

**UNITED STATES – CONTINUED SUSPENSION OF
OBLIGATIONS IN THE EC – HORMONES DISPUTE**

Report of the Panel

Addendum

This addendum contains Annex D to the Report of the Panel to be found in document WT/DS320/R. The other annexes can be found in the following addenda:

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ANNEX D

REPLIES OF THE SCIENTIFIC EXPERTS TO QUESTIONS POSED BY THE PANEL

A. GENERAL DEFINITIONS

1. **Please provide brief and basic definitions for the six hormones at issue (oestradiol-17 β , progesterone, testosterone, trenbolone acetate, zeranol, and melengestrol acetate), indicating the source of the definition where applicable.**

Dr. Boisseau

1. Oestradiol-17 β is the most active of the oestrogens hormone produced mainly by the developing follicle of the ovary in adult mammalian females but also by the adrenals and the testis. This 18-carbon steroid hormone is mainly administered as such or as benzoate ester alone (24 or 45 mg for cattle) or in combination (20 mg) with testosterone propionate (200 mg for heifers), progesterone (200 mg for heifers and steers) and trenbolone (200 mg and 40 mg oestradiol-17 β for steers) by a subcutaneous implant to the base of the ear to improve body weight and feed conversion in cattle. The ear is discarded at slaughter.
2. Progesterone is a hormone produced primarily by the corpus luteum in the ovary of adult mammalian females. It is administered to cattle, steers, usually at 200 mg in combination with oestradiol-17 β or oestradiol benzoate (usually 20 mg) by a subcutaneous implant to the base of the ear to improve body weight and feed conversion in cattle. The ear is discarded at slaughter.
3. Testosterone is a hormone produced primarily in the testes of adult mammalian males. This 19-carbon steroid has potent androgenic properties. It is administered as testosterone propionate (200 mg) in combination with oestradiol-17 β or oestradiol benzoate (20mg) by a subcutaneous implant to the base of the ear to improve body weight and feed conversion in cattle. The ear is discarded at slaughter.
4. Melengestrol acetate is an orally active synthetic progestogen about 30 times as active as progesterone. It is used to improve body weight and feed conversion in female beef cattle. It is fed at daily doses of 0.25-0.50 mg per heifer usually 90-150 days prior to slaughter.
5. Trenbolone acetate is a synthetic steroid with anabolic properties several fold above that of testosterone. It is administered alone (300 mg for heifers) or in combination with oestradiol-17 β (20 mg for calves and 40 mg for steers), by a subcutaneous implant to the base of the ear to improve body weight, feed conversion and nitrogen retention in cattle. It is administered to cattle 60-90 days or more before the intended date of slaughter. The ear is discarded at slaughter.
6. Zeranol, is a natural mycooestrogen derived from zearalenone produced by different species of fusarium molds. This non-steroidal anabolic agent is administered to cattle either alone (36 mg) or in combination with trenbolone acetate (140 mg) by subcutaneous implant to the base of the ear to improve body weight and feed conversion in cattle.

Dr. Boobis¹

7. Oestradiol-17 β is the most potent mammalian oestrogenic hormone. It is produced in the ovary, placenta, testis, and possibly the adrenal cortex (*ChemIDPlus Advanced, National Library of Medicine* (<http://chem.sis.nlm.nih.gov/chemidplus>))

8. Oestradiol-17 β is the most potent form of mammalian oestrogenic steroids. In humans, it is produced primarily by the cyclic ovaries and the placenta. It is also produced by the adipose tissue of men and postmenopausal women (*PubChem, National Library of Medicine* (<http://pubchem.ncbi.nlm.nih.gov/>))

9. Progesterone is the principal progestational hormone of the body, secreted by the corpus luteum, adrenal cortex, and placenta. Its chief function is to prepare the uterus for the reception and development of the fertilized ovum. It acts as an antioviulatory agent when administered on days 5-25 of the menstrual cycle (*ChemIDPlus Advanced*).

10. Progesterone is the major progestational steroid that is secreted primarily by the corpus luteum and the placenta. Progesterone acts on the uterus, the mammary glands and the brain. It is required in embryo implantation, pregnancy maintenance, and the development of mammary tissue for milk production. Progesterone, converted from pregnenolone, also serves as an intermediate in the biosynthesis of gonadal steroid hormones and adrenal corticosteroids (*PubChem*).

11. Testosterone is a potent androgenic steroid and major product secreted by the Leydig cells of the testis. Its production is stimulated by luteinizing hormone from the pituitary. In turn, testosterone exerts feedback control of the pituitary LH and FSH secretion. Depending on the tissues, testosterone can be further converted to dihydrotestosterone or oestradiol (*PubChem*).

12. Trenbolone acetate is a synthetic steroid that has been used as an anabolic agent in veterinary practice. (*Martindale: The Complete Drug Reference* (2006), *Pharmaceutical Press, London*).

13. Zeranol is a naturally occurring metabolite of the mycotoxin zearlenone which is produced by a number of *Fusarium* fungal species. The commercial formulation contains specifically the α -isomer. Zeranol is a non-steroidal anabolic agent. (*JECFA (1988a). Toxicological Evaluation of Certain Veterinary Drug Residues in Food: WHO Food Additives Series 23, WHO, Geneva, Switzerland*).

14. Zeranol is a nonsteroidal oestrogen that has been used for the management of menopausal and menstrual disorders. It has also been used as a growth promoter in veterinary practice (*Martindale: The Complete Drug Reference*).

15. Melengestrol acetate (MGA) is an orally active 6-methyl progesterone acetate with reported glucocorticoid activity and effect on estrus (*PubChem*).

16. Melengestrol acetate is a progestogen that is used as an animal feed in beef heifers to improve feed efficiency, increase the rate of body-weight gain, and suppress oestrus (*Martindale: The Complete Drug Reference*).

Dr. Guttenplan

17. Oestradiol-17 β : an estrogenic sex hormone, which in the female, functions in the ovarian cycle and maintains uterine health. In males it inhibits the synthesis of testosterone. A member of a

¹ A full list of references cited in responses from Dr. Boobis can be found in Attachment 1.

class of compounds called steroids (which, chemically, have three 6-membered rings and one 5-membered ring).

18. Progesterone: a steroidal anti-estrogen; used as a contraceptive and to correct abnormalities in the menstrual cycle.
19. Testosterone: a steroidal androgenic sex hormone, which in the male leads the production of sperm components. It is also important in promoting the development of secondary sex characteristics.
20. Trenbolone acetate: a synthetic anabolic (growth-stimulating) hormone, often used in cattle.
21. Zeranol: a synthetic nonsteroidal **growth promoter often used in cattle**.
22. Melengestrol acetate: a synthetic steroidal growth promoter often used in cattle. Also used for estrus synchronization in cattle.

(Opinion of SCVPH, 1999 (US Exhibit 4 part 1))

2. **Please provide definitions for the following terms as they relate to the hormones at issue, indicating the source of the definition where applicable: anabolic agents, steroids, steroidal oestrogens, parent compounds/metabolites, catechol metabolites, mitogenicity, mutagenicity, androgenic/oestrogenic activity, genotoxicity, genotoxic potential, carcinogenicity, and tumorigenicity. In your replies, please be sure to identify and describe any relevant differences between the terms.**

Dr. Boobis

Anabolic agent

23. The building up in the body of complex chemical compounds from smaller simpler compounds (e.g., proteins from amino acids), usually with the use of energy. Cf.: catabolism, metabolism. *Stedman's Medical Dictionary (2000), Lippincott Williams & Wilkins, Philadelphia, PA*
24. Testosterone, or a steroid hormone resembling testosterone, which stimulates the growth or manufacturing of body tissues. *Taber's Cyclopedic Medical Dictionary - 20th Ed (2005), F. A. Davis Company, Philadelphia, PA*
25. Anabolism: The processes of metabolism that result in the synthesis of cellular components from precursors of low molecular weight. *IUPAC(1997). Compendium of Chemical Terminology, 2nd Edition (<http://www.iupac.org/publications/books/author/mcnaught.html>)*

Steroids

26. A large family of chemical substances, comprising many hormones, body constituents, and drugs, each containing the tetracyclic cyclopenta[a]phenanthrene skeleton *Stedman's Medical Dictionary*.

Steroidal oestrogens

27. (Steroidal) compounds that produce the behaviour estrus ("the portion or phase of the sexual cycle of female animals characterized by willingness to accept the male"). *Hughes, C (1996). Are the*

differences between estradiol and other estrogens merely semantical? (Letter to the Editor). J Clin Endocrinol Metab 81:2405.

28. A more biochemical definition might be: compounds with a steroid structure that possess endocrine effects qualitatively similar to those of oestradiol-17 β and that act through oestrogen receptors.

Parent compounds/metabolites

29. When related to exogenous compounds, the parent is the compound to which an individual is exposed. The relationship between parent compound and metabolite is that the parent serves as a substrate for biotransformation (enzymatic conversion) to yield a product that is chemically distinct from the parent, a metabolite (*A. Boobis*). With respect to metabolites of veterinary drugs, it is possible that the residue in meat comprises, at least in part, one or more metabolites of the drug used to treat the animals. Ingestion of such metabolites can lead to their metabolism in human subjects. Hence, there will be a parent/metabolite relationship even for such compounds.

30. Metabolite: Any intermediate or product resulting from metabolism. National Library of Medicine (1993). Glossary for Chemists of Terms Used in Toxicology (<http://www.sis.nlm.nih.gov/enviro/glossarymain.html>; Pure Appl Chem, 1993, 65, 2003-2122)

31. Metabolism: in a narrower sense, of drugs, one mechanism of clearance, is the irreversible biochemical transformation of a compound to another chemical (metabolite). The metabolite is usually more polar (water-soluble) and, therefore, more readily excreted, than the parent compound; thus, metabolism facilitates drug excretion. *Absorption Systems (2006). Glossary Terms* (<http://www.absorption.com/Site/Glossary/Default.aspx>)

Catechol metabolites

32. Any intermediate or product resulting from metabolism (enzymatic transformation) containing the core structure benzene-1,2-diol IUPAC (1993). *A Guide to IUPAC Nomenclature of Organic Compounds, Blackwell Science, Oxford, UK*

Mitogenicity

33. The property of an agent whereby it induces mitosis and cell proliferation. Mitosis is the process by which a cell nucleus divides into two daughter nuclei, each having the same genetic complement as the parent cell: nuclear division is usually followed by cell division (*NLM Glossary for Chemists of Terms Used in Toxicology*).

Mutagenicity

34. Ability of a physical, chemical, or biological agent to induce heritable changes (mutations) in the genotype in a cell as a consequence of alterations or loss of genes or chromosomes (or parts thereof).

35. Mutation: Any relatively stable heritable change in genetic material that may be a chemical transformation of an individual gene (gene or point mutation), altering its function, or a rearrangement, gain or loss of part of a chromosome, that may be microscopically visible (chromosomal mutation); mutation can be either germinal and inherited by subsequent generations, or somatic and passed through cell lineage by cell division. *NLM Glossary for Chemists of Terms Used in Toxicology*

Androgenic activity

36. Having the property to interact with androgen receptors in target tissues to bring about the effects similar to those of testosterone. Depending on the target tissues, androgenic effects can be on sexual differentiation; male reproductive organs, spermatogenesis; secondary male sex characteristics; libido; development of muscle mass, strength, and power.

37. Capacity to promote the development and maintenance of male sex characteristics. National Library of Medicine, Genetics Home Reference (<http://ghr.nlm.nih.gov/ghr/glossary/Glossary>)

Oestrogenic activity

38. Biological activity similar to that of an oestrogen.

39. Oestrogens cause the thickening of the lining of the uterus and vagina in the early phase of the ovulatory, or menstrual, cycle; in lower animals cyclical oestrogen secretion also induces oestrus, or "heat". The oestrogens are also responsible for female secondary sex characteristics such as, in humans, pubic hair and breasts, and they affect other tissues including the genital organs, skin, hair, blood vessels, bone, and pelvic muscles. *The Columbia Electronic Encyclopedia (2003), Sixth Edition, Columbia University Press, New York City, NY*

40. Oestrogenic activity can arise through several possible mechanisms, by mimicking natural oestrogens and interacting with oestrogen receptors, by affecting oestrogen-sensitive pathways by some other mechanism and by altering the levels of endogenous oestrogens, by changing the rate of synthesis or degradation. *Lintelmann J, Katayama A, Kurihara N, Shore L, and Wenzel A (2003). Endocrine disruptors in the environment (IUPAC Technical Report) Pure Appl Chem, 75, 631–681. Miyamoto J and Burger J (Editors) (2003). Special Topic Issue on the Implications of Endocrine Active Substances for Humans and Wildlife. Pure Appl Chem, 75: 1617-2615.*

Genotoxicity

41. Ability to cause damage to genetic material. Such damage may be mutagenic and/or carcinogenic. *NLM Glossary for Chemists of Terms Used in Toxicology*

42. Mutagenicity is a form of genotoxicity. However, not all genotoxicity is necessarily mutagenicity. Examples include adduction to DNA and damage to DNA that does not lead to heritable change. Whilst adduction can lead to mutation, the presence of adducts *per se* is a measure of genotoxicity and not of mutagenicity.

Genotoxic potential

43. Of a compound, it possesses characteristics such that it might be capable of causing genotoxicity (usually *in vivo*), based on considerations such as the results of tests *in vitro*. It remains to be determined whether genotoxicity is indeed expressed *in vivo*, i.e. that the potential is realized (*A. Boobis interpretation of usage by JECFA and elsewhere*).

Carcinogenicity

44. Process of induction of malignant neoplasms by chemical, physical or biological agents.

45. Malignant neoplasm: a population of cells showing both uncontrolled growth and a tendency to invade and destroy other tissues; a malignancy is life-threatening.

