmental compartments (e.g., freshwater, seawater, land, or air) may be exposed to these contaminants. This conclusion requires further work in two areas before it can be accepted. There needs to be further ecotoxicological testing of the relative sensitivity of different terrestrial and aquatic organisms to growth-promoting hormones used in beef production. There also needs to be further environmental chemistry to determine the pathways taken by these hormones once they are released into the environment, to examine whether it is likely that they will reach aquatic systems.

(ii) *The environmental impact of anabolic steroids is potentially great.* This conclusion is based upon the findings from Study 14 (Schiffer *et al.* 2001) on the degradation kinetics of excreted trenbolone acetate (TBA) and melengestrol acetate (MGA) under different manure storage conditions. The study showed that both hormones are excreted in faeces and can be detected in soil

for up to several months when contaminated dung that has been stored for 4.5 to 5.5 months is applied. There was evidence that both trenbolone and MGA adsorb strongly to soil. The authors speculated that various physical or biological processes could eventually remove these hormones from soil, but no work was done to determine which, if any, of these removal processes is most likely. This was a well-performed study, but it did not demonstrate, or seek to demonstrate, that either of these hormones had an adverse impact on the environment. It simply demonstrates that there is a pathway for these hormones from beef cattle, through dung and into soil. A pathway from soil into either terrestrial or aquatic organisms, and subsequent biological effects in these organisms would need to be demonstrated before one could state that there is an environmental impact.

(iii) Further studies to determine the biological and chemical stability of such steroids in soil and water are warranted. This conclusion is also based on Study 14 (Schiffer *et al.* 2001) and agrees with Schiffer *et al.*'s conclusions. This is an appropriate conclusion to reach; clearly the conclusion stated in (ii) above cannot be supported until these stability studies are done.

(iv) Little information is available on the endocrine disrupting potential of the metabolites of MGA. This is also based on Study 14 (Schiffer *et al.* 2001).

(v) Surface water downstream from a cattle feedlot was contaminated with oestrogenic and androgenic compounds, but the identity of these could not be established. Fish morphology near to cattle feedlots showed signs of endocrine disruption but, once again, the specific cause of this could not be identified. These are based on Study 15, which has apparently not been published in the peer-reviewed literature, except for a brief summary in Jégou *et al.* (2001). The conclusions are also based on Study 16, which has only been published briefly as part of a review (Orlando and Guillette 2001). These published papers do not provide sufficient information to judge the quality of the work, although the researchers involved are acknowledged leaders in the field.

8.2 Conclusions and recommendations

The three environmental studies cited by the SCVPH opinion are important initial efforts to understand the environmental risks posed by use of growth-promoting hormones in beef production. However, it is clear, as acknowledged by SCVPH, that these studies do not provide strong evidence that growth-promoting hormones used in beef production are the cause of oestrogenic and androgenic activity in water below feedlots, or of de-masculinisation of fishes. Application of Hill's criteria or Koch's postulates to these results suggests that much more work needs to be done before uncertainties are reduced to a level at which an evidence-based decision can be made. In particular, the identity of the substances responsible for such effects needs to be established, probably through Toxicity Identification Evaluation procedures, and a more extensive set of sites should be investigated so that problems of pseudoreplication are avoided.

Study 14 has been reported fully in the peer-reviewed literature and shows that at least two of the hormones, or their metabolites, may be found in soil after dung spreading. However, pathways from soil (where these compounds may be strongly bound and therefore unavailable) to sensitive biological receptors, have not been established. In contrast to Study 14, the rather limited published results from Studies 15 and 16 show that endocrine disrupting substances seem to be present, and may be exerting biological effects, at river sites near to cattle feedlots. However, the substances responsible for these effects have not been identified, so a pathway from effects on environmental receptors to application of hormones in beef remains un-established.

The Working Group believes results from these studies are insufficient to demonstrate cause and effect. Research to establish a source-pathway-receptor linkage is required. The SCVPH should provide specific criteria for such research so that their decision is based upon a fair interpretation of the precautionary principle. It is assumed that if the re-introduction of growth-promoting substances for use within the EU were considered in the future, then a full environmental risk assessment would need to be conducted according to good current scientific practice

9 Other considerations

9.1 Formal risk assessment of hormonally active substances

The Working Group discussed whether it would be possible to undertake a formal risk assessment on hormonally active compounds from the available data on residue concentrations and consumption data. The substances of concern all have ADIs and thus, in theory, it would be possible to compare any estimated dose with the current ADIs (see Appendix A). To this end, the FSA are able to provide reliable data on food consumption for toddlers and adults that include dairy, meat and aquaculture products. The information on residue concentrations for the growth-promoting substances (whether natural or synthetic) in bovine meat and meat products, together with the data on UK food consumption, would in the future enable a total body dose to be calculated should this be required. This would require a better understanding of residue levels from both proper and improper use of the hormonal substances and, in the light of the newer scientific information, whether or not new ADIs might be required.

It is arguably also important to distinguish the natural hormones, which, if present in food, simply supplement those already circulating in the body, from the synthetic analogues which may have subtle differences in receptor binding, metabolism etc. For natural hormones it could be argued that the traditional approach of deriving ADIs by applying safety factors to NOELs is excessively conservative. The risks following ingestion of natural hormones in food which simply supplement those already circulating in the body can only truly be assessed from human data and should be put into context with the intake from other sources, the dose relative to normal circulating levels and the human evidence of the adverse effects of elevated circulating hormone levels. If the evidence suggests that any chronic increase in oestrogen levels will tend to increase the risk of breast cancer, as seems to be the case, we need to derive a view of an acceptable level of increase in that risk, and prioritise the sources of exposure for control against that value. Essentially this might mean advice to avoid any contribution of oestrogens from the diet for anyone already taking supplementary oestrogens.

The Working Group therefore believes that there is a need to gain a much better understanding of the impact of very small increases in oestrogen levels in human populations. If it is demonstrated that the contribution from meat from oestrogen-treated animals is not increased above that from untreated animals then the actual treatment is an irrelevance in the debate.

Synthetic hormones (and lipoidal esters) cannot be treated in the same way, since we do not have enough information about their interaction with natural hormones in humans, and probably the only way to arrive at an estimate of a safe human dose is to obtain more data in experimental animals. It would also be important to attempt to model the interaction of synthetic hormones with natural oestrogens in order to explore the possibility of their increasing or decreasing the incidence of tumours in humans.

9.2 Ban on over thirty-month cattle

The Working Group considered the effect of the end of the ban on cattle over 30 months of age entering the food chain. It noted that this would lead to a massive increase in the endogenous concentrations of oestradiol in meat reaching the consumer. Although it was also noted that this increase would be to pre-ban levels. This in itself would likely dwarf any increase in hormone levels reaching the consumer as a result of the use of growth-promoters, were they to be re-introduced. However, set against this consideration was the argument that, if 17 β -oestradiol increases the risk of breast cancer as previously discussed, then any avoidable increase, however small, would have to be viewed as undesirable.

The view was also expressed that other factors beyond our control are likely (and probably already do) have a bigger effect on hormone concentrations in food than the use of growth promoters, e.g. a

change in the type of sire used in the national dairy herd. The point was also raised that, if we were to continue to exclude growth promoters on the grounds that it would raise the exposure of hormones to the consumer, then logically one should consider excluding meat from pregnant animals and possibly those in oestrus.

JECFA (2000a) concluded in their evaluation of the numerous studies using authorised doses of the three natural steroids either alone or in combination that the hormone concentrations in edible tissues and blood were sometimes statistically significantly higher than the corresponding values found in concurrent controls but were always within the physiological range of these substances in bovine animals. The highest concentrations of progesterone and 17β -oestradiol are found in lactating and pregnant cows whereas extremely high concentrations of testosterone are found in bulls; the concentrations in treated calves and steers are significantly less than these natural levels.

The excess contribution of the residues to the ADIs set by JECFA is <4% for oestrogens, approx. 0.003% for progesterone and 0.2% for testosterone. Bearing in mind that post-pubertal humans produce very much larger quantities of these hormones, the margin of safety for adults consuming meat from treated animals is very high. The pre-pubertal child produces less natural steroids but will always consume less than the respective ADIs.

10 Conclusions and recommendations

10.1 Current scientific evidence for or against adverse effects

The previous sections have discussed the current new evidence relating to mutagenicity, carcinogenicity and endocrine disrupting effects of the hormonally-active substances and for humans who may be consuming meat from treated animals. Most of the evidence relates to 17β -oestradiol and the following key features are considered relevant to this and any future hazard or risk assessment:

- 17β -estradiol can be activated to catechol oestrogens and then oxidized to form semiquinones. The metabolites may also generate potentially mutagenic oxygen radicals by redox cycling.
- There is good evidence for the formation of DNA adducts from metabolites of 17β -oestradiol *in vitro* and *in vivo*.
- Synthesized oestrogen metabolite adducts are pre-mutagenic in sub-cellular test systems.
- There is some evidence for mutagenic potential (induction of chromosome damage and mutations) for some metabolites of oestradiol in mammalian cells *in vitro*.
- The evidence for induction of chromosome aberrations and gene mutations *in vivo* is poor and is derived from non-standard studies.
- There are, however, reasonable arguments against the carcinogenicity of 17β -oestradiol being due solely to epigenetic processes.
- It would be prudent to consider oestradiol and its metabolites as a complete carcinogen whilst more substantial evidence for its mode of action is obtained.
- Despite its possible genotoxicity, it is reasonable to consider that 17β -oestradiol may have a threshold for carcinogenicity due to the presence of homeostatic feedback mechanisms, the requirement for activation pathways to exceed inactivation pathways and the presence of antioxidants *in vivo*.

When it came to the current evidence base for 17β -oestradiol, however, in spite of certain data gaps, the view of most of the Working Group was that there is ample information to show that zootechnical and therapeutic uses of 17β -oestradiol do not pose any risk to humans unless an active implant site is ingested.

In relation to the other hormones considered, a number of points emerged in the Working Group discussions. One view was that in regard to the five other hormones (testosterone, progesterone, trenbolone, zeranol and MGA), one could agree with the SCVPH assessment, as expressed in Directive 2003/74/EC of the European Parliament and the Council of 22 September 2003, “that the current state of knowledge does not make it possible to give a quantitative estimate of the risk to consumers.” However, the majority of the Working Group felt that, in spite of the acknowledged data gaps and uncertainties, the available evidence on genotoxicity, tumorigenicity, hormonal activity and endocrine disrupting effects was supportive of the view that eating meat from animals treated with these five hormones was unlikely to be harmful to human health.