46. Neoplasm: new and abnormal formation of tissue as a tumour or growth by cell proliferation that is faster than normal and continues after the initial stimulus that initiated the proliferation has ceased. *NLM Glossary for Chemists of Terms Used in Toxicology*

Tumourigenicity

47. Process of inducing tumours, i.e. any abnormal swelling or growth of tissue, whether benign or malignant. *NLM Glossary for Chemists of Terms Used in Toxicology*

48. Hence, whilst a carcinogen produces tumours (which are malignant), tumourigenic agents do not necessarily produce malignant neoplasia.

Dr. Guttenplan

49. Anabolic agents: agents promoting build-up - in animals, usually muscle mass, in biochemicals, building larger molecules from smaller ones.

50. Steroids: Metabolites of cholesterol, containing three 6-membered rings and one 5-membered ring.

51. Steroidal oestrogens: Estrogens that contain the steroidal ring system.

52. Parent compounds/metabolites: in a chemical conversion, the initial chemical is called the parent compound and the product, the metabolite.

53. Catechol metabolites: Catechols are compounds containing a benzene ring with two hydroxyl groups on the benzene ring. When they are converted to a different compound, a catechol metabolite results.

54. Mitogenicity: Relating to or causing cell division.

55. Mutagenicity: Relating to or causing a change in DNA composition. May also relate to a change in protein structure

56. Androgenic activity: acting like a male sex hormone.

57. Oestrogenic: acting like a female sex hormone.

58. Genotoxicity: Relating to or causing damage to DNA.

59. Genotoxic potential: The possible ability of an agent to cause damage to DNA.

60. Carcinogenicity: Relating to or causing a process leading to cancer.

61. Tumorigenicity: Relating to or causing the formation of tumors. This term refers to tumor formation, whereas carcinogenicity may also refer to the process by which tumors are induced. (*Codex Microbiological RA*).

B. RISK ASSESSMENT TECHNIQUES

3. **Please identify any international guidance documents relevant to the conduct of a risk assessment with respect to veterinary drug residues. Since when have they been available? Please also indicate if there is any relevant ongoing work at Codex.**

Dr. Boisseau

62. To my knowledge, there is no international guidance document relevant to the conduct of a risk assessment with respect to veterinary drug residues. Currently, there is no Codex guidance document relevant to the conduct of a risk assessment with respect to veterinary drug residues. The situation is similar in the European Union. The CVMP has assessed all the pharmacologically active substances used in veterinary medicine without any written guideline about risk assessment.

63. I have proposed some 15 years ago to CCRVDF (Codex Committee on Residues of Veterinary Drugs in Food) to develop and adopt a guidance about risk management including a risk assessment policy. In its last session held in May 2006 in Cancun, Mexico, CCRVDF has decided to propose to the Codex Committee on General principles (CCGP) and to the Codex Commission a draft project concerning a rationale about the risk analysis to be implemented by CCRVDF. This draft project includes two parts: (1) a procedure with the interactions between CCRVDF, responsible for risk management, and JECFA (Joint Expert Committee on Food Additives) responsible for risk assessment, with, in annex, the format to be used by member states for establishing a risk profile; (2) the principles of a risk assessment policy.

Dr. Boobis

International guidance documents

64. The following guidance documents relevant to the conduct of a risk assessment with respect to veterinary drug residues are available:

WHO (2001): Residues of veterinary drugs in food (current version Jan 2001).

WHO procedural guidelines for the Joint FAO/WHO Expert Committee on Food Additives

WHO (1996): Residues of veterinary drugs in food (current version August 1996) Guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives

Residues of veterinary drugs in food (Sept 2002)

FAO (2002a) procedural guidelines for the Joint FAO/WHO Expert Committee on Food Additives

Procedures for Recommending Maximum Residue Limits – Residues of Veterinary Drugs in Food (1987-1999) (FAO, 2000a)

Environmental Health Criteria (EHC) 70: Principles For The Safety Assessment Of Food Additives And Contaminants In Food (IPCS, 1987)

Environmental Health Criteria (EHC) 104: Principles For The Toxicological Assessment of Pesticide Residues In Food (IPCS, 1990)

65. Also available are relevant sections from General Consideration Items in JECFA reports, which document guidance developed by JECFA over the years and are provided as an ongoing update to its risk assessment procedures (relevant volumes of WHO Technical Report Series).

66. Codex publishes a Procedural Manual that contains generic guidance on risk analysis and risk assessment policies. This is updated regularly, the latest version (15th) having been published in 2005: *Codex Alimentarius Commission (CAC) (2005). Procedural Manual, Fifteenth edition, WHO and FAO, Rome, Italy* (ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_15e.pdf).

67. Codex is currently developing a risk assessment policy for recommending maximum residue limits for veterinary drugs in food. To my knowledge this is still in the drafting stage (see JECFA, 2006a).

CCRVDF (2005). Risk Management Methodologies, Including Risk Assessment Policies in the Codex Committees on Residues Of Veterinary Drugs in Foods (ftp://ftp.fao.org/codex/Ccrvdf16/rv16_10e.pdf).

JECFA (2006a). Summary and Conclusions of Sixty-sixth meeting (Residues of veterinary drugs), Rome, 22-28 February 2006 (<http://www.who.int/ipcs/food/jecfa/summaries/summary66.pdf>)

4. The European Communities states that there is "no Codex standard specifically on the risk assessment of effects of residues of veterinary drugs" but a general one on microbiological assessment. Is this correct? Which guidelines or principles have been used by JECFA in the conduct of its risk assessments with respect to the hormones at issue? [see para. 192 of EC Rebuttal Submission (US case)].

Dr. Boisseau

68. The European Communities is right when it says that "there is no Codex standard specifically on the risk assessment of effect of residues of veterinary drugs". In the conduct of its risk assessment with respect to the hormones at issue, as for all the other pharmacologically active substances used in veterinary medicine, JECFA has followed the general rationale used by all the countries which have assessed the safety of veterinary drug residues. This rationale has been internationally harmonised through scientific conferences and it is possible to say that there was an international non written agreement on this rationale. Nevertheless, the International Programme on Chemical Safety (IPCS) has sponsored in the 1980s the preparation and the publication of the Environmental Health Criteria (EHC) monograph No 70 entitled " Principles for the safety assessment of food additives and contaminants in foods". Then, JECFA has, in its meetings, regularly developed and consolidated the principles of this monograph EHC No 70 but it has never published the outcome of this work in any official document or monograph on risk assessment of veterinary drug residues, the only exception being for microbiologicals.

Dr. Boobis

Codex standards for risk assessment

69. It is not clear what is meant by the EC assertion that there is "no Codex standard specifically on the effects of residues of veterinary drugs", but a general one on microbiological assessment. It is certainly true that there is no detailed guidance manual from Codex on the assessment of the effects of residues of veterinary drugs. However, there are guiding principles in place, that have been in existence since before 1999. These relate to the procedures for risk assessment, the implications and meaning of an ADI (Acceptable Daily Intake) and procedures for setting MRLs. As indicated above, JECFA was guided by a number of relevant documents in its risk assessment procedures. JECFA developed an approach to the risk assessment of residues of antimicrobials, which was novel and not covered in detail in such guidance. Specific guidance was therefore developed by JECFA and adopted by Codex. In contrast, the approaches used in the assessment of the hormones followed

established risk assessment principles for toxicologically (as opposed to microbiologically) active compounds.

Dr. Guttenplan

70. It is correct that there is "no Codex standard specifically on the risk assessment of effects of residues of veterinary drugs".

71. A monograph "TOXICOLOGICAL EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD" WHO Food Additives Series: 43, Prepared by the Fifty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) describes the data used to determine the ADI for estradiol, progesterone and testosterone. The principles of risk assessment (described below) were used in determining ADI's for estradiol, progesterone, and testosterone.

5. Please briefly describe the three components of a risk analysis exercise (risk assessment, risk management and risk communication) and explain how they differ.

Dr. Boisseau

72. The following brief description of the three components of a risk assessment exercise is given with respect to veterinary drugs residues likely to be present in food of animal origin.

73. Risk assessment is a procedure run by persons having the relevant scientific and technical expertise. It is intended to determine the likelihood and the gravity of any unexpected unwanted effect for the consumer which may result from the ingestion of veterinary drugs residues likely to be present in food of animal origin. Only scientific data, relevant with regard to assessing this risk, have to be taken into consideration in this procedure. In the Codex procedure, JECFA is responsible for conducting the risk assessment for veterinary drug residues.

74. Risk management is a procedure run by persons having political or administrative responsibilities. It is intended to protect consumers from any problem of public health associated with veterinary drugs residues likely to be present in food of animal origin. Other criteria than scientific ones, such as economical, sociological, cultural etc., can be taken into consideration in this procedure. Usually, this procedure leads to regulatory and/or administrative decisions.

The risk management procedure usually implies four steps:

- (1) Risk evaluation
 - identification of a food safety problem
 - establishment of a risk profile
 - ranking of the hazard for risk assessment and risk management priorities
 - establishment of a risk assessment policy
 - commissioning of risk assessment
 - consideration of the results of the risk assessment
- (2) Assessment of the different possible risk management options
 - identification of the different possible risk management options
 - selection of the preferred risk management option
 - final risk management decision
- (3) Implementation of the risk management decision

(4) Monitoring and review

- assessment of the effectiveness of the measures taken
- review of risk management and /or risk assessment as necessary

75. The risk management procedure has been considered by a joint FAO/WHO expert consultation in 1997.

76. In the Codex procedure, Codex is responsible for conducting the risk management for veterinary drug residues.

77. Even if communication between persons responsible for the risk assessment and the risk management is desirable and useful, scientific persons running the risk assessment procedure must be in the position to perform their work without any influence from the persons having political or administrative responsibilities. In order to guarantee their independence, these scientific persons, very often, carry out their work within independent agencies, at national or regional level. JECFA is an expert committee independent from the Codex. It carries out, among others, the risk assessment for veterinary drug residues on the request of Codex. Codex, including the CCRVDF and the Codex Commission, is, together with the member states, involved in the risk management. Risk assessors have to publish the conclusions of the risk assessment they have performed. JECFA does through monographs on toxicology and residues published respectively by WHO and FAO. Risk assessors may, in their conclusions, address some recommendations to the persons/bodies responsible for the risk management but they have not the power to take any regulatory or administrative decision.

78. Risk communication is an interactive process of exchange of informations and opinions on the potential risks associated with veterinary drug residues likely to be present in food of animal origin, among

- (1) Risk assessors
- (2) Risk managers
- (3) Other interested parties such as
 - consumers
 - veterinarians
 - technicians in animal husbandry
 - animal owners
 - animal health industry
 - food processing industry

Risk communication should, among others,

- promote awareness and understanding of the specific issues under consideration during the risk analysis
- promote consistency and transparency in formulating risk management options/recommendations
- provide a sound basis for understanding the risk management decisions proposed

- improve the overall effectiveness and efficiency of the risk analysis

79. Scientific persons in charge of the risk assessment procedure are responsible of the communication on the issues associated with the risk assessment and the persons having political or administrative responsibilities are responsible of the communication on the issues associated with the risk management.

80. The risk communication has been considered by a joint FAO/WHO expert consultation.

Dr. Boobis

Components of risk analysis

81. The three components of a risk analysis exercise can be described as follows:

Risk Assessment

82. A process intended to calculate or estimate the risk to a given target organism, system, or (sub)population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system.

*From IPCS (2004). Risk Assessment Terminology, WHO, Geneva
(<http://www.iseaweb.org/ipcsterminologyparts1and2.pdf>)*

Risk Management

83. The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options.

84. Risk management comprises three elements: risk evaluation; emission and exposure control; and risk monitoring.

From Codex Alimentarius Commission(2005). 15th Procedural Manual

Risk Communication

85. The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

From Codex Alimentarius Commission (2005). 15th Procedural Manual

86. Risk assessment is a scientific process in which the data are evaluated and on this basis, together with weight of evidence and expert judgment a conclusion is reached as to the nature of the hazards, the potential risk to exposed individuals and the extent to which exposure (measured or estimated) approaches those levels considered to be without appreciable risk. The output of the risk assessment is a health based guidance value, the allowable daily intake (ADI), in the case of a veterinary drug residue in food. An important aspect of risk assessment is to identify and describe the uncertainties associated with the evaluation. The MRL is an exposure level that it compatible with

both health protection and good veterinary practice. The ADI does not determine the MRL recommended by JECFA for consideration by Codex. However, in the risk characterization stage of risk assessment, comparison of exposure based on GVP (good veterinary practice) resulting in levels at the MRL, with the ADI establishes whether the exposure is adequately protective. If not, the risk assessment may be refined, or the conclusion may be that it is not possible to establish an MRL such that exposure would be consistent with public health protection.

87. Risk management is not a scientific process but a procedure whereby policies are established with respect to the use and acceptability of, in this case, a veterinary drug, compatible with protection of the public, good veterinary practice and efficacy and ensuring fair trade. Hence, the output of a risk assessment is one input to risk management decision-making. However, it is not the only one, as indicated above. Issues such as the veterinary need for the product and the security of the food supply may also be considerations. Normally, the risk manager accepts the output of the risk assessor, as this is the conclusion of the scientific experts in the field. There should be a clear separation between risk assessment and risk management. That is not to say that there should be no communication, but that the conclusions of the risk assessors should be their own, uninfluenced by any policy needs of the risk manager. Similarly, the risk manager should accept the conclusions of the risk assessor, unless there is a transparent reason to challenge them. If the risk manager chooses a course of action that is more, or less, precautionary than that justified on the basis of the risk assessment, the reasons for this should be clear and distinct from the risk assessment.

Dr. Cogliano

88. Risk assessment is the use of scientific data to describe the adverse effects of exposure to hazardous agents. Risk communication is the art of explaining these risks to different audiences. Risk management is the process of considering a risk along with other factors (for example, legal mandates, technical feasibility, cost, equity, and social norms) and making a decision about whether and how to mitigate the risk. The three are separate activities carried out for separate purposes.