As a rider to these statements, it should be noted that they are based on the assumption that the consumer is exposed to no greater concentrations of residues than those arising from “correct” or “recommended” use of hormones. The likely misuse of growth-promoting substances is noted elsewhere in this report.

A number of additional points were made by members of the Working Group:

- In spite of the 17 additional studies funded by the Commission, little progress has been made to determine the safety of hormone growth promoters.
- One member felt strongly in support of the findings of the SCVPH – that much more work needs to be done before the safety of the six substances under consideration can be assured and approval given for their growth-promotional purposes.
- At the time the 1999 VPC Subgroup reported, they were unable to support the conclusions reached by the SCVPH “that risks associated with the consumption of meat may be greater than previously thought.” However, whilst the last 1999 VPC Subgroup reported that “none of the publications reviewed provide any substantive evidence that oestradiol was mutagenic/genotoxic”, the more recent evidence does indicate that:
 - a) metabolites of oestradiol do have the potential to be genotoxic, *in vitro* and *in vivo*; and
 - b) steroid metabolites previously considered to be nothing more than inactivation products, may have patho-physiological actions themselves.

10.2 Overall conclusions and recommendations

1. The Working Group were of the view that human exposure to residues of hormonally-active substances, including growth promoters in meat, could exert biological effects if exposure is at a sufficiently high level. Therefore, the two key issues are:
 - (i) determination of the dose-response induction of biological effects by the hormonally-active substances in test animals and, ideally, humans in order to identify a Lowest Observable Effect Level (LOEL), and
 - (ii) determination of the level (and range) of the additional human exposure and uptake from eating meat from treated animals.
2. These determinations should be made in adults and in developing (fetal/neonatal) animals and humans to identify the most sensitive index of effect. These effects would be in addition to those occurring naturally due to endogenous hormones.
3. The research so far has provided some, but not all the basic, but essential information outlined above. Without it, no definitive conclusions can be drawn; although the weight of available evidence suggests that likely levels of human exposure to hormonally-active

substances in meat from treated animals would not be sufficient to induce any measurable biological effect.

4. Specifically, it is very unlikely that the presence of 17β -oestradiol and its metabolites in meat from treated animals would significantly increase the risk of adverse effects in consumers. This is due to their low concentrations in comparison to those arising from endogenous production and from other dietary sources. Any increase would be likely to be small in the context of the whole food basket.

5. In reaching these conclusions, the Working Group expressed a number of qualifications and reservations based on the current lack of evidence of a risk to humans. These included:

- all scientific judgements made by the Working Group were based on the assumption that the consumer is exposed to residues at no greater concentrations than those that would be caused by the “correct” or “recommended” use of the exogenous hormones, be it for growth promotion or other zootechnical uses or therapeutic purposes;
- the Working Group understands that misuse of hormonally-active substances for growth-promotion is more likely than misuse for estrus synchronisation or therapeutic uses; and
- substances with hormonal action may be used in combination, both legally and illegally, while the toxicological and safety factors available (e.g. MRLs and ADIs) only relate to single substances.
- the Working Group had to decide what to do in the absence of information or where there was uncertainty of interpretation of information. One Member expressed the view that for the substances under consideration, there was a large element of uncertainty, so the precautionary principle must assume the primary consideration. The many uncertainties associated with the current lack of knowledge could be addressed by further research where this was both feasible and affordable. The Working Group was unanimous that all uncertainties must be made clear, especially those that were considered crucial in the risk assessment process.

6. As has been noted in this report, and acknowledged in the SCVPH Opinion, there are important gaps in the evidence base that preclude producing definitive risk assessments for 17β -oestradiol or the other five hormonally-active substances. Not all data gaps are equally important for the purposes of risk assessment and the Working Group highlighted a number that could improve future risk assessments. As an example, it would be helpful if the CVMP and JECFA could make available data on pharmacokinetics and metabolism of assessed compounds that were supplied in manufacturers’ dossiers. This openness and transparency would allow greater public scrutiny of the facts and confidence in the hazard and risk assessments produced.

7. The Working Group felt that none of the basic issues could be addressed without a structured approach. There is a need to establish precisely the:

- relationships between the potential use of growth-promoters (including over-use) and concentrations of residues in meat;
- levels of exposure in consumers (i.e. taking account of intake, absorption, bioavailability and metabolism); and
- dose-response relationships for the effects of the hormonally-active substances (and their metabolites) in experimental animals or in humans.

- further data on lipoidal oestrogens, possible bioaccumulation and possible synergistic effects of cocktails of hormonal substances would also be desirable

8. The Working Group noted specific needs:

- To establish in humans a detailed dose-response curve that relates exposure to specific hormonally-active substances to the amount of meat consumed from treated animals.
- To establish in experimental animals the relationship between intake of hormonally-active substances, or their metabolites, and target-organ effects (selecting the likely most sensitive target organ depending on the nature of the activity of the compound). This study to be conducted for adults and then fetal and/or neonatal exposure to be considered.
- To consider lipoidal esters of oestrogen in future studies of the possible passage of oestrogen in implants through cattle to humans. The bioavailability and metabolism of lipoidal esters following ingestion should be investigated to allow the biological significance of the oestrogens to be assessed.
- To carry out studies to confirm whether the ADI for pre-pubertal boys could be exceeded if they consumed a standard 500g portion of meat from an animal that had been treated with a number of hormonal implants. If confirmed this would be of concern.
- To establish an independent laboratory test to confirm that meat has not been derived from animals produced with the aid of growth promoting hormone implants.

Acknowledgements

The Working Group would like to thank Dr Karin Koller for her invaluable assistance in editing the Report. The Working Group would also like to thank Mr. David Webb for his secretarial and administrative support and production of the historical background and glossary sections of the report.

References and Bibliography

- Arnold D (1999) Estradiol-17 β , progesterone and testosterone. In: Residues of some veterinary drugs in animals and foods, 52nd JECFA meeting, FAO Food and Nutrition Paper 41-12. Available [Feb 2005] at: [ftp://ftp.fao.org/es/esn/jecfa/vetdrug/41-12-estradiol 17%DF progesterone testosterone.pdf](ftp://ftp.fao.org/es/esn/jecfa/vetdrug/41-12-estradiol%20progesterone%20testosterone.pdf)
- Ben-Rafael Z, Mastroianni L, Jr., Meloni F, Lee MS & Flickinger GL (1986) Total estradiol, free estradiol, sex hormone-binding globulin, and the fraction of estradiol bound to sex hormone-binding globulin in human follicular fluid. *J Clin Endocrinol Metab*, 63, 1106-1111
- Barham BL., Brooks JC, Blanton JR, Herring AD, Carr MA, Kerth CR, and Miller MF, (2003) Effects of growth implants on consumer perceptions of meat tenderness in beef steers. *Journal of Animal Science* 81:3052-3056.
- Beral V. Million Women Study Collaborators, Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet* 2003; 362: 419-27.
- Bolt, H.M., Janning, P., Michna, H. & Degen, GH. (2001). Comparative assessment of endocrine modulators with oestrogenic activity: I. Definition of a hygiene-based margin of safety (HBMOS) for xeno-oestrogens against the background of European developments. *Arch Toxicol* 74, 649-662.
- Braun, M.M., Ahlbom, A., Floderus, B., Brinton, L.A. & Hoover, R.N. (1995). Effect of twinship on incidence of cancer of the testis, breast and other sites (Sweden). *Cancer Causes & Control* 6, 519-524.
- Campbell-Thompson M, Reyher KK, Wilkinson LB (2001) Immunolocalization of estrogen receptor α and β in gastric epithelium and enteric neurons. *J Endocrinol* 171: 65-73.
- Cavalieri E, K Frenkel, J G Liehr, E Rogan and D Roy (2000) Estrogens as endogenous genotoxic agents – DNA adducts and mutagens. *J. Natl. Cancer. Monographs*, 27, 75-93.
- Chakravarti D, O C Mailander, K-M Li, S Higginbotham, H L Zhang, M L Gross, J L Meza, E L Cavalieri and E G Rogan (2001) Evidence that a burst of DNA depurination in SENCAR mouse skin induces error-prone repair and forms mutation in the H-ras gene. *Oncogene*, 20, 7945-7953.
- Chang L, Heitkemper MM (2002) Gender differences in irritable bowel syndrome. *Gastroenterology* 123: 1686-1701.
- Chiumello G. Guarneri MP. Russo G. Stroppa L. Sgaramella P. Joffe M. Thonneau P. Andersson A-M. Myers P. Toppari J. Hiff J. De Muinck Keizer-Schrama S. Kulin H. Daxenberger A. Bourguignon JP (2001). Accidental gynecomastia in children. *APMIS. Supplementum*. Vol. 109(103) S203-S209
- Chlebowski, R. T., Hendrix, S. L., Langer, R. D., Stefanick, M. L., Gass, M., Lane, D., Rodabough, R. J., Gilligan, M. A., Cyr, M. G., Thomson, C. A., Khandekar, J., Petrovitch, H., McTiernan, A. (2003). Influence of Estrogen Plus Progestin on Breast Cancer and Mammography in Healthy Postmenopausal Women: The Women's Health Initiative Randomized Trial. *JAMA* 289: 3243-3253
- Cosnes J, Carbonnel F, Carrat F, Beauverie L, Gendre JP (1999) Oral contraceptive use and the clinical course of Crohn's disease: a prospective cohort study. *Gut* 45: 218-222.
- COT (Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment) (2003). Phytoestrogens and health. Foods Standards Agency, London [Online: http://www.food.gov.uk/science/ouradvisers/toxicity/COTwg/wg_phyto/].
- Crisp, T.M., Clegg, E.D., Cooper, R.L., Wood, W.P., Anderson, D.G., Baetcke, K.P., Hoffmann, J.L., Morrow, M.S., Rodier, D.J., Schaeffer, J.E., Touart, L.W., Zeeman, M.G. and Patel, Y.M. (1998). Environmental endocrine disruption: an effects assessment and analysis. *Environ Health Perspect* 106 Suppl 1: 11-56.
- CSTEE. (1999). *CSTEE Opinion on Human and Wildlife Health Effects of Endocrine Disrupting Chemicals, with Emphasis on Wildlife and on Ecotoxicology Test Methods*. Report of the Working Group on Endocrine Disrupters of the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) of DG XXIX, Consumer Policy and Consumer Health Protection, European Commission, March 1999.
- CVMP (1999). Report of the CVMP on the Safety Evaluation of Steroidal Sex Hormones in Particular 17 β -oestradiol, Progesterone, Altrenogest, Flugestone acetate and Norgestomet in the Light of New Data/Information made available by the European Commission (EMEA/CVMP/885/99 Restricted Report).