Dr. Guttenplan

89. Risk Assessment is defined as the scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization. It basically attempts to evaluate risk.

90. Risk Management is defined as the process of weighing policy alternatives in the light of the results of risk assessment and, if required, selecting and implementing appropriate control options, including regulatory measures. It basically refers to dealing with the hazard, generally to reduce risk.

91. Risk Communication is defined as the interactive exchange of information and opinions concerning risk and risk management among risk assessors, risk managers, consumers and other interested parties. It is basically concerned with making known the risks to interested and/or affected parties. (Codex Microbiological RA).

6. **Please briefly describe the four steps of a risk assessment (hazard identification, hazard characterization, exposure assessment and risk characterization) as identified by Codex, indicating any relevant sources.**

Dr. Boisseau

92. The brief description of the four steps of a risk assessment procedure is given with respect to veterinary drugs residues likely to be present in food of animal origin. These four steps are:

(1) hazard identification, (2) hazard characterization, (3) exposure assessment, (4) risk characterization

93. The goal of the hazard identification is (1) to identify all the residues of the veterinary drugs under review likely to pose problems of health to consumers. The residues of concern for this substance imply both the parent compound and all the pharmacologically active metabolites derived from this parent compound; (2) to determine the concentrations of all these residues in the different edible tissues and products derived from animals treated by this veterinary drug; (3) to determine the evolution over the time of the concentrations of all these residues in the different edible tissues and products after animals have been treated by this veterinary drug; (4) to identify the marker residue to be used for the monitoring of residues in order to be sure that the animal derived food intended to the human consumption does not contain concentrations of residues exceeding the MRLs established for this veterinary drug.

94. The goal of the hazard characterization is to assess qualitatively and quantitatively all the adverse effects associated with the residues of veterinary drugs which may negatively impact the health of consumers or the environment. An important component of this step is to ascertain whether or not it is possible to establish a dose-effect relationship and a threshold which is the quantity of residues under which no adverse effect towards the health of consumers can be expected. The outcome of this step is, when possible, to establish a NOAEL (No Observed Adverse Effect Level) from the scientific data base available and to derive an ADI (Acceptable Daily Intake) from this NOAEL using an appropriate safety factor, the value of which depends on the toxicological profile of the residues. The NOAEL is the highest quantity of the veterinary drug at issue which is not associated with any adverse effect in toxicity tests carried out in animals or in studies carried out in humans. The ADI represents the maximum amount of residues of concern for the veterinary drug under review which can be daily ingested by consumers over a life time without any risk for their health. NOAELs and ADIs are expressed in mg or µg/kg/day.

95. The goal of the exposure assessment is to assess quantitatively the exposure of consumers to the residues of the veterinary drugs under review through the consumption of food of animal origin. This exposure is determined through a standard food basket determined by JECFA which encompasses mainly 500g muscle, 100g liver, 50g kidney, 50g fat, 1.5l milk and 100g eggs.

96. In general terms, the goal of the risk characterization is to assess qualitatively and quantitatively the likelihood and the gravity of a given hazard for a human population exposed to this hazard. This assessment is based on the conclusions of the three former steps of hazard identification, hazard characterization and exposure assessment. In the specific case of veterinary drug residues, this step does not exist as the goal of the risk analysis for these compounds is not to assess qualitatively and quantitatively the likelihood and the gravity of the adverse effects for the health of consumers associated with the veterinary drug residues they are exposed to through the animal derived food but to protect consumers' health from any adverse effect associated with these residues. In order to do so, MRLs are established, when possible. MRLs represent the highest concentrations of the residues of concern which can be accepted for the different edible tissues and products derived from the animals treated by the veterinary drug under review so that the quantity of residues daily ingested by consumers does not exceed the established ADI. This establishment of MRLs, aimed at providing an efficient protection of consumer health, is, therefore, a component of the risk management. Thus, when JECFA proposes MRLs to CCRVDF, it is also involved in a risk management component of the risk analysis procedure but, as these MRLs have to be adopted by Codex (CCRVDF and Commission), Codex is really, together with the member states, the body responsible for the risk management.

Dr. Boobis

Four steps of risk assessment

97. The four stages of risk assessment are as follows (*IPCS (2004). Risk Assessment Terminology*):

Hazard Identification

98. The identification of the type and nature of adverse effects that an agent has an inherent capacity to cause in an organism, system, or (sub)population. This has been described as the intrinsic toxicological properties of the compound.

Hazard Characterisation

99. The qualitative and, wherever possible, quantitative description of the inherent property of an agent or situation having the potential to cause adverse effects. This should, where possible, include a dose–response assessment and its attendant uncertainties. It also entails determining whether or not there is a threshold for the toxicological effect, i.e. a dose below which no effect occurs.

Exposure Assessment

100. The qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant (*Codex Alimentarius Commission (2005)*).

Risk Characterisation

101. The qualitative and, wherever possible, quantitative determination, including attendant uncertainties, of the probability of occurrence of known and potential adverse effects of an agent in a given organism, system, or (sub)population, under defined exposure conditions.

Dr. Guttenplan

102. Hazard identification is defined as the identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods. It is concerned with recognizing potential harmful agents. Hazard characterization is defined as the qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with the hazard. Exposure assessment is defined as the qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant. It basically attempts to estimate the quantity of the agent to which individuals or populations are exposed. Risk characterization is defined as the process of determining the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment. For instance the risk of lung cancer in smokers is 1 in 10. (Codex Microbiological RA).

7. **Please comment on the EC statement made in para. 140 of the EC Replies to Panel Questions that "which ever approach of a risk assessment is followed, they are all based on a deterministic approach to risk characterization [and that they] have serious limitations in non-linear situations, such as in the current case regarding hormones". Are these situations, in your view, addressed by the risk assessment guidance currently available from the Codex Alimentarius Commission? Have they been addressed in the**

1988 and 1999 JECFA risk assessments of these hormones? [see Canada's comments in para. 72 of its Rebuttal Submission]

Dr. Boisseau

103. The EC statement in para. 140 of the EC Replies to Panel questions indicating that "which ever approach of a risk assessment is followed, they are all based on a deterministic approach to risk characterization (the level of exposure amounts proportionally to the level of risk for a given hazard) (and therefore they) have serious limitations in non linear situations, such as in the current case regarding hormones" refers to the genotoxic effect of oestradiol-17 β and expresses the view that, for this hormone, a threshold should not be set. This situation is addressed by the risk assessment guidance currently under discussion within the CCRVDF. In 1987 and 1999, at the time of the assessment of oestradiol-17 β , there was no risk assessment guidance available on this issue. Nevertheless, JECFA was perfectly aware about this kind of non linear situations. Thus, in 1987 and 1989, although the relevant data bases were not complete, JECFA considered that, for compounds such as Chloramphenicol, associated with aplastic anemia, and genotoxic nitroimidazole compounds such as Dimetridazole and Ronidazole, it was not possible to establish an effect/dose relation, and decided to base its conclusions on a qualitative risk assessment and did not recommend any ADI for these compounds.

104. In its 32nd session held in 1987, JECFA did not address this kind of non linear situation for oestradiol-17 β because it concluded that the tumorigenic effect associated with this compound was related to its hormonal activity and that it was therefore possible to consider a threshold in this case.

105. If, in 1999, the 52nd JECFA recognized that oestradiol-17 β "has a genotoxic potential", it concluded nevertheless that "the carcinogenicity of oestradiol-17 β was probably a result of its interaction with hormonal receptors". Therefore, it did not take into consideration a non linear situation in its risk assessment and decided to confirm its conclusions made in 1987 and to establish an ADI of 0-0.05 μ g/kg of body weight.

Dr. Boobis

Deterministic risk assessment

106. This question presupposes a specific outcome of the risk assessment, that there is no threshold for the toxicological effects of the hormones. The JECFA risk assessment concluded that the dose-response relationship for all of the endpoints was non-linear and that there was a threshold dose below which there was no appreciable risk over a lifetime of exposure. Hence, a deterministic approach, via the establishment of ADIs, was appropriate according to the procedures followed by the Committee. Should the Committee have concluded that the dose-response relationship was linear and that there was no dose below which there was no appreciable risk, there would have been two options. These would have been to have declined to establish an ADI on the basis that no exposure would be acceptable. The second would have been to establish a margin of exposure below which exposures would have been judged to pose a minimal (though non-zero) risk. Such an approach has recently been formalised (*IPCS (2005). Draft ECH, Principles for Modelling Dose-Response for the Risk Assessment of Chemicals, WHO, Geneva, Switzerland*) and was utilized by JECFA for its evaluation of certain contaminants in 2005 (*JECFA (2006b). Evaluation of Certain Food Contaminants, WHO Technical Report Series 930, WHO, Geneva*). In practice, it is likely that as veterinary drug residues in food are avoidable by not using the drug, the Committee would have declined to establish an ADI.

8. Please describe the procedure followed by JECFA in the identification of ADIs and the development of recommendations on MRLs. Please identify and describe any steps that

are taken in the risk assessment process to build a margin of safety into the final recommendation.

Dr. Boisseau

107. The procedure followed by JECFA for establishing ADIs and recommending MRLs includes three steps.

108. Establishment of a no observed adverse effect level (NOAEL) . This NOAEL is established after JECFA has considered the data obtained from all the available in vivo toxicity studies run in laboratory animals and from all the epidemiological studies and observations carried out in humans. JECFA considers also all the available in vitro tests, such as batteries of mutagenicity tests, which are likely to make easier the understanding of the mechanism of action of the toxicological effects of the veterinary drug under review. For each of these studies, except for in vitro tests, JECFA establishes a NOAEL which is the highest dose of the veterinary drug under review which is not associated with an observed adverse effect in humans or in animals. When the review of all these studies is completed, JECFA adopts, among the different NOAELs established for these studies, the final NOAEL which, once combined with an appropriate safety factor, will lead to the most conservative, the lowest, ADI.

109. Establishment of an ADI (Acceptable Daily Intake) in humans. ADI is the highest quantity of residues of the veterinary drug under review which can be daily ingested over a life time by consumers through animal derived food which will not pose a problem of health. JECFA derives such an ADI from the established NOAEL by using a safety factor. The value of this safety factor depends on the nature of the toxic effect associated with the NOAEL finally adopted by JECFA. If the NOAEL is derived from an in vivo toxicological study run in a laboratory animal, the value of such a safety factor is usually 100 as it associates two 10 safety factors. The first 10 safety factor is for the extrapolation from this laboratory animal to human as it is assumed, for caution reason, that humans may be 10 times more sensitive to this toxic effect than the laboratory animal involved in the study. The second 10 safety factor is for taking into consideration the diversity of humans, resulting from the sex, age, race, which can lead to a different sensitivity with regard to this toxic effect. If the toxic effect associated with the NOAEL finally adopted by JECFA is considered as being serious, as long as it is nevertheless still possible to consider that this toxic effect is compatible with a linear situation and the establishment of a threshold, the value allocated to the safety factor can be higher, up to 1000. On the contrary, if the adverse effect, associated with the NOAEL finally adopted by JECFA, is only derived from observations made in humans, the value of this safety factor, for exemple in the case of a reversible physiological effect, can be 10. In conclusion, the value of an ADI is usually 100 times less than the value of the corresponding NOAEL but may be also much lower.

110. Proposal of MRLs (Maximum Residue Limits). As an ADI is the final end point of the risk assessment procedure, there is a need for an operational tool which offers a practical way to be sure that this ADI will not be exceeded. That is the reason for which MRLs, already defined in my reply to the question No 6, are established so that it is possible for analytical laboratories to check that animal derived food do not contain residues of the veterinary drug under review in such amounts that the established ADI would be exceeded. In order to establish these MRLs for all the different edible tissues and products derived from the animals treated by the veterinary drug under review, JECFA uses a very conservative estimation of the human consumption of these tissues and products which represent an important additional safety factor. This food basket has been already described in my reply to the question No 6. Thus, MRLs are established in such a way that the quantities of residues potentially daily ingested resulting from this theoretical consumption of animal derived food do not exceed the value of the corresponding ADI. In addition, when it is not possible, for a veterinary drug under review, to identify and quantify all the residues associated with the toxic effect of concern, JECFA uses an additional safety factor in considering that all the residues derived from this veterinary drug have the same potential toxicity. On the other hand, all the residues which have not been proven

as being non bioavailable after oral ingestion are considered among the residues of concern. It is of special importance for the three natural hormones which are poorly bioavailable through oral route.

111. In conclusion, in order to build a margin of safety into the final recommendations, JECFA includes at different steps of its risk assessment the following different safety factors:

- (1) establishment of ADI: humans are 10 times more sensitive than the animals involved in the most sensitive toxicity test; some humans may be 10 times more sensitive than others with regard to this toxic effect; the value of the safety factor can be increased in case of some serious adverse effects,
- (2) exposure assessment: the human consumption of animal derived food is definitively overestimated,
- (3) MRLs establishment: all residues, which are not clearly demonstrated that they are not associated with the toxic effect on which the ADI is based, are considered as being as toxic as the metabolite responsible for this toxic effect. All residues, which are not clearly demonstrated as being not bioavailable via oral route, are also included in the daily intake of residues of concern.

Dr. Boobis

JECFA procedure for establishing ADIs and MRLs

112. The procedure adopted by JECFA to establish ADIs is as outlined in the guidance on risk assessment principles (*Codex Alimentarius Commission (2005)*; see also my reply to question 3 above). Specifically, the hazard identification involved a systematic examination of the studies in experimental animals, together with studies in humans, where available and in vitro studies as appropriate. The extent of these varied with the hormone, being much greater with the natural hormones than with the synthetic ones. Human studies comprised epidemiological investigations, clinical trials and experimental studies. This evaluation enabled the range of effects of the compounds to be identified. In the hazard characterization stage, the mode of action and the dose-response curve for the toxicological endpoints were determined, to the extent possible. Understanding the mode of action helped inform the interpretation of the dose response relationship. Hence, once the Committee had concluded on the weight of evidence that the carcinogenic effects observed were most likely due to an endocrine mode of action, the identification of a threshold in the dose-response relationship was consistent with this. The dose at which no effect could be observed for each endpoint was determined (NOAEL) by inspection of the data, and failing that the dose producing the lowest observable adverse effect was identified (LOAEL). These data were used as the starting points (points of departure) for the derivation of the ADIs. To allow for human interindividual variability due either to differences in sensitivity (dynamics) or kinetics, a 10-fold factor was applied. When extrapolating from studies in experimental animals an additional 10-fold factor was applied to allow for possible inter-species differences in dynamics and kinetics. If a LOAEL was used an additional factor of up to 10 was used, depending on dose spacing, the shape of the dose-response curve above the LOAEL, and the magnitude of the response. Finally, where there was an identifiable sub-group who might reasonably be expected to be more sensitive than the group in whom data were obtained, for example children relative to adults, an extra factor was applied. Exposure assessment was based on determining residues in edible tissues after controlled trials in cattle. Using radiolabel, unless there was evidence to the contrary, all radioactive material was assumed to be parent and biologically active (e.g. for MGA (see *JECFA (2000a). Residues of Some Veterinary Drugs in Foods And Animals, FAO Food and Nutrition Paper 41/13, FAO, Rome*). This is a cautious assumption, as often some or even most of the radiolabel is in the form of biologically less active or inactive metabolites (see *JECFA (2004). Residues of Some Veterinary Drugs in Foods And Animals, FAO Food and Nutrition*

Paper 41/16, FAO, Rome). Standard food consumption figures were used for different segments of the population, which again were relative conservative. Using these data the predicted exposure of high consumers was obtained, i.e. theoretical maximum daily intakes (TMDIs). In risk characterization, comparison of the estimated exposure (TMDI) with the ADI showed whether lifetime exposure at the levels predicted would be expected to be associated with any appreciable risk of adverse effects. This was undertaken for different age groups within the population. For all of the hormones under consideration, the estimated daily intake was well below the ADI, and hence use according to GVP would be without appreciable risk. Steps where a margin of safety is built in to the procedure are indicated above. However, to emphasize a few of these: risk assessment is based on the most sensitive endpoint, it assumes high level consumption over a lifetime, it often assumes that all of the residue is as active as the parent, default safety factors are used which are generally conservative.

9. **Please confirm or comment on the following Canadian statement: "it is recognized that JECFA only allocates an ADI for a food additive or veterinary drug under review when JECFA considers that its scientific data base is complete and that there are no outstanding scientific issues". [see para. 68 of Canada Rebuttal Submission]**

Dr. Boisseau

113. The Canadian statement stipulating that "it is recognized that JECFA only allocates an ADI for a food additive or a veterinary drug under review when JECFA considers that its scientific data base is complete and that there is no outstanding scientific issue" is correct.

Dr. Boobis

Influence of completeness of scientific database on establishing an ADI

114. I would qualify the statement that "it is recognized that JECFA only allocates an ADI for a food additive or veterinary drug under review when JECFA considers that its scientific data base is complete and that there are no outstanding scientific issues" as follows: This is certainly normally the case, but there are exceptions. The critical issue is whether a sufficiently cautious default can be adopted in the absence of certain information. For example, there may not be a NOAEL in a study, but it might be judged acceptable to use the LAOEL with an additional safety factor of up to 10. Similarly, the nature of the residue might not be fully defined in which case it would be assumed that it was all as active as the most active moiety, often the parent compound. As often some of the residue will be less active or inactive metabolites, this assumption is generally conservative. Hence, JECFA would require a complete data base unless it could adopt default assumptions that would if anything lead to a more conservative risk assessment than would be the case otherwise.

10. **In paras. 129 and 168 of its Replies to the Panel Questions, the European Communities states that "JECFA's traditional mandate does not allow it to examine all risk management options but restricts it to either propose MRLs or not". Does Codex have risk management options other than (1) the establishment of an MRL, (2) establishment that an MRL is not necessary or (3) no recommendation?**

Dr. Boisseau

115. As already written in my replies to the questions No 5 and 6, JECFA is only responsible for conducting the risk assessment and Codex is responsible for conducting the risk management even if JECFA is also partly involved in the risk management in proposing MRLs to Codex. To my knowledge, Codex has no other risk management options concerning veterinary drug residues than (1) the establishment of a MRL, (2) establishing that a MRL is not necessary, (3) no recommendation.

116. Nevertheless, as already said in my reply to the question No 7, when JECFA decided that it was not possible to propose any ADI for Chloramphenicol and nitroimidazole compounds, it suggested to Codex that efforts should be made to replace or prohibit the use of these veterinary drugs.

11. What should, in your view, be the components of a qualitative risk assessment, compared with a quantitative risk assessment? [see para. 82 of Canada Rebuttal Submission]

Dr. Boisseau

117. A qualitative risk assessment should be based on the following components: (1) hazard identification, (2) hazard characterization and (3) qualitative exposure assessment. A qualitative risk assessment can be applied to a veterinary drug for which it has been demonstrated that (1) according to the hazard identification step, it leads to residues in animal derived food, (2) according to the hazard characterisation step, some of these residues are responsible of an adverse effect (a) which, such as genotoxicity, can not be associated with a relation effect/dose, (b) which can be expressed in humans, (c) for which it is not possible to establish a threshold under which an amount of residues, even very limited, cannot generate this adverse effect in humans, (3) according to the exposure assessment step, consumers are likely to ingest these residues through animal derived food.

118. As already said in my reply to the question No 7, JECFA based its conclusions on such a qualitative risk assessment for chloramphenicol, dimetridazole and ronidazole and did not recommend any ADI for these compounds.

Dr. Boobis

Qualitative versus quantitative risk assessment

119. The risk assessment paradigm is such that it is not appropriate to conduct a qualitative risk assessment *a priori*. This is because such an assessment requires knowledge of hazard and mode of action, either determined experimentally or assumed. Hence, a qualitative assessment might be undertaken after conducting at least part of a conventional risk assessment, when it was apparent, or assumed, that there was no exposure that did not pose some risk and thus establishing a safe dose (an ADI) would not be possible.

120. Hence, a qualitative risk assessment should comprise all four steps of the conventional risk assessment paradigm, but with certain differences. There would still be need of hazard identification and some form of hazard characterization. During hazard characterization, if possible, the mode of action should be determined through mechanistic considerations. The potential relevance of this to human risk should be considered. Where mode of action cannot be established, human relevance is assumed in the absence of evidence to the contrary. Certain modes of action are considered to possess no threshold based on the intrinsic hazard (most notably DNA-reactive genotoxicity). For compounds exhibiting such properties it is assumed that there is no threshold for the response. In such circumstances, current practice in many regions, including WHO and the EU, would be that it would be inappropriate to derive a health based guidance value (ADI), as any exposure would be considered to pose a risk. The need for detailed dose-response analysis would be questionable. However, in a risk assessment, as opposed to risk management, there is still need for scientific rigour. Hence, the conclusion that exposure is irrelevant because of the nature of the effect is a risk management decision. In risk assessment, even if establishment of an ADI is considered inappropriate, it would be of value to risk managers to provide a margin of exposure estimate, to determine how great the risk is likely to be. This would require exposure assessment. This would be of help in considering the relative risk compared with background exposure, particularly for compounds occurring

endogenously. Finally, risk characterization would be necessary to consider the relevance of experimental observations to humans. There may be kinetic or dynamic factors indicating that although theoretically there was no exposure with zero risk, in practice the risk would be minimal and therefore acceptable (e.g. PPR Opinion on daminozide, which contributed to EC decision to approve annex 1 listing of the compound .(PPR (2004). *Opinion of the PPR Panel related to the evaluation of daminozide in the context of Council Directive 91/414/EEC (May 2004)* (http://www.efsa.eu.int/science/ppr/ppr_opinions/453_en.html; *Official Journal L 241* , 17/09/2005 P. 0051 – 0056))

Dr. Cogliano

121. The components of a qualitative risk assessment are (1) a critical review of the pertinent scientific information on an agent and (2) an evaluation of the weight of the evidence that the agent can alter the risk of one or more adverse effects.

122. Paragraph 82 of Canada's Rebuttal Submission seems confused about the role of dose-response analyses in a qualitative assessment. A qualitative risk assessment can consider the presence or absence of dose-response relationships in evaluating epidemiological and experimental information. For example, the *IARC Monographs* do this in their evaluations of whether an agent can alter the incidence of cancer in humans. This is a completely different matter from estimating the dose of an agent that may provoke a specific level of adverse effect. This latter activity is part of quantitative risk assessment and it can be delineated as a separate activity from the qualitative risk assessment.

12. How is scientific uncertainty addressed in risk assessments in general? With respect to the assessment of risks from the consumption of meat treated with the growth promotion hormones at issue, how has scientific uncertainty been considered by JECFA/Codex? How does it differ from the way it has been considered by the European Communities in its assessment of risks from the consumption of meat treated with the growth promotion hormones at issue?

Dr. Boisseau

123. In assessing the risk for human health associated with the exposure to veterinary drug residues, JECFA addresses the scientific uncertainty by using the safety factors listed above in my reply to question 8 describing, among others, how JECFA builds a margin of safety into its final recommendations.

124. For the hormonal growth promoters, JECFA has considered that, given the quality and the quantity of the available data, it was possible to carry out a complete quantitative risk assessment. For establishing ADIs and MRLs for the three synthetic hormones, melengestrol, trenbolone and zeranol, JECFA has implemented the usual procedure regarding the safety factors. For the three natural hormones, oestradiol-17 β , progesterone and testosterone, JECFA has decided that the margin of safety deriving from the values of the established ADIs and from a maximum estimated intake of residue was such that it was not necessary to set up MRLs.

125. For oestradiol-17 β , the European Communities did not consider any scientific uncertainty as it decided that it was not possible, for reasons of principle, to establish an ADI for a genotoxic compound. For the five other hormones at issue, the European Communities did not really consider any scientific uncertainty as it decided that the available data were too limited to allow a complete quantitative risk assessment to be carried out.

Dr. Boobis

Addressing scientific uncertainty in risk assessment

126. Scientific uncertainty is dealt with in a variety of ways in risk assessment. A description of some of the issues can be found in the draft report of the UK VUT (Variability and Uncertainty in Toxicology) working group of the COT (April, 2006) at <http://www.food.gov.uk/multimedia/pdfs/vutdraftreport.pdf>.

127. One way of dealing with uncertainty is to default to the worst case in the absence of evidence to the contrary. Hence, the most sensitive relevant endpoint in the most sensitive species is used as the basis of the risk assessment. In extrapolating to humans a default factor of 10 is used to allow for species differences, which assumes that humans are more sensitive than the experimental species. A further factor of 10 is included for interindividual differences. These differences may be due to gender, genetics, life stage or other factors. However, to some extent such differences have already been taken into account in the choice of endpoint, as this will usually represent the most sensitive lifestage, gender and to some extent genetics by using data from the most sensitive species. Where there are additional uncertainties, such as no NOEL or the absence of a non-critical study, an additional safety factor will be included, and this is almost always conservative, as when the data gaps have been completed, the appropriate safety factor is almost always less than that used to account for these data gaps. The residue may be assumed to be all as active as the most active moiety, which is almost always a conservative assumption. Dietary intake is based on conservative data for food consumption. It is also assumed that all meat that could contain veterinary drug residue will contain the residue and that this will be present at the high end of the range (MRL or other appropriate level). In respect of the ADI, the assumption is that intake will be at this high level for a lifetime, when in reality there will be occasions when little or no meat is consumed or that which is consumed contains less or even no residue. In their risk assessment of the hormones, JECFA applied all of these approaches to dealing with the uncertainty.

128. In dealing with scientific uncertainty much depends on the expert judgment of the risk assessor. Issues such as biological coherence, whether effects are considered compound related, relevance to humans, the reliability of model systems at predicting effects in vivo all impact on the interpretation of the data. Within the EU, it is clear that there are also differences in the interpretation of data, as illustrated by the differing conclusions of the Committee on Veterinary and Medicinal Products - CVMP (1999) and the Scientific Committee on Veterinary Measures relating to Public Health - SCVPH (1999). In part, the EC assessment of the hormones did not go as far as including some of the considerations for uncertainty used by JECFA because of the conclusion that there was insufficient information to determine whether there was a threshold for the carcinogenic effects. However, for some of the compounds this was based on the results of a small number of non-standard tests of genotoxicity, with equivocal or very weak responses. It is not clear whether the EC applied a weight of evidence approach to evaluating the genotoxicity of all of the compounds, taking into account the totality of the available data, as was the case by JECFA.

CVMP (1999). Report of the CVMP on the Safety Evaluation of Steroidal Sex Hormones in particular for 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 (<http://www.emea.eu.int/pdfs/vet/srwp/088599en.pdf>)

SCVPH (1999). Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health: Assessment of potential risks to human health from hormone residues in bovine meat and meat products (http://ec.europa.eu/food/fs/sc/scv/out21_en.pdf)

C. ASSESSMENT OF OESTRADIOL-17 β

13. **To what extent, in your view, does the EC risk assessment identify the potential for adverse effects on human health, including the carcinogenic or genotoxic potential, of the residues of oestradiol-17 β found in meat derived from cattle to which this hormone had been administered for growth promotion purposes in accordance with good veterinary practice? To what extent does the EC risk assessment evaluate the potential occurrence of these adverse effects?**

Dr. Boisseau

A. CARCINOGENIC POTENTIAL OF THE RESIDUES OF OESTRADIOL-17 β

129. There is a general international agreement to recognize that oestradiol-17 β is associated with a carcinogenic potential resulting from its interaction with hormonal receptors.