- DeCosse JJ, Hgoi SS, Jacobson JS, Cennerazzo WJ (1993) Gender and colorectal cancer. *Eur J Cancer Prev* 2: 105-115.
- Endogenous Hormones and Breast Cancer Collaborative Group. (2002) Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *JNCI*, 2002; 94: 606-16.
- European Commission (2003) Directive 2003/74/EC of the European Parliament and of the Council of 22 September 2003 amending Council Directive 96/22/EC concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of beta-agonists. http://europa.eu.int/eur-lex/pri/en/oj/dat/2003/l_262/l_26220031014en00170021.pdf
- Feldman M, Richardson CT, Welsh JH (1983) Sex-related differences in gastrin release and parietal cell sensitivity to gastrin in healthy human beings. *J Clin Invest* 71: 715-720.
- Hammond, G.L. (2002) Access of reproductive steroids to target tissues. *Obstet Gynecol Clin North Amer* 29, 411-423.
- Hankinson SE, Manson JE, Spiegelman D, Willett WC, Longcope C, Speizer FE (1995). Reproducibility of plasma hormone levels in postmenopausal women over a 2-3-year period. *Cancer Epidemiology, Biomarkers & Prevention*. 4(6): 649-54.
- Hawkey CJ, Wilson I, Naesdal J, Langstrom G, Swannell AJ, Yeomans ND (2002) Influence of sex and helicobacter pylori on development and healing of gastroduodenal lesions in non-steroidal ant-inflammatory drug users. *Gut* 51: 344-350.
- Henricks, D.M., Gray, S.L., Owenby, J.J. & Lackey, B.R. (2001). Residues from anabolic preparations after good veterinary practice. *APMIS* 109, 273-283.
- Hoogenboom L A P (2000) Investigation on the metabolism of 17 β -estradiol by bovine hepatocytes, human intestinal and breast cells and the genotoxic and estrogenic properties of the metabolites. Unpublished report, RIKILT Research Institute.
- 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.
- JECFA (1999) Joint FAO/WHO Expert Committee on Food Additives, Fifty-second meeting, Rome, 2-11 1999 [published as WHO, 2000].
- JECFA (2000a) Residues of some Veterinary Drugs in animals and food. 52nd meeting 1999. Estradiol-17 β , Progesterone and Testosterone. FAO Food and Nutrition Paper 41/12, pp 37-90.
- Jégou B, Soto A, Sundlof S, Stephany R, Meyer H, Leffers H. (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.
- Jordan, V.C. (1998). Designer estrogens. *Sci Amer* 279, 60-67.
- Kappel, B., Hansen, K., Moller J. & Faaborg-Andersen, J. (1985). Human placental lactogen and dU-estrogen levels in normal twin pregnancies. *Acta Genet Med Gemello (Roma)* 34, 99-106.
- Kajiser M, Lichtenstein P, Granath F, Erlandsson G, Cnattingius S, Ekblom A (2001). In utero exposures and breast cancer: A study of opposite-sexed twins. *Journal of the National Cancer Institute* 93(1): 60-62).
- Kong L-Y, P Szaniszló, T Albrecht and J G Liehr (2000) Frequency and molecular analysis of hprt mutations induced by estradiol in Chinese hamster V79 cells. *Int J Oncology*, 17, 1141-1149.
- Lange, I.G., Daxenberger, A. & Meyer, H.H.D. (2001). Hormone contents in peripheral tissues after correct and off-label use of growth promoting hormones in cattle: effect of the implant preparations Finaplix-H, Ralgro, Synovex-H and Synovex Plus. *APMIS* 109, 53-65.
- Lange IG, Daxenberger A, Meyer HHD, Rajpert-De Meyts E, Skakkebaek NE, Veeramachaneni DNR (2002) Quantitative assessment of foetal exposure to trenbolone acetate, zeranol and melengestrol acetate, following maternal dosing in rabbits. *Xenobiotica* 32(8) 641-651.
- Lavigne J A, J E Goodman, T Fonong, S Odwin, P He, D W Roberts and J D Yager (2001) The effects of catechol-o-methyltransferase inhibition on estrogen metabolite and oxidative DNA damage levels in estradiol-treated MCF-7 cells. *Cancer Res.*, 61, 7488-7494.
- Le Bizec B et al (2000) Le controle des anabolisants dans la viande. *Annales de Toxicologie Analytique Vol XII no. 1*

- Leffers H, Naesby M, Vendelbo B, Skakkebaek NE, Jorgensen M, Grandjean P, Sippell W, Soto A, Vollmer G, Meyer H (2001). Oestrogenic potencies of Zeranol, oestradiol, diethylstilboestrol, Bisphenol-A and genistein: Implications for exposure assessment of potential endocrine disrupters. *Human Reproduction* 16(5): 1037-1045.
- Liehr J C (2001) Genotoxicity of the steroidal oestrogens oestrone and oestradiol: possible mechanism of uterine and mammary cancer development. *Human Reproduction Update*, 7, 273-281.
- Lindsay D G (1985) Zeranol – a ‘Nature-Identical’ Oestrogen? *Fd Chem. Toxic.* 23, 8, 767-774
- Mader TL, (1997) Carry-over and lifetime effects of growth promoting implants. OSU Conference: Proc. Impact of implants on performance and carcass value of beef cattle, pp 88-94.
- Mastri C, Mistry P & Lucier GW (1985) *In vivo* oestrogenicity and binding characteristics of α -zearalanol (P-1496) to different classes of oestrogen binding proteins in rat liver. *J Steroid Biochem*, 23, 279-289
- Maume, D., Deceunick, Y., Pouponneau, K., Paris, A., Le Bizec, B. & Andre, F. (2001). Assessment of estradiol and its metabolites in meat. *APMIS* 109, 32-38.
- Metzler M, Pfeiffer E (2001) Genotoxic potential of xenobiotic growth promoters and their metabolites. *APMIS* 109, 89-95.
- Nagel SC, vom Saal FS & Welshons WV (1998) The effective free fraction of estradiol and xenoestrogens in human serum measured by whole cell uptake assays: physiology of delivery modifies estrogenic activity. *Proc Soc Exper Biol Med*, 217, 300-309
- Noller, K.L. (1983). In utero diethylstilbestrol exposure: Structural and epithelial abnormalities. *Cervix Low Female Gen Tract* 1, 75-82.
- Orlando EF, Guillette Jr. LJ. (2001). A re-examination of variation associated with environmentally stressed organisms. *Human Reproduction Update* 7:765-272.
- Platter WJ, Tatum JD, Belk KE, Scanga JA, and Smith GC, (2003). Effects of repetitive use of hormonal implants on beef carcass quality, tenderness, and consumer ratings of beef palatability. *Journal of Animal Science* 81; 984-996.
- Richold M (1988) The genotoxicity of trenbolone, a synthetic steroid. *Arch. Toxicol*, 61, 249-258.
- Scheffler JM, Buskirk DD, Rust SR, Cowley JD, and Doumit ME, (2003), Effect of repeated administration of combination trenbolone acetate and oestradiol implants on growth carcass traits, and beef quality of long-fed Holstein steers. *Journal of Animal Science* 81; 2395-2400.
- Schiffer B, Daxenberger A, Meyer K, Meyer HHD. (2001). The fate of trenbolone acetate and melengesterol acetate after application as growth promoters in cattle: environmental studies. *Environmental Health Perspectives* 109:1145-1151.
- SCVP (1999) Scientific Committee on Veterinary measure relating to Public Health. 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 (30 April 1999). Available [DATE] at: http://europa.eu.int/comm/food/fs/sc/scv/out21_en.html
- SCVPH (2000) Scientific Committee on Veterinary measure relating to Public Health. Review Of Specific Documents Relating To The SCVPH Opinion Of 30 April 99 On The Potential Risks To Human Health From Hormone Residues In Bovine Meat And Meat Products. Available [DATE] at: http://europa.eu.int/comm/food/fs/sc/scv/out33_en.pdf
- SCVPH (2002) Scientific Committee on Veterinary measure relating to Public Health. 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). Available [DATE] at: http://europa.eu.int/comm/food/fs/sc/scv/out50_en.pdf
- Sharpe, R.M. (2003). The ‘oestrogen hypothesis’ – where do we stand now” *Int J Androl* 26, 2-15.
- Sharpe, R.M. & Franks, S. (2002) Environment, lifestyle and infertility - an inter-generational issue. *Nature Medicine (Supplement 10)*: s33-s40
- Singleton GH & Dobson H (1995). A survey of the reasons for culling pregnant cows. *Vet Record* 136(7): 162-165.

- Shrimanker K, Salter LJ & Patterson RL (1985) Binding of steroid hormones and anabolic agents to bovine sex-hormone binding globulin. *Horm Metab Res*, 17, 454-457
- Smith IE, Dowsett M (2003). Aromatase inhibitors in breast cancer. *New England Journal of Medicine*. 348(24): 2431-2442
- Swerdlow, A.J., De Stavola, B.L., Swanwick, M.A., & Maconochie, N.E.S. (1997). Risks of breast and testicular cancers in young adult twins in England and Wales: evidence on prenatal and genetic aetiology. *Lancet* 350, 1723-1728.
- Terashima I, Suzuki N, Shibutani S (2001) Mutagenic properties of estrogen quinone-derived DNA adducts in Simian Kidney cells. *Biochemistry*, 40, 166-172.
- Toppiari, J., Larsen, J.C., Christiansen, P., Giwercman, A., Grandjean, P. et al. (1996). Male reproductive health and environmental xenoestrogens. *Environ Health Perspect* 104 (Suppl 4), 741-803.
- Tsutsui, T, Tamura Y, Hagiwara M, Miyachi T, Hikiba H, Kubo C, Barrett JC (2000a) Induction of mammalian cell transformation and genotoxicity by 2-methoxyestradiol, an endogenous metabolite of estrogen. *Carcinogenesis*, 21, 735-740.
- Tsutsui, T, Tamura Y, Yagi E, Barrett JC (2000b) Involvement of genotoxic effects in the initiation of estrogen-induced cellular transformation: Studies using Syrian Hamster Embryo cells treated with 17 β -estradiol and eight of its metabolites. *Int. J. Cancer*, 86, 8-14.
- Tuvemo, T. & Proos, L.A. (1993). Girls adopted from developing countries: a group at risk of early pubertal development and short final height. Implications for health surveillance and treatment. *Ann Med* 25, 217-219.
- Virdis, R., Street, M.E., Zampolli, M., Radetti, G., Pezzini, B. et al. (1998). Precocious puberty in girls adopted from developing countries. *Arch Dis Child* 78, 152-154.
- WHO (2000) Joint JECFA/WHO Food Additive Series 43; Toxicological evaluation of certain veterinary drug residues in food: Estradiol-17 β , progesterone and testosterone. World Health Organization, Geneva. Available at: <http://www.inchem.org/documents/jecfa/jecmono/v43jec05.htm>
- WHO (2002) Global Assessment of the State-of-the-Science of Endocrine Disruptors (International Programme on Chemical Safety, WHO/PCS/EDC/02.2), Geneva, Switzerland, World Health Organization, Available [August 2004] at: http://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/
- Witorsch, R.J. (2002). Low-dose in utero effects of xenoestrogens in mice and their relevance to humans: an analytical review of the literature. *Food Chem Toxicol* 40, 905-912.
- Yagi E, Barrett JC, Tsutsui T (2001) The ability of four catechol estrogens of 17 β -estradiol and estrone to induce DNA adducts in Syrian hamster embryo fibroblasts. *Carcinogenesis*, 22, 1505-1510.
- Yared E, McMillan TJ, Martin FL (2002) Genotoxic effects of oestrogens in breast cells detected by the micronucleus assay and the Comet Assay. *Mutagenesis*, 17, 345-352.
- Yen, S, Martin, P; Burnier, A; Czekala, N; Greaney, M and Callantine, M (1975) Circulating estradiol, estrone and gonadotropin levels following administration of orally active 17 β -estradiol in postmenopausal women. *Journal of Clinical Endocrinology and metabolism*, 40, 518-521.

Glossary

Altered redox status – An alteration in the balance between oxidation and reduction as a result of alterations in enzyme or cofactor levels.

Aneugens acting by inhibition of spindle formation – aneugens are chemicals which alter the number of chromosomes in a cell. They may do this by altering the formation of the spindle which is responsible for distributing chromosomes into the daughter cells during cell division. This may be a step in the process of carcinogenesis or, if it occurs in germ cells, it may give rise to birth defects.

Aromatase inhibitors – Drugs that act by lowering the amount of oestrogen made in the body after menopause. Lowering the concentration of oestrogen can slow or stop the growth of cancer that needs oestrogen to grow. Arimidex, Femara, and Aromasin are brand names of this type of drug.

Bioavailable – Able to be absorbed into the body and reach target cells.

BMI – Body Mass Index is a formula that uses weight and height to estimate body fat and gauge health risks due to carrying too much weight. It is not definitive as the two measures used – height and weight – do not accurately measure the amount of lean or fat that a person has.

Bone marrow micronucleus test – an assay which measures the induction of chromosome damage in red blood precursor cells in the bone marrow of rodents. Broken or whole chromosomes which have been separated from the rest of the chromosome set during cell division are visualized as small staining bodies (micronuclei) in blood cells which lack nuclei.

Catechol-O-methyl transferase (COMT) – The enzyme which adds a methyl group to catechols.

Clastogenicity – Induction of chromosome breakage. This is believed to be an important step in carcinogenesis and can also contribute to birth defects.

Comet assay – An assay which detects breaks in the single strands of DNA or the loss of bases from DNA. The method is based on the differential movement of DNA after electrophoresis from cells containing damaged DNA compared to undamaged cells. It indicates that DNA damage has occurred, but it may not be indicative that this damage would result in permanent changes to the genetic material.

Cryptorchidism – means ‘concealed testicle’ where one or both of the testes have not descended into the scrotum by a particular age.

Cytochalasin B – A chemical added to cells to stop the cell membrane dividing so that the cells become binucleate i.e. they have two nuclei within one cytoplasm.

Cytogenetics assay – An assay for testing whether a chemical is able to cause chromosome breakage or rearrangements. It is used as a screen for chemicals able to induce cancer.

Cytotoxicity – the degree to which a substance is toxic to living cells.

Depurinating – able to remove the purine bases (one of the two sorts of building blocks) in DNA.

DNA adducts – a modification to the DNA by the addition of a reactive chemical moiety to a base. DNA adducts indicate that a substance is able to interact with the genetic material. They may however be repaired without any long term consequences to the cell.

Endocrine – system of glands that secrete (produce) hormones.

Endogenous – endogenous hormones are those produced by the animal.

Endometritis – is an inflammation and/or irritation of the endometrium (lining of the uterus)

Exogenous – exogenous hormones are from an outside source, such as might be found in meat that forms part of our diet.

Feedlot – these are intensive systems of rearing beef, used in the USA. Rather than grazing in fields, the bovines are kept penned on concrete or in dirt corrals.

G to T transversions – Refers to changes in the composition of DNA (mutations) involving the replacement of a guanine base with a thymine base. The result may be an alteration in the product of the DNA causing a change in the function of a protein.

GC/MS – Gas chromatography/ mass spectrometry.

Genotoxic – Able to induce damage to the genetic material. This may be repaired without consequences for the affected cell or it may result in a permanent change (=mutation).

Gestagen – Hormonally active substances that have a similar effect to progesterone. An example is allyl trenbolone. Gestagens can be used to control and synchronise oestrus.

Glucuronidation – The addition of glucuronic acid to a substrate. It generally, but not always, results in a decrease in biological activity and toxicity.

GSTu3 – A type of glutathione transferase enzyme, responsible for the addition of a glutathione group to various substrates. This generally, but not always, results in a decrease in biological activity and toxicity.

Gynaecomastia – breast development in males.

Hepatocytes – The main structural component of the liver. They are specialized epithelial cells that are organized into interconnected plates called lobules.

HPLC – High pressure liquid chromatography.

HPRT locus in V79 cells: – the gene which encodes the enzyme hypoxanthine-guanine phosphoribosyl transferase. Mutations in this enzyme result in an inability to take up a toxic substance added to cell culture such that only mutants will be able to grow. This allows a method of scoring mutations induced by chemicals. V79 cells are an established cell line, originally obtained from a hamster and extensively used in genotoxicity assays.

in vitro – literally means *in glass*. It refers to experiments performed on biological processes outside the living organism.

in vivo – refers to experiments performed on biological processes in a living organism.

L5178Y cells – a cell line which was derived from a mouse lymphoma and which is used in gene mutation assays. It has a single mutant gene at the thymidine kinase locus and mutations in the remaining intact gene result in resistance to a toxic agent added to the cell culture. In this way it can be used to assess the ability of a chemical to induce gene mutations.

lacI loci – A gene, originally obtained from bacteria, which is used for measuring the ability to induce gene mutations.

Metal homeostasis – The ability of a cell to maintain normal levels of metals and metal ions so that essential pathways which require metal ions and metal containing components are not perturbed.

Methotrexate resistance – Methotrexate is a chemical which is poisonous to mammalian cells. Cells which are mutated to methotrexate resistance can be selected for in culture by the addition of this agent.

Micronuclei – Small cellular bodies which contain DNA which are either derived from chromosomes by chromosome breakage or which result from the loss of a whole chromosome during cell division.

Microsatellite assay – A non-standard genotoxicity assay where chromosome breakage is detected.

Multinucleate cells – Cells with many nuclei (not a normal occurrence except for some cells such as muscle.)

Mutagenicity – changes to the genetic material which result in permanent changes. Depending on which genes are affected the result may have significant long-term effects.

Negative feedback – in relation to hormones, it means that when exogenous hormones are administered, the body reacts by reducing the amount of endogenous hormones that are available.

OECD guideline compliance – the Organization for Economic Cooperation and Development (OECD) provides guidelines on how testing should be performed for the evaluation of safety of substances. Substances seeking regulatory approval are expected to have testing performed to these guidelines.

Oligodeoxyribonucleotides adducted with hydroxyoestrogen moieties – Short segments of DNA which have had modified oestrogen molecules joined to them. These are then inserted into longer segments of DNA and used in an assay to determine whether these modified DNA nucleotides will result in mutations.

Phase 1 and Phase 2 enzymes. The first step of metabolism of a foreign molecule, Phase 1, involves the addition of a functional group. The major types are oxidation, reduction, hydrolysis, hydration and dehalogenation. It may lead to a more active molecule. The second phase involves conjugation with another substance, such as glucuronic acid or glutathione and may lead to a less active substance which can be excreted from the body.

Phytoestrogens – are naturally occurring compounds found in plants; these compounds structurally or functionally similar to 17- β oestradiol or exert oestrogenic effects.

Placebo-controlled – a standard test technique where a treatment is compared to a seemingly similar treatment, that contains no active ingredient.

Postpartum anoestrus – the period after giving birth where there is no cyclical ovarian activity.

Precautionary principle – is a phrase from the late 1980s that indicates that if the consequences of an action, especially use of a technology are unknown but may have a high risk of a negative outcome, then until better knowledge is obtained the technology should not be used.

Prostaglandins – a group of extremely active substances in the body that affect many organs. Certain prostaglandins have a role in stimulating the uterine contractions of labour and birth

Purulent vaginitis – a Inflammation of the vagina characterised by pain and a discharge of pus.

Pyometra – an infection of the uterus.

SENCAR mice – a strain of mice commonly used for studies of susceptibility and resistance to the induction of skin tumours.

SHBG – Sex hormone binding globulin: A glycoprotein, synthesized in the liver, which binds testosterone and 5 alpha-dihydrotestosterone strongly, and estradiol somewhat less strongly. Circulating testosterone is mainly bound to protein - primarily SHBG, but also to albumin and cortisol-binding globulin. Since variations in the carrier protein levels may affect the concentration of testosterone in circulation, SHBG levels are commonly measured as a supplement to total testosterone determinations. The "free androgen index" (FAI), calculated as the ratio of total testosterone to SHBG, has proved to be a useful indicator of abnormal androgen status in conditions such as hirsutism.

SHE assay – A genotoxicity assay which uses primary cells derived from Syrian hamster embryo cells. The morphology of the cells is scored after treatment with a chemical and the incidence of cells which appear to have "transformed" (i.e. have defined morphological changes which are thought to be related to carcinogenesis) is scored.

Somatic – any cells of a plant or animal other than the reproductive cells.

Therapeutic - therapeutic uses of hormonal substances relate to a product being applied to an animal with a clinical condition.