130. For example, in its fifty second session held in 1999, JECFA noted that "in long term studies in carcinogenicity in animals, reviewed at its thirty second meeting, oral and parenteral administration of oestradiol-17 β increased the incidence of tumors only in hormone dependent tissues including the kidney of male Syrian hamsters" and concluded that "the carcinogenicity of oestradiol-17 β is most probably a result of its interaction with hormonal receptors". Considering also epidemiological studies on women who took oestrogens, either alone or in combination with progestogens and androgens, JECFA concluded that "the available data suggest that the increase of cancers of the breast and the endometrium observed in women receiving post menopausal oestrogen replacement therapy is due to the hormonal effect of oestrogens. Therefore, JECFA has considered appropriate to establish a NOAEL on the basis of the changes in several hormone dependent parameters in post menopausal women and to derive from this NOAEL an ADI using two safety factors of 10, one to account for normal variation among individuals and a second one to protect the sensitive human populations.

131. In its 1999 report, CVMP concluded also that "hormonal carcinogens in humans and experimental animals are characterized by (1) tumorigenic action typically in various endocrine-responsive organs and/or tissues and (2) the need for a prolonged exposure to high concentrations before tumorigenic effects become apparent".

132. In its 1999 report, SCVPH concluded also that "whether it is clear that exogenous oestrogens, present in oral contraceptives or used in hormonal replacement therapy in women, are responsible for an increase risk of endometrial cancer and, to lesser extent, some increased risk of breast cancer, there is no direct evidence on the consequences of the contribution of exogenous oestradiol-17 β originating from the consumption of treated meat".

B. GENOTOXIC POTENTIAL OF THE RESIDUES OF OESTRADIOL-17 β

133. The amounts of substance needed to be used in toxicological studies in general are, by far, higher than the levels of residues likely to be present in food derived from animals treated by veterinary drugs. If these studies would have been carried out with the very little amounts of substances such as those corresponding to the residue levels in food, they would have always led to negative results. That is the reason for which these studies are, practically, always carried out with the parent substances and not with the residues and it is assumed that the residues derived from the parent substances have the same toxicological potential as these parent substances. As far as they are concerned, genotoxicity tests are mainly carried out in order to understand the mechanism of the carcinogenic effects, if any, of the substance under review and even the in vivo tests, because it is obvious for the in vitro studies, are not scheduled to determine a dose-response relationship and to

establish a threshold. Therefore, when genotoxicity tests give positive results, it is only possible to conclude that the parent substance itself has been shown genotoxic in the conditions of these tests and that its residues, given their very low levels in animal derived food, may have also a genotoxic potential.

134. There is currently some general agreement on the fact that oestradiol-17 β is associated with a genotoxic effect.

135. Thus, although it recognized that oestradiol-17 β does not lead to positive results in all the classical tests which have been used to demonstrate its genotoxicity and its mutagenicity (oestradiol-17 β did not cause gene mutations in vitro and gives, in some other assays, sporadic but unconfirmed positive results), JECFA, in its fifty second session held in 1999, concluded "that oestradiol-17 β has a genotoxic potential".

136. In its 1999 report, the Committee for Veterinary Medicinal Products (CVMP) of the European Medicine Agency (EMA) released the following conclusions: "oestradiols and/or their synthetic analogues are devoid of the ability to induce gene mutations or chromosomes aberrations in vitro. With regard to the studies of Rajah and Pento (1995) and Thibodeau et al. (1998), those are considered inconclusive and, therefore, additional experiments are needed before making any statement that oestradiol-17 β induces MTX resistance and/or HPRT-deficient gene mutations. Tsutsui and Barret and Tsutsui et al. hypothesised that oestradiols are capable of inducing aneuploidy, followed by malignant transformation and the studies of Abul-Hajj et al., Paquette, and Anderson et al. may suggest that oestradiol-17 β and/or its metabolites induce DNA damage or genomic instability. However, the demonstration remains to be made that the observed indicator effects are representative of mutagenesis at the gene or chromosome level and also occur in somatic cells in vivo. This is not likely in the view of the following: earlier studies had mostly indicated that hormones do not induce micronuclei or other chromosomes aberration types in vivo. With the exception of the study reported by Dhillon and Dhillon, the recent data confirm the earlier findings and clearly indicate that hormones and/or their synthetic analogues are not associated with genotoxicity properties in the bone marrow micronucleus assay in vivo.

137. The sub-group of the UK Veterinary Product Committee (VPC) concluded in its 1999 report that "there is currently no positive results from internationally accepted test systems which indicate that the hormones considered in the report are genotoxic".

138. In its 2002 opinion, SVCPH reported a series of new assays in which oestradiol-17 β and/or its metabolites induce positive results but it has to be noted that all these assays have been carried out in vitro studies with cell cultures and no one in an in vivo study.

139. If there is currently some general agreement on the fact that oestradiol-17 β is associated with a genotoxic effect, there is nevertheless no agreement on the fact that this genotoxic potential could be expressed in vivo in order to give to oestradiol-17 β the capacity to act as a complete carcinogen, responsible of both initiation and promotion of tumours.

140. CVMP, quoting JECFA(1999) and IARC(1999) concluded that the potential genotoxic properties of the compounds(hormones and in particular oestradiol-17 β) would not be expressed in vivo and/or not play a role in the tumorigenic activity. Therefore, it does mean that, even it has been considered that oestradiol-17 β has a genotoxic potential, the tumorigenic activity of this hormone is not associated with its genotoxic potential but with its hormonal activity.

141. If SCVPH, in its 1999 report, expresses its concern in concluding that "Finally, in consideration of the recent data on the formation of genotoxic metabolites of oestradiol suggesting oestradiol-17 β acts as complete carcinogen by exerting tumour initiating and promoting effects ... no

quantitative estimate of the risk related to residues in meat could be presented", it provides no data indicating that oestradiol-17 β is associated with the increase of tumours in tissues or organs which are not hormone dependent.

142. In conclusion, the EC risk assessment did not support that residues of oestradiol-17 β , despite the genotoxic potential of this hormone, can initiate and promote tumours in humans.

C. OTHER ADVERSE EFFECTS ON HUMAN HEALTH

143. In its 1999 Opinion, SCVPH has also identified that hormonally active substances could be associated with other adverse effects concerning, for example, the intrauterine and perinatal development, the growth and puberty in humans and the immune system. Nevertheless, these data have not been used by the European Communities to conduct any quantitative risk assessment likely to lead, for these effects associated with the hormonal properties of growth promoters, to the establishment of thresholds and ADIs different from those proposed by JECFA.

Dr. Boobis

EC risk assessment of hormone residues in meat

144. The EC has not identified the potential for adverse effects on human health of residues of oestradiol found in meat from treated cattle. This is because the analysis undertaken was focused primarily on hazard identification. There was little in the way of hazard characterization, and no independent exposure assessment was undertaken. Data from the JECFA evaluation were used, together with speculative assumptions about misuse or abuse of the product. No adequate assessment of exposure following use according to GVP was undertaken. Hence, it was not possible to complete the risk characterization phase of the assessment. The EC's evaluation essentially stopped once it was concluded that the effects of the hormone were such that there were no thresholds (genotoxic carcinogenicity and hormonal effects). There was no attempt to estimate the potential occurrence of adverse effects in humans following exposure to levels of the hormones found in meat from treated animals.

Dr. Guttenplan

145. I believe the EC has done a thorough job in identifying the potential for adverse effects on human health of oestradiol-17 β found in meat derived from cattle to which this hormone had been administered. They have identified a number of potential adverse effects of oestradiol-17 β in humans. They have established metabolic pathways relevant to these effects, and have examined mechanisms of these effects. In addition they have performed thorough studies of residue levels in cattle, and the environment. The evidence evaluating the occurrence of adverse effects is weak. Animal models are very limited and the target organs do not coincide well with the target organs in humans. There are basically no epidemiological studies comparing matched populations consuming meat from untreated and hormone-treated cattle. Thus, little can be inferred about the potential occurrence of the adverse effects, the potential for adverse effects seems reasonable. (JECFA Meeting 52-WHO-FAS 43, SCVPH Opinions 1999, 2002).

14. In your view, does the risk assessment undertaken by the European Communities on oestradiol-17 β follow the Codex Guidelines on risk assessment, including the four steps of hazard identification, hazard characterization, exposure assessment and risk characterization with respect to oestradiol-17 β ?

Dr. Boisseau

146. The European Communities does not indicate anywhere in its submission that it does not intend to follow the Codex guidelines on risk assessment including the four steps of hazard identification, hazard characterisation, exposure assessment and risk characterisation. On the contrary, the following indicates that the European Communities considers the same approach for assessing the risk associated with the residues of growth promoters. It only claims, on the basis of the opinion released by the Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) in 1999, as the two following opinions of this SCVPH, released successively in 2000 and 2002, did not amend this conclusion adopted in 1999, that it is not possible to carry out a quantitative risk assessment with regard to the six hormones in general and to oestradiol-17 β in particular. For the European Communities, such a quantitative risk assessment cannot be carried out because "In consideration of the recent concerns relating to the lack of understanding of critical developmental periods in human life as well as the uncertainties in the estimates of endogenous hormone production rates and metabolic clearance capacity, in particular in prepubertal children, no threshold and therefore no ADI can be established for any of the six hormones".

147. After re-appraisal of 17 studies launched early in 1998 and recent literature, SCVPH, in its opinion released in 2002, adopted conclusions which do not challenge the Codex guidelines on risk assessment. SCVPH concluded, among others, that (1) "the consequence of the consumption of lipoidal esters of oestradiol-17 β needs to be considered in a risk assessment", (2) "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 site", "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 increase adult breast cancer risk" (These two statements call for refining the exposure assessment to hormone residues).

Dr. Boobis

Adherence of EC assessment to Codex risk assessment guidelines

148. As indicated above, the EC risk assessment of oestradiol does not follow the four steps of the Codex risk assessment paradigm. Even if it were concluded that oestradiol is a genotoxic carcinogen, the four steps should have been followed, for the reasons explained in answer to question 11 above, and as described further in the next section.

Dr. Guttenplan

149. The EC has been thorough in following Codex guidelines on hazard identification and very thorough in exposure assessment. The hazard characterization is more limited since there is only one animal model that is well characterized and this is in the hamster kidney. As kidney is not a known target of estradiol in humans the extrapolation to humans is uncertain. The risk characterization is very qualitative at best. There is also a mouse uterus model, but this has not been characterized with respect to dose-response and mechanism. More limited data is available in certain other animal systems and these are older studies with no reports of replication. There are no epidemiological studies comparing cancer incidence or prevalence in populations consuming hormone-treated or untreated meat, and, as indicated above, the hazard characterization is limited. Thus, taken together, the risk assessment has a mixed rating in following the Codex guidelines.

[The references for the two questions above are: para. 77 of EC Replies to Panel Questions and the Opinions in Exhibits US-1, 4, and 17; paras. 194-207 of EC Rebuttal Submission (US case), paras. 115-127 of EC Rebuttal Submission (Canada case), paras. 85-91, 134-153 of EC Replies to Panel Questions; paras. 35-40 US Rebuttal Submission, paras. 72-73 of US Replies to Panel

Questions, paras. 140-160 of US First Submission; paras. 70-111 of Canada Rebuttal Submission and paras. 88-106 of Canada First Submission]

D. CONSUMPTION OF MEAT CONTAINING HORMONES

(a) Carcinogenicity

15. Does the identification of oestradiol-17 β as a human carcinogen indicate that there are potential adverse effects on human health when it is consumed in meat from cattle treated with hormones for growth promotion purposes? Does your answer depend on whether good veterinary practices are followed? [see paras. 206-207 of EC Rebuttal Submission (US case), para. 121 of EC Rebuttal Submission (Canada case), para. 97-98 of EC Replies to Panel Questions, paras. 76-77, 150 and 155-156 of US First Submission, paras. 35-40 and 46 of US Rebuttal Submission]

Dr. Boisseau

150. Considering my reply to question 13, it is legitimate to conclude that (1) the carcinogenic potential of oestradiol-17 β results from its hormonal activity, (2) it is possible to establish a NOAEL and, by using an appropriate safety factor, to derive from this NOAEL an ADI which represents the highest quantity of oestradiol-17 β causing in humans no hormonal effect and therefore no carcinogenic effect. On these grounds, it is possible to conclude, in agreement with JECFA, that oestradiol-17 β , even it has been recognized as being able to generate tumours, is not likely to produce adverse effects on human health when it is consumed in meat from cattle treated with hormones for growth promotion purposes.

151. My reply depends on the efficient implementation of good veterinary practices. It has to be clearly understood that if these good veterinary practices are not implemented or if the conditions of use of the veterinary drugs in animal husbandry are different from those which have been taken into consideration by JECFA in its risk assessments, all the work carried out since years by both JECFA and Codex to establish MRLs to guarantee the hygienic quality of animal derived food and to protect human health with regard to veterinary drug residues is meaningless.

Dr. Boobis

Relevance of carcinogenicity of oestradiol-17 β

152. The entire basis of risk assessment is based on the fact that there is a relationship between dose and effect. This is true even for compounds for which there is no threshold in their dose-response curve. Hence, the greater the dose the greater the risk. The corollary is that the lower the dose, the lower the risk. A key consideration in the risk assessment is whether there is a threshold in the dose-response. If not, whilst risk declines with dose, it does not reach zero until there is no exposure (zero dose). However, in the case of oestradiol, the issue is complicated by the fact that the compound is produced naturally in the body. Hence, an additional factor in the risk assessment of this compound is whether the levels from consumption of meat from treated animals impacts on the circulating levels of the hormone. If not, then there should be no change in risk.