Thresholded / non-threshold – substances that can exert an effect may not do so unless a critical concentration is reached. This is a threshold. Substances that are non-thresholded may cause an effect at any concentration.

Trout SBP – a steroid binding protein in trout.

Weight gain stasis – weight is stable.

Zootechnical - zootechnical uses of hormonal substances relate to a product being applied to a healthy animal to synchronise oestrus, terminate unwanted gestation, prepare donors and recipients for the implantation of embryos, and improve fertility.

Appendix A: Tables of ADIs set for hormonally active substances

ADIs set by JECFA

Substance	ADI (µg/kg bw)	Safety Factor applied	Basis set	When set (Meeting/year)
17β-oestradiol	0 - 0.05	100	Hormone-dependant parameters in post-menopausal women	52/1999
Progesterone	0 – 30.0	100	Uterine changes in women	52/1999
Testosterone	0 – 2.0	1000	Restoration of sexual function in eunuchs	52/1999
Melengestrol acetate	0 - 0.03	200	Effects on the menstrual cycle of monkeys	54/2000
Trenbolone acetate	0 - 0.02	100	14-week no-hormonal effect study in pigs, supported by maturation of vaginal epithelial cells in ovariectomised female monkeys	34/1989
Zeranol	0 - 0.50	100	Maturation of vaginal epithelial cells in ovariectomised female monkeys	32/1987

Assessments of hormonally active substances by the CVMP

Substance	ADI (µg/kg bw)	Safety Factor applied	Basis set	When set (year)
17β-oestradiol			For zootechnical and therapeutic use, no ADI or MRL need be established	1994
Progesterone			For zootechnical and therapeutic use, no ADI or MRL need be established	1994
Testosterone				
Melengestrol acetate				
Allyl trenbolone	0.04	100	No hormonal effect level in monkeys and pigs (This being lower than the toxicological NOEL observed in a 2-generation reproduction study with rats)	1999
Zeranol				

Appendix B: The 17 EU-funded studies and related publications

Study Number	Title of the study Principal worker Institute Reports and Publications
1	Presence of estrogen in meat (delivery of samples)
	No publications to be done
2	Hormones as growth promoters: genotoxicity and mutagenicity of Zeranol & Trenbolone
	Dr Manfred Metzler Institute of Food Chemistry, University of Karlsruhe <i>Unpublished contract report:</i> Hormones as growth promoters: genotoxicity and mutagenicity of Zeranol & Trenbolone <i>Published paper:</i> Metzler M & Pfeiffer E (2001) Genotoxic potential of xenobiotic growth promoters and their metabolites APMIS 109:89-95
3	Metabolic pathways of estrogens as steroidal growth promoting agents
	Dr Alain Paris Institut National de la Recherche Agronomique, Laboratoire des Xenobiotiques <i>Unpublished contract report:</i> Estradiol-17 β , Metabolic pathways of estrogens used as steroidal growth promoting agents
3a	Dr Daniel Maume Laboratoire Des Dosages Hormonaux, Nantes France <i>Unpublished contract report:</i> Metabolic pathways of estrogens used as steroidal growth promoting agents <i>Published paper :</i> Maume, D., Deceunick, Y., Pouponneau, K., Paris, A., Le Bizec, B. & Andre, F. (2001). Assessment of estradiol and its metabolites in meat. APMIS 109, 32-38.
3b	Dr LAPG Hoogenboom Department of Food Safety and Health, State Institute for Quality Control of Agricultural products (RIKILT) <i>Unpublished contract report:</i> Investigations on the metabolism of 17 β estradiol by bovine hepatocytes, human intestinal and breast cells, and the genotoxic and estrogenic properties of the metabolites <i>Published paper:</i> 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: 101-7, 2001

4	Metabolites of melengestrol acetate, trenbolone acetate & zeranol in bovine & humans
	Dr Manfred Metzler Institute of Food Chemistry and Toxicology, University of Karlsruhe, Germany <i>Unpublished contract report:</i> Metabolism of melengestrol acetate and trenbolone; (publication foreseen).
5	Application of anabolic agents to food producing animals- health risks through disregard of requirements of good veterinary practice
	Andreas Daxenberger Institut für Physiologie, D-85350 Freising- Weihenstephan, Germany <i>Unpublished contract report:</i> Application of anabolic agents to food producing animals - health risks through disregard of requirements of good veterinary practice. <i>Published papers:</i> 1) Daxenberger A, Meyer K, Hageleit M, Meyer HHD (1999) Detection of melengestrol acetate residues in plasma and edible tissues of heifers, Vet Quart 21:154-158. 2) Daxenberger A, Lange IG, Meyer K, Meyer H (2000). Detection of anabolic residues in misplaced implantation sites in cattle Journal of AOAC International 83(4); 809-819 3) Daxenberger A, Hageleit M, Kraetzl W-D et al (2001) Suppression of tandrostenone in entire male pigs by anabolic preparations. Livestock Production Science 69: 139-144 4) Hageleit M, Daxenberger A, Meyer HHD (2001) A sensitive enzyme immunoassay (EIA) for the determination of Melengestrol acetate (MGA) in adipose and muscle tissues. Food Additives and Contaminants 18(4): 285-291 5) 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 the bovine gestagens receptor APMIS 108(12): 838-46 6) Hageleit M, Daxenberger A, Kraetzl W-D, Kettler A, Meyer HHD (2000) Dose dependent effects of melengestrol acetate (MGA) on plasma levels of estradiol, progesterone and luteinizing hormone in cycling heifers and influences on oestrogen residues in edible tissues APMIS 108(12): 847-854 7) Lange IG, Daxenberger A, Meyer HHD (2001) Hormone contents in peripheral tissue after correct and off-label use of growth promoting hormones in cattle: Effect of the implant preparations Finaplix-H®, Ralgro®, Synovex-H® and Synovex Plus® APMIS 109(103) 53-65 8) 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(5): 345-55
6 and 7	Analysis of 500 samples for the presence of growth promoters
	R.W Stephany and F Andre National Institute of Public Health and the Environment RIVM - Bilthoven- The Netherlands <i>Unpublished contract report:</i> Results of 'hormone' residue analysis of bovine liver originating in the USA and imported into the EU as pet food. <i>Published papers:</i> 1) Stephany RW, Gregor K, Stephany R, McLachlan J, Risbridger G (2001) Hormones in meat: Different approaches in the EU and in the USA. APMIS. Supplementum. Vol. 109(103) S357-S364 2) Stephany RW (2000) : Abstract of a lecture: Hormones found in meat samples from regular controls within the EU and from US imports Chemical awareness; issue 9, July 5th 3) Marchand, P, le Bizec B, Gade, C et al (2000) Ultra trace detection of a wide range of anabolic in meat by gas chromatography coupled to mass spectrometry. J Chromatography A, 867: 219-233 4) le Bizec B, Marchand P, Andre F (2000) Le contrôle des anabolisants dans la viande (The survey of anabolic agents in meat.) Annales de Toxicologie Analytique, vol.XII, no.1 : 56-63

8	Comparison of assay methods
	<p>Joachim Liehr The Stehlin Foundation for Cancer Research, Houston, Texas, USA <i>Unpublished contract report:</i> Estrogen in Meat: Comparison of Assay Methods <i>Published papers:</i> 1) Kong L-Y, Szaniszló P, Albrecht T, Liehr J (2000) Frequency and molecular analysis of hprt mutations induced by estradiol in Chinese hamster V79 cells. <i>Int J Oncology</i> 17:1141-1149 2) Cavaliere E, Frenkel K, Liehr JG, Rogan E, Roy D (2000) <i>Estrogens as endogenous genotoxic agents-DNA adducts and mutations</i> <i>J Nat Cancer Inst Monographs</i> 27, 75-93 3) Jefcoate CR, Liehr JG, Santen RJ et al (2000) Tissue-specific synthesis and oxidative metabolism of estrogens, <i>J Nat Cancer Inst Monographs</i> 27, 95-112 4) Newbold RR, Liehr JG (2000) Induction of uterine adenocarcinoma in CD-1 mice by catechol estrogens <i>Cancer Research</i> 60:235-237 5) Liehr JG (2001) Genotoxicity of the steroidal estrogens estrone and estradiol: possible mechanism of uterine and mammary cancer development. <i>Human Reproduction Update</i> 7(3): 273-281</p>
9	Bioassay of estrogenic/antiestrogenic compounds
	<p>Dr Remy le Guevel and Dr Farzad Pakdel Equipe d'Endocrinologie Moléculaire de la Reproduction, UPRES -A CNRS 6026 Université de Rennes <i>Unpublished contract report:</i> Bioassay for screening and determination of estrogenic potency of Chemicals used as Growth Promoters. <i>Published paper:</i> Le Guevel R, Pakdel, F (2001) Assessment of oestrogenic potency of chemicals used as growth promoter by in-vitro methods. <i>Human Reproduction</i> 16:1030-1036</p>
10	Interaction of xenobiotics with sex hormone binding globulin; impact on endogenous steroid transport, bioavailability, mechanism of action
	<p>Dr Florence le Gac Station Commune de recherche en Ichtyophysiologie, Biodiversité, et Environnement (SCRIBE) <i>Unpublished contract report:</i> Interaction of xenobiotics with sex hormone binding globulin (SHBG/SBP), a study contract with EC concerning the "assessment of the effects of hormones on human health and the environment". [The Principal Investigator has not yet indicated name of journal and publication date]</p>
11	Reproductive sequelae of developmental exposure of rabbits to trenbolone, zeranol & MGA; emphasis on differential & neoplastic transformation of germcells
	<p>Dr E. Rajpert-De Meyts M.D. Dept of Growth & Reproduction, Copenhagen University Hospital and Animal Reproduction and Biotechnology, Colorado State University <i>Unpublished contract report:</i> Reproductive sequelae of developmental exposure of rabbits to trenbolone, zeranol & MGA; emphasis on differential & neoplastic transformation of germcells <i>Published paper:</i> G. Lange, A. Daxenberger, H. H. D. Meyer, E. Rajpert-De Meyts, N. E. Skakkebak, D. N. R. Veeramachaneni. (2002) Quantitative assessment of foetal exposure to trenbolone acetate, zeranol and melengestrol acetate, following maternal dosing in rabbits. <i>Xenobiotica</i> 32(8): 641-651.</p>

12	Long term effects in children to estrogenized meat
	<p>Professor Guiseppe Chiumello, Head of the Centre for Infant and, Adolescent Endocrinology, Life and Health University, San Raffaele Scientific Institute, Milan, Italy</p> <p><i>Unpublished contract report:</i> Long-term effects in children exposed to estrogen-contaminated meat: a retrospective study.</p> <p><i>Published paper:</i> Chiumello G, Guarneri MP, Russo G et al (2001) Accidental gynecomastia in children. APMIS 109 (suppl.103):S203-9</p>
13	Androgen exposures in utero, risk of breast cancer
	<p>Dr Magnus Kaijser Dept of Medical Epidemiology, Karolinska Institutet. Stockholm, Sweden</p> <p><i>Unpublished contract Report:</i> In utero exposures and breast cancer. A study of female twins and male co-twins.</p> <p><i>Published paper:</i> Kaijser M, Lichtenstein P, Franath F et al (2001) <i>In Utero</i> Exposures and Breast Cancer : A study of opposite-sexed twins. J Nat Cancer Institute 93(1): 60-62</p>
14	Endocrine disrupting activity of anabolic steroids used in cattle
	<p>Dr Andreas Daxenberger Institute of Physiology, Technical University Munich</p> <p><i>Unpublished contract Report:</i> Endocrine disrupting activity of anabolic steroids used in cattle</p> <p><i>Published papers:</i></p> <p>1) Bauer ERS, Daxenberger A, Petri T et al (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 progesterin receptor APMIS 108: 838-46</p> <p>2) Schiffer, B., Daxenberger, A., Meyer, K., and Meyer H.H.D, (2001) The fate of trenbolone acetate and melengestrol acetate after application as growth promoters in cattle - environmental studies. Environmental Health Perspectives 2001,109, .11, 1145-1151.</p>
15	Screening water samples for estrogenic &androgenic anabolic chemicals
	<p>PI Ana M Soto M.D. Tufts University, School of Medicine, Dept of Cell Biology, Boston, USA</p> <p><i>Unpublished Contract Report:</i> Anabolic hormones in water draining feedlots</p> <p><i>Published paper:</i> Soto <i>et al</i>, 2004 Androgenic and Estrogenic Activity in Water Bodies Receiving Cattle Feedlot Effluent in Eastern Nebraska, USA Environmental Health Perspectives, 2004 112(3) 346-52. Some results can be found in APMIS 109-suppl.103;pp 551-6, 2001 General discussion on " Existing guidelines for the use of meat hormones and other food additives in Europe and USA"</p>
16	Endocrine disrupting effects of cattle farm effluent on environmental sentinel species
	<p>Prof Louis Guillette Dept of Zoology, University of Florida</p> <p><i>Unpublished Contract Report:</i> Endocrine disrupting effects of cattle feedlot effluent on environmental sentinel species</p> <p><i>Published paper:</i> Orlando EF, Guillette LJ (2001) A re -examination of variation associated with environmentally stressed organisms. Human Reproduction Update,7(3):.265-272</p>

17	Human cells exposed to the estrogenic compound zeranol
	<p>Dr Henrik Leffers Rigshospitalet, Copenhagen, Denmark.</p> <p><i>Unpublished Contract Report:</i> Human cells exposed to the estrogenic compound zeranol: dose-dependant effects on gene expression.</p> <p><i>Published paper:</i> Leffers H, Naesby M, Vendelbo B et al (2001) Oestrogenic potencies of zeranol, estradiol, diethylstilboestrol, Bishpenol-A, and genistein: Implications for exposure assessment of potential endocrine disrupters. Human Reproduction 16: 1037-1045</p>

Appendix C: Metabolism and pharmacokinetics of growth promoting hormones

This account of some aspects of the metabolism of pharmacokinetics of the growth promoting hormones has been included to supplement the information given in the main report and is provided for those who require further information. It is also helpful to assist explanation of the other reproductive and therapeutic uses of these substances. Some information, for completeness, is also included on progesterone and testosterone.

1. Estradiol

1.1 Oestradiol ('Oestradiol' or 'Estradiol'; E_2), more properly called 17- β Oestradiol or Oestradiol-17- β , is the most potent of the physiological oestrogens in mammals, including humans, cattle and sheep. It is readily converted in the body by 17 β -hydroxysteroid dehydrogenase to oestrone ('Estrone'; E_1), which also has oestrogenic activity, and then to various hydroxy and sulphated metabolites (see figure), principally oestrone-3-sulphate, which circulates at a much higher concentration than oestradiol itself. As there is ready metabolic conversion of the sulphate back to oestrone, and as that in turn is readily converted to oestradiol, the totality of oestrogenic activity is related to the total circulating concentration of oestradiol, oestrone and oestrone-3-sulphate (Dollery, 1999a; Kuhn *et al*, 1999; Liehr, 2001; Lippert *et al*, 1999).

The general metabolism of oestradiol in cattle and sheep resembles that in other species (Lohne, 1997). A concise review of the pharmacokinetics and other properties of oestradiol is given in JECFA (2000), including much information about its pharmacology and general metabolism, disposition and elimination.

Further information about the kinetics of hormones released from implants in cattle is given by Arnold (Arnold, 1999), where there are experimental results for proprietary implants of oestradiol variously with or without testosterone, progesterone and trenbolone. In general terms, release from the implants was slow, as intended, the resulting plasma levels were also very low and so was the concentration of the implanted hormones in muscle. The concentrations in fat tended to be up to about 10-fold higher than in muscle. Tissue concentrations and their behaviour with time paralleled the number of implants inserted.

Oral absorption of oestradiol and oestrone is good, except after high doses when it is greatly reduced (Kuhn *et al*, 1993), but the quantity reaching the systemic circulation is limited by the extent of its first-pass metabolism in the intestines and liver to oestrone by 17 β -hydroxysteroid dehydrogenase, and to various hydroxy metabolites, some of which subsequently become glucuronidated or sulphated; oral bioavailability of oestradiol in women is ~5-6% (Kuhn *et al*, 1999) and 8.3% in the rat (Schleicher *et al*, (1998). The plasma (composite) elimination half-life of oestradiol in humans is ~13-20h (Kuhn *et al*, 1999). In the rat the clearance was high at 154mL/min/kg body weight (Schleicher *et al*, 1998). The glucuronides and sulphates are excreted in urine and bile and may undergo partial desulphation and deglucuronidation by bacteria in the intestines (Dollery, 1999a; Kuhn *et al*, 1999); see general account of oestradiol metabolism in JECFA (2000).

17 β -hydroxysteroid dehydrogenase is a member of the alcohol dehydrogenase family of widely distributed enzymes. It is constitutively expressed in the intestine and liver, probably as a major defence against ingested oestrogenic substances (Lippert *et al*, 1999; Reed and Purohit, 1999).

Hydroxylation depends on the action of several specialised members of the cytochrome P450 family of mono-oxygenase enzymes, especially members of the CYP 3A4 and 1A2 families.

Binding of oestradiol and oestrone to plasma proteins in all species is high ~98-99% (Carr, 1998; Dollery, 1999a; Kuhn *et al*, 1999).

Oestradiol passes from the circulation into milk (CVMP, 1999a) and it is likely that catechol oestrogens (see Section 1.2 below) will also enter milk from the breast to the extent they are present in the circulation for may be formed locally in mammary tissue.

1.2 Hydroxylation of oestradiol in humans also leads to formation of 2-OH and 4-OH (A-ring) and 16-OH (D-ring) oestradiol metabolites (Reed and Purohit, 1999; JECFA, 2000). The 2- and 4-OH derivatives (2- and 4-OH-E₁ and E₂) chemically are 'catechol' oestrogens.

Catechol oestrogens may undergo redox cycling under certain circumstances, potentially generating free radicals capable of damaging DNA directly by binding to it covalently and also by the actions of oxygen or hydroxy free radicals (Cavalieri *et al*, 2000; JECFA, 2000); Jefcoate *et al*, 2000; Liehr, 2001). 2-hydroxylation in the liver depends on CYP3A4, CYP1B1 forms the 4-OH derivative, and the enzyme responsible for 16-hydroxylation has not been characterised (Lippert *et al*, 1999; Reed and Purohit, 1999). Extrahepatic oxidation, also producing 2- and 4-OHE₂, depends on other isoforms of P450 (Jefcoate *et al*, 2000).

There is evidence from a variety of experiments that 4-OHE₁ and -E₂ are able to produce DNA damage and mutations and that they have been associated with tumour formation in various animal experiments. The 2-OH form lacks this potential (Jefcoate *et al*, 2000; Liehr, 2001). The relative quantitative importance of the 2- and 4-hydroxylation pathways in different species and tissues has not been widely examined but there is some analytical evidence that the 2OH route may be predominant (Jefcoate *et al*, 2000).

The catechol oestrogens undergo rapid metabolism by the widely distributed, detoxifying enzyme catechol-O-methyl transferase (COMT) to inactive methoxy compounds (Reed and Purohit, 1999). The quantitative importance of this route of detoxification *in vivo* is not known.

The biological importance of the competing pathways of catechol oestrogen formation and detoxification are discussed in the sections of this report dealing with the genotoxicity and carcinogenicity of estradiol. From the viewpoint of biochemical metabolism it seems reasonable to suggest that 2-OHE₂ and 4-OHE₂ are probably formed *in vivo* and that DNA adducts may also be produced to some extent. However, the consequence of this is likely to be depurination, a very common event in cells, and for which there is an efficient repair mechanism. When the normal defence mechanisms against reactive intracellular metabolites and the catabolic pathways for the catechol oestrogens are considered as well as the physiological DNA repair mechanisms, it seems unlikely that the potential genotoxicity of the catechol oestrogens will add appreciably to tumour risk. There is also no mechanistic explanation for the apparent restriction of tumours linked to this mechanism to the breast when the activation and defence mechanisms are present in many other tissues in the body. The potential genotoxicity of oestrogens is also not consistent with well-known effect of pregnancy in reducing the incidence of breast cancer in women when oestrogen levels in pregnancy are several-fold higher than those in post-menopausal period.