153. JECFA concluded that whilst oestradiol is a human carcinogen, its mode of action is such that there would be no appreciable risk of cancer at exposures up to the ADI. The risk of cancer at exposures above the ADI would depend on the duration of exposure, which would need to be relatively prolonged (in the order of years rather than months) and on the magnitude of the exposure. It is likely that at exposures slightly above the ADI, the risk would be minimal. However, it is not possible to estimate with any accuracy at which level of exposure risk would become significant.

This would also vary with the individual. Exposure from meat of cattle treated according to GVP would be substantially below the ADI and hence the threshold for any carcinogenic effects. If GVP is not followed, then whether there is a carcinogenic risk would depend on whether the ADI is exceeded and by what margin. However, even if the ADI is exceeded, this would have to be on a regular basis. As indicated above, the occasional exposure above the ADI, such as might occur if GVP is not followed, would not be associated with any increase in risk of cancer.

Dr. Cogliano

154. The identification of oestradiol-17 β as a human carcinogen indicates that there are potential adverse effects on human health when oestradiol-17 β is consumed in meat from cattle treated with hormones for growth promotion purposes. This answer does not depend on whether good veterinary practices are followed. It depends on the presence of the hormone in the meat that people consume.

Dr. Guttenplan

155. If potential is taken to mean possible, then an adverse effect cannot be ruled out, but it is unlikely if good veterinary practices are followed. If good veterinary practices are not followed, the potential for adverse effects may be significant. (JECFA Meeting 52-WHO-FAS 43, SCVPH Opinions 1999, 2002).

16. Does the scientific evidence relied upon in the SCVPH Opinions support the conclusion that carcinogenic effects of the hormones at issue are related to a mechanism other than hormonal activity? [see para. 148 of the EC Replies to Panel Questions and paras. 35-40 and 46 of US Rebuttal Submission]

Dr. Boisseau

A. OESTRADIOL-17 β

156. Considering my reply to the question 13, the scientific evidence relied upon in the SCVPH Opinions does not support the conclusion that carcinogenic effects of oestradiol-17 β are related to a mechanism other than hormonal activity.

B. PROGESTERONE

157. In its thirty second session, JECFA concluded that "Although equivocal results have been reported for the induction of single-strand DNA breaks and DNA adducts have been seen in vivo and in vitro in some studies, progesterone was not mutagenic ... progesterone has no genotoxic potential". It concluded also that "these effects on tumour production occurred only with doses of progesterone causing obvious hormonal effects ... the effects of progesterone on tumour production was directly related to its hormonal activity".

158. In its 1999 report, SCVPH concluded, about the carcinogenicity of progesterone, that "At present, the data are insufficient to make any quantitative estimate of the risk arising from the exposure to residues in meat" Therefore, the scientific evidence relied upon in the SCVPH Opinions does not support the conclusion that the carcinogenic effects of progesterone are related to a mechanism other than hormonal activity.

C. TESTOSTERONE

159. In its thirty second session, JECFA concluded that the increase of the incidence of prostatic and uterine tumours observed in rodents treated with high doses of testosterone resulted from the

hormonal activity of testosterone". In its fifty second session held in 1999, JECFA concluded that "In mammalian cells, no chromosomal aberrations, mutations or DNA adducts were found following treatment with testosterone ... testosterone has no genotoxic potential".

160. In its 1999 report, SCVPH concluded, about the carcinogenicity of testosterone, that, given the limited data on genotoxicity and on carcinogenicity in humans, no conclusive quantitative estimate of the risk arising from the excess intake with meat from treated animals can be made. Therefore, the scientific evidence relied upon in the SCVPH Opinions does not support the conclusion that the carcinogenic effects of testosterone are related to a mechanism other than hormonal activity.

D. MELENGESTROL

161. In its fifty fourth session, JECFA concluded from the review of a range of assays in vitro and in vivo that melengestrol acetate is not genotoxic. It also agreed upon the fact that "no firm conclusion could be drawn about the carcinogenic potential of melengestrol acetate in ICR mice ... the increased incidence of malignant tumors in the highest-dose group of prepuberal C3Han/f mice was assumed to be due not to a direct carcinogenic effect of melengestrol acetate but to the promoting effect of increased prolactin concentrations".

162. In its 1999 report, SCVPH concluded, about the carcinogenicity of melengestrol, that "in view of the lack of data on mutagenicity/carcinogenicity and on DNA interactions and in consideration of carcinogenicity studies conducted only in one animal species, these data are inadequate to assess the carcinogenic potential of melengestrol. Therefore, the scientific evidence relied upon in the SCVPH Opinions does not support the conclusion that the carcinogenic effects of melengestrol are related to a mechanism other than hormonal activity.

E. TRENBOLONE

163. In its thirty second session held in 1987, JECFA concluded from carcinogenic studies in animals that "the liver hyperplasia and tumours in mice ... and the slight increase in the incidence of islet-cell of the pancreas of rats arose as a consequence of the hormonal activity of trenbolone". In its thirty fourth session held in 1989, JECFA, having reviewed a comprehensive battery of short term tests, concluded that " it was unlikely that trenbolone acetate was genotoxic" and decided to confirm its previous conclusion to base the evaluation of trenbolone acetate and its metabolites on their non-hormonal-effect.

164. In its 1999 report, SCVPH concluded, about the carcinogenicity of trenbolone, that "in consideration of the lack of in vitro short term assays on mutagenicity and genotoxicity of other trenbolone metabolites other than α -trenbolone and in consideration of the equivocal results of the transformation assays and the in vivo studies, the available information is insufficient to complete a quantitative risk assessment". Therefore, the scientific evidence relied upon in the SCVPH Opinions does not support the conclusion that the carcinogenic effects of trenbolone are related to a mechanism other than hormonal activity.

F. ZERANOL

165. In its thirty second session held in 1987, JECFA concluded that zeranol and its metabolites, zearalanone and taleranol, were not mutagenic in a number of tests in bacterial and mammalian systems even if it has noted that zeranol gives a positive result in the Rec-assay and taleranol gives a positive result in the test with Chinese hamster ovary cells in the absence of activation but a negative result with activation. After having reviewed the carcinogenicity studies in animals, JECFA concluded that " the tumorigenic effect of zeranol was associated with its oestrogenic properties".

166. In its 1999 report, SCVPH concluded, about the carcinogenicity of zeranol, that "in consideration of the lack of data on mutagenicity/genotoxicity and the clear evidence for an induction of liver adenomas and carcinomas in one animal species, no assessment of the possible carcinogenicity of zeranol can be made". Therefore, the scientific evidence relied upon in the SCVPH Opinions does not support the conclusion that the carcinogenic effects of zeranol are related to a mechanism other than hormonal activity.

167. In conclusion, considering my reply to question 13 above, the scientific evidence relied upon in the SCVPH Opinions does not support the conclusion that carcinogenic effects of oestradiol-17 β are related to a mechanism other than hormonal activity. On the other hand, considering the conclusions of JECFA and the fact that SCVPH bases always its reservations on the lack of data more than on data establishing the genotoxicity and the capacity of the five other hormones (progesterone, testosterone, melengestrol, trenbolone and zeranol) to act as complete carcinogens, it can be said that the scientific evidence relied upon in the SCVPH Opinions does not support the conclusion that the carcinogenic effects of these five hormones are related to a mechanism other than hormonal activity.

Dr. Boobis

Mechanism of carcinogenicity of oestradiol-17 β

168. There is no doubt that some of the hormones in dispute are genotoxic and mutagenic in some assays *in vitro*. However, the conduct and interpretation of these assays requires expert judgment. Some endpoints are prone to artefactual positives, for example due to cytotoxicity and even a true positive may be a reflection of the non-physiological conditions of the *in vitro* system (*Greenwood et al, 2004; Kirkland et al, 2005*). Hence, the guidelines on genotoxicity testing require confirmation of an *in vitro* positive using an appropriate *in vivo* assay (*CVMP, 2004*). An additional factor is the testing of metabolites or putative metabolites. *In vitro* it is possible to test these unopposed by detoxication or excretion processes. However, *in vivo*, it is often the situation that some metabolites are not formed in sufficiently high concentrations for a sufficient period of time to cause any genotoxicity. A key example is the formation of reactive oxygen species. Whilst this is an established mechanism for mutagenicity *in vitro*, there is very little evidence for such an effect *in vivo* (*Bianco et al, 2003; Brusick, 2005*). Further, there is a threshold for this mechanism, due to the efficiency of endogenous antioxidant systems (*Aria et al, 2006; Russo et al, 2004*). This is because endogenous production of reactive oxygen species during intermediary metabolism is substantial, and hence efficient protective systems have evolved to maintain the integrity of the cells (*Russo et al, 2004*). For these reasons, *in vitro* studies implicating metabolites in the mode of action of a carcinogen should be supported by mechanistic studies *in vivo*. In particular, evidence for the formation and genotoxic effects of such metabolites should be sought *in vivo*. Whilst there is adequate evidence that some of the hormones are genotoxic in some *in vitro* assays, there are data supporting mechanisms other than direct reactivity with DNA. The possibility of redox cycling of some metabolites, with the generation of reactive oxygen species that can give rise to 8-hydroxylation of guanine has been discussed above (see also *Yagi et al, 2001*). Redox cycling may give rise to adducts by other mechanisms, such as formation of aldehydes (*Lin et al, 2003*). There are clear thresholds for these interactions (see above). The evidence is against any direct interaction of oestradiol or its metabolites with DNA (*Chen et al, 2005; Hurh et al, 2004; Huez et al, 2004*). Oestradiol can cause genotoxicity by effects other than direct or indirect interaction with DNA. These include induction of micronuclei (*Fischer et al, 2001*) and promotion of DNA instability (*Stopper et al, 2003*), both of which exhibit thresholds.

169. The carcinogenic effects of oestradiol appear to be a consequence of its endocrine activity. Some of the evidence for this is the target tissues, which are hormonally responsive, the concordance of carcinogenic effect with oestrogenic potency, the absence of reliable evidence for genotoxicity, including DNA binding, in target tissues (see above). It is notable that specific antagonism of the

oestrogen receptor in women with drugs such as tamoxifen, markedly reduces the risk of oestrogen-related cancers, such as of the breast in those with high risk factors due to endocrine status (Fisher *et al*, 2005). This suggests that the carcinogenic effects of oestradiol are mediated, to the extent that can be estimated from such studies, by activation of the oestrogen receptor. The importance of the oestrogen receptor (ER α) in the carcinogenic effects of oestradiol is reinforced by the results of experimental studies in genetically engineered mice (Tilli *et al*, 2003).

170. As indicated above, the studies in which positive results were obtained for the genotoxicity of oestradiol and upon which the conclusions of the EC regarding mechanism were based, should have been evaluated on a weight of evidence basis. Several of the studies suffered from significant limitations and there were a number of well conducted studies on a variety of endpoints that should have been included in such an evaluation.

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Dr. Guttenplan

171. The SCVPH Opinions (SCVPH Opinions 1999, 2002) do indicate that a mechanism other than hormonal activity is possible, "In acknowledging the recent findings on the metabolism based genotoxicity of 17- β oestradiol (see chapter 2.5 of the report) it has to be stated that the assumption that the carcinogenic potential is exclusively related to the hormonal activity is no longer valid." However, the US and Canada cite other reports indicating that genotoxic effects of estrogens are unlikely. It should also be noted that more recent reports support a role for a genotoxic mechanism by which hormones contribute to cancer (SCVPH Opinion, 2002).

17. Could you comment on Canada's statement that "the studies commissioned by the European Communities also failed to find evidence of "catechol metabolites" – that is the oestradiol metabolites identified as the source of the genotoxic potential – in meat from treated animals"? What would be the implication of an absence or presence of catechol metabolites? [see para. 102 of Canada Rebuttal submission, EC Exhibit 51A]

Dr. Boisseau

172. The Canada statement that " the studies commissioned by the European Communities also failed to find evidence of " catechol metabolites "in meat from treated animals" seems right if the study reported in the Exhibit EC-51A is considered. It is written in this report, page 15, that, in an in vivo metabolic study, "(1) the presence of methoxy-oestrogens that derive by catechol-O-methyltransferase activity from catechol oestrogens was demonstrated neither in liver nor in kidney... (2) Residues ... are scarcely detectable 12 days after injection of oestradiol-17 β , that could be explained by a fast turn over of metabolites covalently bound to macromolecules, if really present, which should be different from catechol oestrogens adducts in proteins ... (3) However, glutathione

or glucuronide conjugates of catechol oestrogens could be present at very low concentrations in liver or kidney extracts and could correspond to sub-minor peaks we have isolated without being able to identify them due to the too low amounts we have purified ... Nevertheless, in urine of one steer, we have identified a glucuronic acid derivative of a methoxy-estrone as a minor metabolite, which demonstrates that catechol oestrogen biosynthesis activity is present although very weak.... (4) no trace of catechol oestrogen adducts could be detected at the same time in this fraction " page 16, that, in in vitro studies, "No metabolites coming from the catechol oestrogen biosynthesis could be isolated" page 18, that "metabolic studies performed in vivo ...and in vitro ...failed to demonstrate a significant aromatic hydroxylation activity that would lead to catechol oestrogen derived metabolites.

173. In conclusion, (1) it can be said that this study could not find evidence of metabolites coming from the catechol oestrogen biosynthesis. Nevertheless, it cannot be excluded that such catechol oestrogen biosynthesis may exist although being very weak, (2) if the amount of catechol metabolites would have been demonstrated as being significant, which is not the case, the genotoxic potential of these metabolites would have to be taken into consideration in assessing the genotoxicity potential of oestradiol-17 β .