1.3 Veterinary treatment of animals may employ the more pharmaceutically convenient valerate, benzoate or hydroxybenzoate esters of oestradiol. They are readily hydrolysed to the parent oestradiol after absorption [CVMP, 1999a]

2. **Trenbolone**

Trenbolone is a semi-synthetic gestagen introduced because of its greater metabolic stability than the physiological progesterone. It is normally implanted subcutaneously as the acetate salt, a prodrug, from which slow release occurs followed by rapid hydrolysis to the parent compound

(Lohne, 1997). After SC implantation in cattle, the peak plasma trenbolone occurs after about 30 days; trenbolone acetate 200mg resulted in peak levels of

The metabolism of trenbolone in cattle is principally via oxidation and epimerization to 17- α - and - β OH trenbolone, and in the rat and rabbit mainly to the corresponding 16- α compounds (Pottier *et al*, 1981; JECFA, 1988; Lange *et al*, 2002). The α -epimers are much weaker gestagens than the 17- β derivatives, which are themselves much weaker gestagens than trenbolone. The importance of 17- α epimerisation in man is disputed (Heitzman *et al*, 1984 cited in Lohne, 1997). As the principal oxidative step relies on the physiological 17- β steroid dehydrogenase, there will be considerable metabolism of ingested trenbolone e.g. in the diet by humans (de Boer *et al*, 1991; Lohne, 1997).

Various distant metabolites of trenbolone and its OH-forms are mainly excreted in bile in cattle and sheep, and in urine in man (Spranger and Metzler, 1995 cited in Lohne, 1997; Lohne, 1997). Investigation of the fate of trenbolone in dung from treated heifers showed that the majority was excreted as the 17- α epimer, which has little gestagenic activity (Schiffer *et al*, 2001).

The metabolism of oral trenbolone in humans has been examined (de Boer *et al*, 1991; Spranger and Metzler, 1991 cited in Lohne, 1997). There was rapid and extensive formation of various metabolites, which were speedily and quantitatively cleared in urine, principally epitrenbolone (17- α) glucuronide. Ye *et al* (1994) demonstrated trenbolone itself, epitrenbolone and hydroxy and other metabolites in human urine.

Some ingested trenbolone may become covalently bound to protein but its chemical nature is not known and its toxicological importance has yet to be demonstrated (Metzler, 1989).

Allyltrenbolone is also used in the pig and horse as an orally active gestagen. Details have not been published but it is likely that its overall metabolism is similar to that of trenbolone (CVMP, 1999b).

Orally ingested trenbolone in the heifer showed little accumulation in the blood stream and was rapidly cleared from the body (half life of elimination < 24h), even after daily administration for several weeks (JECFA, 1988). The half-life of elimination after SC implantation of trenbolone acetate in the heifer was ~26 days (JECFA, 1988). In a relay toxicity study, in which rats were fed meat from heifers previously treated with trenbolone, oral uptake was very limited (3-7%) and most of the dose was excreted unabsorbed in faeces (JECFA, 1988). Trenbolone can cross the placenta to reach the fetus from the mother late in pregnancy; depending on the tissue examined the concentration ratio dam:fetus is about 8:1 (Lange *et al*, 2001)

Co-implantation of trenbolone acetate and 17- β oestradiol in cattle almost doubled the period in which there was a raised plasma E2 level (JECFA, 1988).

3. **Melengestrol**

Melengestrol is a potent, synthetic gestagen (used as the acetate) licensed for use as a growth promoter for cattle in the USA, where it is administered as a feed additive.

Relatively little has been published about its pharmacokinetics and metabolism. It is stated to be cleared rapidly from the body of treated heifers (Krzeminski *et al*, 1981; Lauderdale *et al*, 1977; Lohne, 1997) but may be present for longer in humans (Lohne, 1997). A recent small experiment in heifers demonstrated residues in fat and other tissues (Daxenberger *et al*, 1999); after feeding three-times the recommended dose the residue in fat was 29ppb, which exceeded the US regulatory limit of 25ppb.

It was absorbed after oral administration to women, the half-life of elimination was 3.5 days and a variety of hydroxy, glucuronide and sulphated metabolites was found in urine (Cooper *et al*, 1967).

In the pregnant rabbit fed melengestrol acetate, there was a 2-3-fold higher concentration in the fetus than in the dam (Lange *et al*, 2001).

4. **Zeranol**

Zeranol (a-zearalanol) is a semisynthetic oestrogen structurally related to the fusarial mycotoxin zearalenone (Lindsay, 1985). Implants are used in the USA as growth promoters in cattle and pigs.

Zeranol is rapidly metabolised in the body to zearalenone and then to α - and β -zearanol in proportions that differ in different species (Mirocha *et al*, 1981; Migdalof *et al*, 1983; Baldwin *et al*, 1983; Lindsay, 1985; Bories and Suarez, 1989). There is a predominance of free zearalenone in the rat (Mirocha *et al*, 1981). Glucuronide and sulphate conjugates of the latter are excreted in urine as well as zearalenone. Humans show urinary excretion principally of the glucuronides of zearalenone and a-zearalanol (Mirocha *et al*, 1981). Zearalenone and a-zearalanol can be further metabolised to various hydroxy compounds, and to glucuronide conjugates (Thouvenot *et al*, 1981).

The parent zeranol and its proximate metabolites zearalenone and α - and β -zearanol all show oestrogenic activity, but they are 100- to 1000-times less active than oestradiol after oral administration because of the extent of first pass metabolism.

Natural infection of grain with *Fusaria spp* will result in the formation of zearalenone and α - and β -zearanol and small amounts of zeranol, too (Lindsay, 1985; Lohne, 1997; Kennedy *et al*, 1998), which may be detected in animals.

Zeranol from a SC implant in the pregnant rabbit was found in 2-6-fold higher concentration in the fetus than in the dam (Lange *et al*, 2001).

Zearalenone can enter bovine milk from the blood stream (Mirocha *et al*, 1981).

5. **Progesterone**

Progesterone is readily absorbed from the gastrointestinal tract but it undergoes speedy first-pass metabolism there and in the liver; its bioavailability in man is about 25% (Dollery, 1999b; JECFA, 2000). Subsequent metabolic clearance is also rapid; the half-life of elimination is about 1h.

The rapidity of its clearance is due partly to uptake by fat but mainly to oxidation in the liver to hydroxy derivatives, which are glucuronidated prior to excretion in urine.

6. **Testosterone**

The extent of first pass metabolism of testosterone and its simple esters by the intestinal wall and liver is such that oral administration of anything less than an heroic dose will have no effect (Dollery, 1999c; JECFA, 2000). The metabolism involves oxidation of the 17-OH group and reduction of ring A to form androstenedione and other derivatives, which have very little hormonal activity; there is also limited aromatisation to E2 (Dollery, 1999c; JECFA, 2000).

The formal bioavailability of oral testosterone has not been reported but it is generally considered to be negligible and there would be rapid clearance of any testosterone absorbed from the GI tract (JECFA, 2000).

References

Baldwin RS, Williams RD, Terry MK 1983. Zeranol: a review of the metabolism, toxicology, and analytical methods for detection of tissue residues. *Regul Toxicol Pharmacol*, 3, 9-25.

- de Boer D, Bernal G, van Ooyen RD, Maen RAA 1991. The analysis of trenbolone and the human metabolites of trenbolone acetate by gas chromatography/mass spectrometry and gas chromatography/tandem mass spectrometry. *Biol Mass Spectrom*, 20, 459-466
- Bories G, Suarez AF 1989. Profiling of free and conjugated [3 H]zeranone metabolites in pig plasma. *J Chromatogr*, 489, 1919-197.
- Carr BR 1998. Disorders of the ovary and female reproductive tract. In Wilson JD, Foster DW, Kronenberg HM, Larsen PR eds. *Williams Textbook of Endocrinology*, 9th ed, Saunders, Philadelphia, pp 751-817.
- Cavalieri E, Frenkel K, Liehr JG, Rogan E, Roy D 2000. Estrogens as endogenous genotoxic agents-DNA adducts and mutations. *J Nat Cancer Inst Monogr*, 27, 75-93.
- Cooper JM, Elce JS, Kellie AE 1967. The metabolism of melengestrol acetate. *Biochem J*, 104 (3) p57P-58P.
- CVMP 1999a. Report of the CVMP on the safety Evaluation of Steroidal Sex Hormones. EMEA/CVMP/885/99. Avail at: <http://www.emea.eu.int/pdfs/vet/srwp/088599en.pdf>
- CVMP 1999b. Altrenogest. Summary Report. EMEA/CVMP/175/96-FINAL.
- Daxenberger A, Meyer K, Hageleit M, Meyer HHD 1999. Detection of melengestrol acetate residues in plasma and edible tissues of heifers. *Vet Quart*, 21, 154-158.
- Dollery CT ed 1999a. Estradiol. *Therapeutic Drugs*. 2nd ed., Vol 1, pp E58-64. Churchill Livingstone, Edinburgh.
- Dollery CT 1999b. Progesterone. *Therapeutic Drugs*. 2nd ed., Vol 2, pp P29-31. Churchill Livingstone, Edinburgh.
- Dollery CT 1999c. Testosterone [esters]. *Therapeutic Drugs*. 2nd ed., Vol 2, pp T56-60. Churchill Livingstone, Edinburgh.
- Heitzman RJ, Carter A, Dixon SN, Harwood DJ, Phillips M 1984. Recent studies of pharmacokinetics and residues of anabolic agents in beef cattle and other farm animals. In *Manipulation of Growth in farm Animals*, ed Roche JF and Callaghan D eds. P 1, Martinus Nijhoff, Boston. [not seen, cited from Lohne, 1997].
- JECFA 1988. Trenbolone Acetate. WHO Food Additive Series 23. Pp 73-121. CUP, Cambridge.
- JECFA 2000. Toxicological Evaluation of Certain Veterinary Residues in Food. WHO Food Additive Series: 43. WHO, IPCS, Geneva, Pp 1-90.
- Jefcoate CR, Liehr JG, Santen RJ, Sutter TR, Yager JD *et al* 2000. Tissue specific synthesis and oxidative metabolism of estrogens. *J Nat Cancer Inst Monogr*, 27, 95-112.
- Kennedy DG, Hewitt SA, McEvoy JDG, Currie JW, Cannavan A *et al* 1998. Zeranone is formed from *fusarial* spp. Toxins in cattle *in vivo*. *Food Add Contam*, 15, 393-400.
- Krzeminski LF, Cox BL, Gosline RE 1981. Fate of radioactive melengestrol acetate in the bovine. *J Agric Food Chem*, 29, 387-391.
- Kuhnz W, Blode H, Zimmerman H 1999. Pharmacokinetics of exogenous natural and synthetic estrogens and antiestrogens. *Hdbk Exp Pharmacol*, eds M. Oettel and E. Schillinger. Vol 135/1, pp 261-310. Springer-Verlag, Berlin.
- Lange IG, Daxenberger A, Meyer HHD, Rajpert-de Meyts E, Skakkebaek NE, Veeramachaneni DNR 2001. Quantitative assessment of fetal exposure to trenbolone acetate, zeranol and melengestrol acetate, following maternal dosing in rabbits. *Xenobiotica*, 32, 641-651
- Lange IG, Daxenberger A, Schiffer *et al* (2002) Sex hormones originating from different livestock production systems: fate and potential disrupting activity in the environment. *Anal.Chim Acta* 473: 27-37.
- Lauderdale JW, Goyings LS, Krzeminski LF, Zimbelman RG 1977 Studies of a progestagen (MGA) as related to residues and human consumption. *J Tox Environ Hlth*, 3, 5-33.
- Lindsay DG 1985. Zeranol-a 'nature-identical' oestrogen. *Fd Chem Toxicol*, 23, 767-774.
- Liehr JG 2001. Genotoxicity of the steroidal oestrogens oestrone and oestradiol: possible mechanism of uterine and mammary cancer development. *Acta Path Microbiol Pathol Scand*, 109, supplement, S519-527.

- Lippert TH, Seeger H, Mueck AO 1999. Metabolism of endogenous estrogens. *Hdbk Exp Pharmacol*, eds M. Oettel and E. Schillinger. Vol 135/1, pp243-260. Springer-Verlag, Berlin.
- Lohne KP 1997. Natural sex steroids and their xenobiotic analogs in animal production. *Crit Rev Fd Sci Nutr*, 37, 93-209.
- Metzler M 1989. Metabolism of some anabolic agents: toxicological and analytical aspects. *J Chromatogr*, 489, 11-21.
- Migdalof BH, Dugger HA, Heider JG, Coombs RA, Terry MK 1983. Biotransformation of zearanol: disposition and metabolism in the female rat, dog, monkey and man. *Xenobiotica*, 13, 209-221.
- Mirocha CJ, Pathre SV, Robison TS 1981. Comparative metabolism of zearalenone and transmission into bovine milk. *Fd Cosmet Toxicol*, 19, 25-30.
- Pottier J, Cousty C, Heitzman RJ, Reynolds JP 1981. Differences in the biotransformation of a 17 β -hydroxylated steroid trenbolone acetate, in rat and cow. *Xenobiotica*, 11, 489-500.
- Reed MJ, Purohit A 1999. Estrogen transforming enzymes. *Hdbk Exp Pharmacol*, eds M. Oettel and E. Schillinger. Vol 135/1, pp 223-241. Springer-Verlag, Berlin.
- Schiffer B, Daxenberger A, Meter K, Meyer HHD 2001. The fate of trenbolone acetate and melengestrol acetate after application as growth promoters in cattle: environmental studies. *Environ Hlth Perspect*, 109, 1145-1151.
- Schleicher F, Tauber U, Louton T, Schunack W 1998. Tissue distribution of sex steroids: Concentration of 17 β -oestradiol and cyproterone acetate in selected organs of female Wistar rats. *Pharmacol Toxicol*, 82, 34-39.
- Spranger B, Metzler M 1995. Disposition of 17 β -trenbolone in humans. *J Chromatogr*, 564, 485.
- Thouvenot D, Morfin R, Di Stefano S, Picart D 1981. Transformation of zearalenone and α -zearalanol by homogenates of human prostate glands. *Eur J Biochem*, 121, 139-145.
- Ye L, Zhang CJ, Zhang YZ, Liu X 1994. Trenbolone and Its metabolites in human urine by GC/MS analysis. *Acta Pharmaceutica Sinica*. 29, 61-67 [in Chinese].
- Zimbelman RG, Lauderdale JW, Sokolowski JH, Schalk TG 1970. Safety, and pharmacologic evaluations of melengestrol acetate in cattle and other animals. *J Am Vet Med Assoc*, 157, 1528-36

Appendix D: Membership and Expertise of the VPC Working Group on Hormones

Professor Leonard Stephen Levy OBE, BSc, MSc, PhD, FFOM (Chairman)

Specialism: Toxicology and Carcinogenesis

Professor Levy is currently Head of Toxicology and Risk Assessment at the Medical Research Council Institute for Environment and Health based at the University of Leicester. He is an occupational environmental toxicologist. He trained in cancer research at the Institute of Cancer Research at the University of London and has held teaching and research appointments at the Universities of Aston and Birmingham. He is currently engaged in providing risk assessments to humans from a wide range of environmental and occupational substances. He was appointed to the Veterinary Products Committee in May 2001.

Dr Andrew Bradley MA, VetMB, DCHP, DipECBHM, PhD, MRCVS (RCVS Specialist in Cattle Health and Production)

Department of Clinical Veterinary Science, University of Bristol - Specialism: Large Animal Veterinary Medicine

Dr Bradley is a Senior Lecturer in Ruminant Production Medicine at the University of Bristol and is an RCVS Specialist in Cattle Health and Production. He is Director of the University of Bristol Farm Animal Practice. His area of research is dairy production medicine, in particular bovine mastitis. Dr Bradley is keen to maximise animal welfare whilst maintaining productivity and food safety using an evidence-based approach. He is a member of the Veterinary Products Committee.

Professor Anthony Dayan LLB, MD, FRCP, FRCPath, FFOM, FFPM, FIBiol

Specialism: Mechanistic Toxicology

Professor Anthony Dayan was formerly Professor of Toxicology at Queen Mary and Westfield College, University of London. He has previously been a member of the Veterinary Products Committee, the Medicines Commission and the Committee on Toxicity. His principal scientific interests have included immunotoxicology and functional mechanisms of toxicity.

Professor Mitch Dowsett BSc, PhD

Specialism: Endocrinology

Professor Dowsett is Professor of Biochemical Endocrinology, Head of the Academic Department of Biochemistry at the Royal Marsden Hospital and Institute of Cancer Research, London. His area of research is breast cancer with particular emphasis on hormonal analyses. His laboratory has provided the oestrogen analyses for regulatory submissions for several new drugs for breast cancer treatment and has conducted analyses from several large epidemiological studies over the last few years. From 2001 – 2003 Mitch was the Chairman of the British Breast Group.

Dr Leigh Henderson BSc, PhD, DIBT

Specialism: Genetic Toxicology

Dr Henderson is an independent consultant providing advice on general, genetic and occupational toxicology to the consumer goods and chemicals industry. She is also the external programme advisor to the UK Food Standards Agency on colon cancer and diet. She is a EUROTOX registered toxicologist and is a UK-nominated genetic toxicology expert to the OECD. She has conducted

research in the areas of veterinary cytogenetics, transplacental genotoxicity, DNA repair and development of new tests for genotoxicity. Dr Henderson is a member of the Veterinary Products Committee.

Professor Ed Houghton

Specialism: Analytical Chemist

Professor Houghton joined HFL (formally the Horseracing Forensic Laboratory) in 1974 as a Senior Scientific Officer to establish a Mass Spectrometry Unit. Ed is currently a Director of HFL and Chief Scientist, he has also been appointed as a Visiting Professor in the Department of Chemistry and Physics at Nottingham Trent University. Ed is a fellow of the Royal Society of Chemistry and the Association of Official Racing Analysts and Veterinarians. He has published extensively on drug metabolism and mass spectrometry.

Dr W John McCaughey MA, MS, MVB, PhD, MRCVS, FRAgS

Specialism: Veterinary Public Health

Dr McCaughey was Deputy Chief Veterinary Research Officer in the Veterinary Science Division of the Department of Agriculture, Northern Ireland. Until his retirement he was also Senior Lecturer in the Faculty of Agriculture and Food Science in the Queen's University, Belfast, where he continues to lecture. He holds a part-time consultancy in cattle breeding in the Agricultural Research Institute for Northern Ireland. Dr McCaughey is a past President of the Association of Veterinary Teachers and Research Workers, of the North of Ireland Veterinary Association and is an honorary member of the Association of Veterinary Surgeons Practising in Northern Ireland. His interests centre on the detection and control of veterinary drug residues in food animals, the detection of natural toxins and on the reproductive performance of farm animal species. He was first appointed to the Veterinary Products Committee in 1998.

Professor Jim Parry

Specialism: Genetic Toxicology

Professor Parry is Professor of Genetics at the University of Wales Swansea where he heads a research group focusing on the mechanisms of chemically induced mutation. He has a particular interest in the influence of low doses of chemical upon the fidelity of chromosome segregation. Professor Parry is currently a member of the Food Standards Agency's Advisory Committee for Wales and the Medical and Toxicology Panel of the ACP.

Professor Richard Sharpe

Specialism: Reproductive Sciences

Professor Sharpe heads one of the research teams in the MRC Human Reproductive Sciences Unit in the area of male reproductive health. He has research interests in all aspects of male reproduction and endocrinology, from molecular to clinical, from fetal life through to adulthood. His main research interest is in the role of fetal development in the aetiology of adult human male reproductive dysfunction. He serves/has served on numerous government and other expert committees, grant awarding bodies etc. He was until recently Editor of the International Journal of Andrology and is currently on the Editorial board of Human Reproduction and the Journal of Endocrinology. He is an Academician of the European Academy of Andrology.

Mr John Verrall DBA

Specialism: Pharmacology and Risk Assessment

Mr Verrall was a pharmaceutical chemist for 50 years, 35 of which he worked in the pharmaceutical industry and was involved in product development, marketing and general management. He has taken a particular interest in the use, misuse and abuse of products in their veterinary and animal health applications and also in risk assessment/risk management and the application of the Precautionary Principle. A former Chairman of the Farm and Food Society, he is now a member of The Food Ethics Council – an independent council for ethical standards in food and agriculture. He represents the Council on the Codex Consumer Group of the Food Standards Agency and at Consumer and Stakeholders' Liaison meetings with the Veterinary Medicines Directorate. He has been a member of the VPC since 2002.

Expertise called upon by the Working Group

Dr Mark Crane BSc, PhD¹²

Specialism: Environmental Risk Assessment

Dr Crane is an independent environmental consultant, providing advice and training to government and industry. He is an ecotoxicologist interested in the effects of agricultural and industrial chemicals on a wide range of biological systems. He currently works on the risk assessment of contaminated sediments, surface waters and groundwaters, the derivation of environmental quality standards, and the effects of endocrine modulators on aquatic invertebrates. He is a member of the Environmental Panel of the Advisory Committee on Pesticides. He was appointed to the Veterinary Products Committee in 2000.

¹² Dr Crane was not a member of the Working Group. He was invited to give his expertise on the environmental effects of the use of hormonal growth promoters by correspondence to the VPC Working Group on Hormones. He had full access to all documentation, including the SCVPH 2002 Opinion and was consulted over the draft presented to the VPC.