Dr. Boobis

Relevance of catechol metabolites

174. The analytical data certainly show that levels of catechol metabolites in meat from treated animals were below the limits of detection of the method. This is consistent with the rapid detoxication and elimination of these metabolites in vivo. The implications for the risk assessment of oestradiol would depend on the underlying assumptions for the carcinogenic effects of the compound. For the catechols to be significant it would be necessary for these to be responsible for the carcinogenic effects of oestradiol, it would be necessary for there to be no threshold for their effects and if there were it would be necessary for intake to exceed this threshold. Oestradiol is itself carcinogenic at high doses in human subjects. Hence, there is no need for exposure to preformed catechols for a carcinogenic effect. If these are necessary for the carcinogenicity, sufficient can be formed in vivo. However, as indicated above, there is no good evidence implicating catechols in the carcinogenic effects of the hormones. Further, also as discussed above, any genotoxicity of these compounds due to redox cycling would be militated against by endogenous anti-oxidant systems. Hence, whilst the absence of detectable catechols in meat from treated animals is reassuring, even if they were detected at low levels, it would not impact on the risk assessment.

Dr. Cogliano

175. The presence of catechol metabolites would support the potential for adverse effects to occur. The absence of catechol metabolites could imply either (1) that detectable levels of catechol metabolites were not formed from the parent compound or (2) that some level of catechol metabolites was formed that the test methods were not sufficiently sensitive to detect it.

Dr. Guttenplan

176. It is true that only very small amounts of catechol metabolites were detected in meat from treated animals. However, significant levels of estradiol and estrone were detected. These can be metabolized in humans to catechols (*Rogan EG. Badawi AF. Devanesan PD. Meza JL. Edney JA. West WW. Higginbotham SM. Cavalieri EL. Relative imbalances in estrogen metabolism and conjugation in breast tissue of women with carcinoma: potential biomarkers of susceptibility to cancer. Carcinogenesis. 24(4):697-702, 2003*). In contrast to humans, cattle do not efficiently metabolize estradiol to catechols. The latter explains the very low levels of catechols in meat. Thus,

the lack of catechols in meat does not imply that meat from estrogen-treated cattle is without risk for genotoxicity.

- 18. Please comment on the US argument that the European Communities fails to demonstrate through scientific evidence that oestradiol-17 β is genotoxic. Would your reply have been different at the time of adoption of the EC Directive in September 2003? If so, why? [see paras. 118-119 of EC Rebuttal Submission (US case), paras. 123-124 of EC Rebuttal Submission (Canada case), paras. 87-91 and 153-156 of US First Submission, paras. 35-40 and 46 of US Rebuttal Submission, and paras. 90-97 of Canada Rebuttal Submission]**

Dr. Boisseau

177. This issue regarding the genotoxic potential of oestradiol-17 β has been already addressed in my reply to the question 13. In addition, I would like to comment the content the para 118 and 119 of the EC Rebuttal Submission (US case). It is true that JECFA, considering the outcome of its assessment, did not think necessary in 1988 to establish an ADI for the three natural hormones. Later on, not because JECFA has amended its assessment regarding these three hormones but in order to present in a more convincing way the outcome of its assessment, it decided, in its fifty second session held in 1999, to establish an ADI for each of the three natural hormones and to indicate that the estimated intake of residues accounts respectively for 2%-4% of the ADI for oestradiol-17 β , 0,03% of the ADI for progesterone and 0,05% of the ADI for testosterone. On the other hand, taking into consideration my reply to the question number 8, it has to be reminded that this theoretical estimated intake of residues is all the more conservative that it disregards the very poor oral bioavailability of these hormones.

178. My reply would not have been different at the time of adoption of the EC Directive in September 2003.

Dr. Boobis

Genotoxicity of oestradiol-17 β

179. This issue has been discussed in detail in response to question 15. To reiterate, whilst there are reliable studies demonstrating the genotoxicity of oestradiol in certain in vitro tests, the evidence is against any genotoxicity in vivo. Some, if not all, of the genotoxicity observed in vitro would be expected to exhibit a threshold, particularly that involving reactive oxygen species. My reply to this question would have been the same at the time of adoption of the EC Directive in September 2003.

Dr. Cogliano

180. The EC does demonstrate through scientific evidence that oestradiol-17 β is genotoxic. The issue, though, is whether this genotoxicity would occur at levels found in meat residues. The EC's last argument (in paragraph 124 of the EC's Rebuttal Submission, Canada case) that oestradiol-17 β is carcinogenic by a combination of both genotoxicity and cell proliferation is not contradicted by earlier arguments made by Canada and the US. On the other hand, it has not been established by the EC that genotoxicity and cell proliferation would be induced by levels found in meat residues added to the pre-existing levels occurring in exposed humans.

Dr. Guttenplan

181. There was scientific evidence cited by the EC in 2003 that oestradiol-17 β is genotoxic, "17 β oestradiol induces mutations in various cultured mammalian cells. The reactive metabolite,

oestradiol-3,4-quinone, also induces mutations in mouse skin *in vivo*. The catechol oestrogen-quinones form DNA adducts in cultured cells and in mouse skin" (footnote 82, Rebuttal Submission (US case). This evidence was stronger compared to previous reports. However the evidence now is much stronger. (Rogan EG. Cavalieri EL. *Estrogen metabolites, conjugates, and DNA adducts: possible biomarkers for risk of breast, prostate, and other human cancers. Advances in Clinical Chemistry. 38:135-49, 2004.*)

19. The European Communities states that "... it is generally recognized that for substances which have genotoxic potential (as is the case with oestradiol-17 β) a threshold can not be identified. Therefore it cannot be said that there exist a safe level below which intakes from residue should be considered to be safe. Therefore the fact that doses used in growth promotion are low is not of relevance". Does the scientific evidence referred to by the European Communities support these conclusions? Would your reply have been different at the time of adoption of the EC Directive in September 2003? If so, why? [see para. 201 of EC Rebuttal Submission (US case), paras. 120-122 of EC Rebuttal Submission (Canada case), paras. 73 and 86-98 of Canada Rebuttal Submission, paras. 87-91 and 153-156 of US First Submission and paras. 35-40 and 46 of US Rebuttal Submission]

Dr. Boisseau

182. The issue regarding the genotoxic potential of oestradiol-17 β has been already addressed in my reply to the question 13. The statement of the European Communities according which "it is generally recognized ... is not of relevance" is correct as long as it refers to the assessment of residues of xenobiotics. The scientific evidence referred to by the European Communities does not demonstrate that this statement can also apply in the case of oestradiol-17 β , progesterone and testosterone as these three natural hormones are produced by both humans and food producing animals. Therefore, even in the absence of any consumption of food coming from animals treated by growth promoting hormones, humans are naturally and continuously exposed to these natural hormones through, among others, (1) their own production of these hormones which may be very high, for exemple in the case of pregnant women, (2) the consumption of meat from non treated cattle, (3) the consumption of meat from other food producing animals, (4) the consumption of milk and eggs. To my knowledge, there is no epidemiological survey indicating that this continuous exposure of humans to these natural hormones results in any identified risk for health.

183. My reply would not have been different at the time of adoption of the EC directive in September 2003.

Dr. Boobis

Genotoxic potential and the absence of a threshold

184. This issue has been addressed in part in responses above. Generally, in risk assessment within the EU and JECFA, for compounds that are carcinogenic by a genotoxic mechanism (or mode of action), it is assumed that there is no threshold and that there is no level below which exposure is considered without risk. Hence, in such circumstances no ADI would be set (as this would imply that there was a "safe" level). However, the important point here is that it is the carcinogenic effect that is of concern, not *in vitro* genotoxicity. Whilst *in vivo* genotoxicity without carcinogenicity may be of concern, carcinogenicity by a mode of action other than genotoxicity, for which there is a demonstrable and biologically plausible threshold, would not fall into this category. Hence, whilst oestradiol may be genotoxic in certain *in vitro* assays, whether this requires a no-threshold approach to risk assessment depends critically upon a) the mechanism for genotoxicity and b) the relevance of the *in vitro* findings for the *in vivo* effects. The EC has accepted that for some mechanisms of

genotoxicity, such as inhibition of spindle assembly, there is a threshold (*EC, 2005a*). Redox active compounds also show a threshold in their genotoxic effects (*Brusick, 2005*). In addition, the EC has accepted that on occasion kinetic factors in vivo may be such that the genotoxic potential of a compound that is positive in vitro is not expressed in vivo at normal exposure levels, and hence there is a de facto threshold (e.g. oral exposure to phenol; *European Chemicals Bureau, 2006*). There is no good evidence that oestradiol is genotoxic in vivo or that it causes cancer by a genotoxic mechanism. Indeed, the evidence is against this. Hence, the scientific evidence does not support the EC on this issue, that the levels of the hormones in meat from treated cattle are not of relevance.

185. My reply to this question would have been the same at the time of adoption of the EC Directive in September 2003.

EC (2005a). Official Journal L 241 , 17/09/2005 P. 0051 – 0056

European Chemicals Bureau (2006). European Union Risk Assessment Report on phenol. CAS No. 108-95-2. EINECS No. 203-632-7. 1st Priority List, Volume 64. EUR 22229 EN

Dr. Cogliano

186. The EC's statement that a threshold cannot be identified reflects their view of genotoxic mechanisms, just as the contrary statement that there is a threshold and that this threshold is above the levels found in meat residues reflects how Canada and the US view genotoxic mechanisms. Neither statement has been demonstrated by the scientific evidence, rather, they are different assumptions that each party uses in their interpretation of the available evidence.

Dr. Guttenplan

187. The data referred to by the EC supports a genotoxic mechanism as well as a hormonal mechanism. It is true that there is no reason to expect a threshold to exist for a genotoxic chemical. Although DNA repair can occur, it presumably is occurring at all doses and the fraction of DNA damage repaired probably does not change at physiological levels, because the repair enzymes are unlikely to be saturated. The statement that, "the fact that doses used in growth promotion are low is not of relevance" is not necessarily true. (para. 118-119 of EC Rebuttal Submission (US case). For any toxin the dose determines the risk. When exposure is very low risk will be very low. However, one can argue about the definition of "low". It should also be noted that at very low levels of genotoxic carcinogens the decrease in risk is more than proportional than the decrease in applied dose.

188. The opinion about genotoxic effects would be less sure in 2003, but the opinion about the existence and significance of thresholds would not change.

20. In your view, how do the European Communities' conclusions above relate to the conclusion by Codex that "establishing an ADI or MRL for a hormone that is produced endogenously in variable levels in human beings was considered unnecessary"? To what extent, in your view, has JECFA's conclusion that oestradiol "has genotoxic potential" affected its recommendations on this hormone?

Dr. Boisseau

189. The European Communities' conclusions referred to in question 19 relate obviously to the conclusion by Codex that "establishing an ADI or MRL for a hormone that is produced endogenously in variable levels in human beings was considered unnecessary". The reply given to the question No 19 explains how it does and why these European Communities' conclusions are questionable.

190. The reply given to question 13 applies also to the second question of this question. JECFA's conclusions that oestradiol-17 β "has genotoxic potential" did not affect its recommendations on this hormone.

Dr. Boobis

Relevance of endogenous occurrence of oestradiol-17 β in its risk assessment

191. The EC's conclusions depend somewhat on the concept of incremental risk. This holds that whether an exogenous exposure is of concern depends on the magnitude of the underlying endogenous or background exposure. Some argue that for a compound with no threshold, even a very modest increment is of concern, whilst others would argue that a small percentage change would not materially affect risk (e.g. *ICRP, 2003*). However, before considering the question of incremental risk, it is pertinent to ask whether low levels of exposure impact on circulating hormone levels at all. The production of oestradiol is under homeostatic control, that regulates its synthesis and degradation (reviewed by *Fotherby, 1996*). In addition, the bioavailability of orally ingested oestradiol is very low (<10%), due to presystemic metabolism (*Kuhnz et al, 1993*). Hence, there should be a range of exposures for which there are compensatory alterations in endogenous levels, thereby maintaining the oestradiol level in the body. There is evidence that low exposures to oestradiol, though above those that are found in meat from treated animals, do not result in any measurable change in the circulating levels of oestradiol (*Mashchak et al, 1986*). Endogenous levels of oestradiol vary with physiological state. Hence, the endocrine effects of a given concentration of oestradiol will vary with the specific physiological state. As a consequence, a modest incremental increase in oestradiol concentration from exogenous exposure (above the ADI) might conceivably perturb endocrine effects, depending on the physiological state. However, non-endocrine effects, such as genotoxicity, will depend on the circulating concentration of oestradiol and will not vary with physiological state. Hence, the natural variations in circulating oestradiol levels should have a much greater effect on any genotoxic response than the much more modest change that could arise from the hormone in meat from treated animals, at any conceivable level arising from its use as a growth promoter. Indeed, this would be the case, regardless of the mechanism of carcinogenesis. Hence, the EC conclusions on the absence of safety at any level of exposure is somewhat at odds with the underlying basis of the Codex conclusion regarding the need for an ADI or MRL.

192. I do not believe that JECFA's conclusion that oestradiol has "genotoxic potential" affected its recommendations on this hormone, which were based on the conclusion that there was a threshold for its carcinogenic effects. JECFA's conclusion regarding genotoxicity was based on positive results in certain in vitro tests, but the evidence was against a mutagenic response in vivo.

Fotherby K (1996). Bioavailability of orally administered sex steroids used in oral contraception and hormone replacement therapy. Contraception, 54:59-69

ICRP (2003). The evolution of the system of radiological protection: the justification for the new ICRP recommendations. J Radiol Prot, 23:129-142

Kuhnz W, Gansau C and Mahler M (1993). Pharmacokinetics of estradiol, free and total estrone, in young women following single intravenous and oral administration of 17 beta estradiol. Arzneimittelforschung (Drug Res), 9:966-973

Mashchak CA, Lobo RA, Dozono-Takano R, Eggena P, Nakamura RM, Brenner PF and Mishell DR Jr (1986). Comparison of pharmacodynamic properties of various estrogen formulations. Am J Obstet Gynecol, 144:511-518

Dr. Cogliano

193. In my view, the EC's conclusions seem to reflect a concern that endogenous hormone levels are variable (the variability of endogenously produced hormone levels is recognized by Codex). In my view, the argument that the EC seems to be making is that a threshold cannot be established for the incremental human exposures that would be found in meat residues, because these additional exposures may not be safe for some parts of the population.

Dr. Guttenplan

194. The European Communities' conclusions above are at variance with those of Codex. Probably JECFA's conclusion that oestradiol "has genotoxic potential" had some effect on the European Communities' conclusions. However as also noted by the EC (SCVPH 2002 Opinion) newer methods of analyses have identified areas of concern, such as developmental effects, since levels of hormones in meat may represent a significant increase in endogenous levels of prepubescent children.

21. Does the scientific evidence referred to by the European Communities demonstrate that the five hormones other than oestradiol-17 β , when consumed as residues in meat have genotoxic potential? Does your answer depend on whether good veterinary practices are followed? Would your reply have been different at the time of adoption of the Directive in September 2003? If so, why? [see, *inter alia*, the SCVPH Opinions and paras. 63, 83, 89-91 and 93 of US First Submission, paras. 131-136 of Canada Rebuttal Submission]

Dr. Boisseau

195. My reply to question 16 applies also to the first question of this question.

196. The fact that good veterinary practices are followed or not has no impact on the genotoxic potential of these hormones.

197. My reply would not have been different at the time of adoption of the directive in September 2003.

Dr. Boobis

Genotoxicity of the five hormones other than oestradiol-17 β

198. There is no evidence that the hormones testosterone or progesterone have genotoxic potential. There is no convincing evidence that trenbolone acetate, MGA and zeranol are genotoxic. They were negative in a range of tests for genotoxicity. They were very weakly positive in a micronucleus test, at high (potentially cytotoxic) concentrations. Trenbolone also produced a low level of DNA adducts measured by 32P-post-labelling (*Metzler and Pfeiffer, 2001*). As indicated above, micronuclei can arise via a non-genotoxic mechanism, particularly at concentrations that may have caused some toxicity. In addition, the 32P-post-labelling assay is not specific, and data cited above suggest that DNA adduction can arise by mechanisms other than direct interaction with DNA. In no case did any of the compounds produce a mutagenic response. These data are insufficient to support the conclusion that these hormones have genotoxic potential in vivo. Thus, there is no evidence that any of the hormones are genotoxic in vivo at the levels found in meat from treated animals. Even if GVP were not followed, the levels of exposure to the hormones would be such that no genotoxicity would be anticipated in vivo.

199. I would have replied the same to this question at the time of adoption of the EC Directive in September 2003.

Metzler M and Pfeiffer E (2001). Genotoxic potential of xenobiotic growth promoters and their metabolites. APMIS, 109:89-95

Dr. Guttenplan

200. There is no conclusive evidence presented by the EC that the five hormones other than oestradiol-17 β , when consumed as residues in meat have genotoxic potential. There is some evidence that certain of the hormones have genotoxic potential, but generally the potential is weak. Testosterone and progesterone are negative in genotoxic assays. Zeranone can induce transformation of breast epithelial cells in culture with efficiency similar to that of estradiol, but the mechanism is not known, and it is negative or marginally active in other assays. Trenbolone is either negative or marginally active in *in vitro* genotoxic assays. MGA is negative in genotoxicity assays. Any genotoxic effects of the five hormones are likely to be minimized by good veterinary practice. My reply for the hormones would not have been different in September 2003 (SCVPH 2002 Opinion).

22. How would you define *in vivo* DNA repair mechanisms? How effective or relevant are *in vivo* DNA repair mechanisms with respect to potential genotoxic effects from residues of the growth promoting hormones at issue when consumed in meat? Does your answer depend on whether good veterinary practices are followed in the administration of these hormones? To what extent does the scientific material referred to by the European Communities take into account these mechanisms in its evaluation of potential occurrence of adverse effects from residues of growth promoting hormones? Would your reply have been different at the time of adoption of the Directive in September 2003 and if so, why? [see paras. 40 and 46 of US Rebuttal Submission, footnote 107 of US First Submission, and para. 89 of Canada Rebuttal Submission]

Dr. Boobis

DNA repair mechanisms

201. DNA repair mechanisms comprise a series of enzymatic systems that recognize different types of damage to DNA and repair it. There are different systems for different types of chemical modifier of DNA (e.g. small alkyl groups, bulky aromatic groups, oxidative damage), the site at which the damage occurs (e.g. O6 or N7 of guanine) and the nature of the damage (e.g. covalent modification, inter-strand cross-linking, single strand breaks) (reviewed in *Dip et al, 2004; Huffman et al, 2005; Sharova, 2005*). It has been estimated that endogenous processes are responsible for considerable oxidative DNA damage, but this rarely causes heritable changes to the cell (*Shigenaga et al, 1989; Pollycove and Feinendegen, 1999*). To a large extent this is because of the evolution of a flexible and very efficient DNA repair process. DNA damage caused by exogenous agents (genotoxins) is repaired by similar mechanisms. Hence, adduction of DNA is detectable at much lower doses than mutation (*Williams et al, 2004*). DNA repair capacity needs to be overwhelmed before mutagenicity increases as a linear function of dose, the lower end of the dose response showing non-linearity (*Williams et al, 2004*). A major difficulty in the risk assessment of such compounds however, is the identification of the threshold for such effects. This is because they occur with low incidence, and experimental studies do not have the statistical power to determine the location of the threshold with any confidence. Thus, whilst recognizing the likelihood for a threshold for even genotoxic effects (*Williams et al, 2004*), the risk assessor is faced with the impossibility of locating it. The conservative solution is to assume that the response is linear and that there is no dose below which exposure is safe (e.g. *UK Committee on Carcinogenicity, 2004*).

202. As indicated above, the evidence is against direct modification of DNA *in vivo* by hormones in meat from treated animals, or by their metabolites produced *in vivo*. Indirect modification could conceivably come about by products of active oxygen. The DNA repair processes for this are amongst the most efficient (Arai *et al*, 2006; Russo *et al*, 2004) and even if such modification did occur, it is anticipated that no heritable change would result, because of DNA repair (Arai *et al*, 2006). This would be true even at the levels of exposure that could arise should GVP not be followed.

203. My reply would have been the same at the time of adoption of the EC Directive in September 2003.

Dip R, Camenisch U, Naegeli H (2004). *Mechanisms of DNA damage recognition and strand discrimination in human nucleotide excision repair. DNA Repair (Amst)*, **3**:1409-1423

Huffman JL, Sundheim O, Tainer JA (2005). *DNA base damage recognition and removal: new twists and grooves. Mutat Res*, **577**:55-76

Sharova NP(2005). *How does a cell repair damaged DNA? Biochemistry (Mosc)*, **70**:275-291

Shigenaga MK, Gimeno CJ, Ames BN (1989). *Urinary 8-hydroxy-2'-deoxyguanosine as a biological marker of in vivo oxidative DNA damage. Proc Natl Acad Sci U S A*, **86**:9697-9701

Pollycove M and Feinendegen LE (1999). *Molecular biology, epidemiology, and the demise of the linear no-threshold (LNT) hypothesis. C R Acad Sci III*, **322**:197-204

UK Committee on Carcinogenicity (2004). *Guidance on a Strategy for the Risk Assessment of Chemical Carcinogens, Department of Health, London* (<http://www.advisorybodies.doh.gov.uk/COC/guideline04.pdf>)

Williams GM, Iatropoulos MJ and Jeffrey AM (2004). *Thresholds for the effects of 2-acetylaminofluorene in rat liver. Toxicol Pathol*, **32**, Suppl 2:85-9.

Dr. Guttenplan

How would you define *in vivo* DNA repair mechanisms?

204. DNA repair *in vivo* refers to the ability of the organism to remove damaged or chemically modified portions of DNA and replace them non-damaged DNA.

How effective or relevant are *in vivo* DNA repair mechanisms with respect to potential genotoxic effects from residues of the growth promoting hormones at issue when consumed in meat?

205. The repair processes involved in DNA damage produced by estrogen metabolites are no different than those involved in DNA damage by many other DNA damaging agents. Most DNA damage by any agent is repaired and there is considerable redundancy in DNA repair, insuring that repair is effective. However, a small fraction of damage inevitably escapes repair. In essence, there is a race for repair and cell division. If cell division occurs before repair then a mutation or cell death may arise. Most DNA repair processes are not saturated in whole animals (including humans) as such high levels would likely accompany extremely toxic levels of carcinogens. Some DNA repair processes are faulty (error-prone). They enable the cell to survive potentially fatal DNA damage, but they increase the levels of mutations in the cell. Increased levels of mutations increase the risk of cancer. There is no reason to assume that DNA repair processes involved in DNA damage produced

by estrogen metabolites are any more or less effective than those involved in repair of other carcinogens.

Does your answer depend on whether good veterinary practices are followed in the administration of these hormones?

206. NO

To what extent does the scientific material referred to by the European Communities take into account these mechanisms in its evaluation of potential occurrence of adverse effects from residues of growth promoting hormones?

207. The scientific material referred to by the European Communities for the most part doesn't address DNA repair. However, since it is not likely to be different for estrogen derived damage than other types of damage it is not really relevant. There is some evidence referred to in the SCVPH Opinions that error-prone DNA repair of certain estrogen derived damage can occur.

Would your reply have been different at the time of adoption of the Directive in September 2003 and if so, why?

208. NO

Lindahl T. Wood RD. Quality control by DNA repair. Science. 286(5446):1897-905, 1999, para. 40 and 46 of US Rebuttal Submission, footnote 107 of US First Submission, and para. 89 of Canada Rebuttal Submission.

23. To what extent is it necessary or possible to take into account the "long latency period" of cancer in the conduct of a risk assessment, which is supposed to assess carcinogenic effects of these hormones when consumed in meat? Have the hormones in dispute been used as growth promoters over a sufficient number of years for an assessment of their long-term effects on human health to be made? [see para. 149 of EC Rebuttal Submission (US Case), para. 143 of EC Rebuttal Submission (Canada case)].

Dr. Boisseau

209. I don't think possible/useful to take into account the "long latency period" of cancer in order to assess properly and specifically the carcinogenic effects of residues of natural hormones only resulting from the treatment of food producing animals by growth promoting hormones. In my view, epidemiological studies carried out in humans during long enough in order to take into account this "long latency period" will not be able to discriminate, in the case of a possible but limited increase of tumours, between the responsibilities of (1) hormone residues resulting from the treatment of food producing animals by growth promoting hormones, (2) hormone residues resulting from the endogenous production of these animals, (3) other components of the diet including other food additives and contaminants. That is the reason for which, to my knowledge, even the hormones in dispute have already been used as growth promoters over a sufficient number of years, the epidemiological studies in humans already carried out in this domain have failed to identify any relation between the occurrence of hormonally dependent tumours and the consumption of meat containing hormonally active residues resulting from the treatment of cattle with growth promoters.

Dr. Boobis

Latency period for cancer

210. The latency period is an important consideration in risk assessment, both in the design and in the interpretation of studies. Thus, the duration of exposure, either of experimental animals or in epidemiology studies, should be sufficiently long to permit assessment of effects with a long latency period. Most forms of cancer come into this category. The observational studies of humans (e.g. on HRT or oral contraceptives) and the experimental studies in animals covered a sufficiently long period to encompass the latency period for any carcinogenic effects of the hormones (see *IARC, 1999*).

211. The long term studies of the hormones undertaken in experimental animals and in humans, involved much higher doses than would be encountered on consumption of meat from animals treated with growth promoting hormones. The maximum risk from such low levels of exposure, even assuming a linear dose-response relationship for cancer, would be such that it would be necessary to study extremely large populations to detect any increase in cancer incidence, particularly as the most likely cancers are quite common. This is because the lower the risk the greater the number of subjects that are required to detect it, a function of the power of the study which takes account the magnitude of the risk and the difference from the background rate (*Hunter, 1997*). Hence, in the risk assessment of the hormones used as growth promoters, it is questionable whether an increase in risk, even if it existed, could be detected in exposed populations. However, it is still necessary to protect against such a risk. The risk assessment of the hormones conducted by JECFA suggested that there would be no risk at exposure levels up to the respective ADI. Even if duration of exposure were for a sufficiently long period (usually 20-25 years for solid tissue tumours), any increase in risk would probably not be detectable. Hence, a negative result from such an observational study would not resolve the issue.

212. A second issue with respect to the latency is the significance it has for interpretation of the exposure pattern. Where there is a long latency, and regular exposure is necessary before a carcinogenic response is manifest, as appears to be the case for the hormones in question (*Coombs et al, 2005*), occasional exposures above the ADI will not pose any additional risk (*Larsen and Richold, 1999*). Hence, latency is of value in assessing the risks from different exposure scenarios.

Coombs NJ, Taylor R, Wilcken N, Fiorica J and Boyages J (2005). Hormone replacement therapy and breast cancer risk in California. Breast J, 11:410-415

Hunter DJ (1997). Methodological issues in the use of biological markers in cancer epidemiology: cohort studies. IARC Sci Publ, 142:39-46

IARC (1999). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol 72. Hormonal Contraception and Post-menopausal Hormonal Therapy, IARC, Lyon, France

Larsen JC and Richold M (199). Report of workshop on the significance of excursions of intake above the ADI. Regul Toxicol Pharmacol, 30:S2-12.

Dr. Cogliano

213. It is definitely necessary to take into account the latency period of cancer in the conduct of a risk assessment. In this regard, the guidelines for developing *IARC Monographs* state, "Experience with human cancer indicates that the period from first exposure to the development of clinical cancer is sometimes longer than 20 years; latent periods substantially shorter than 30 year cannot provide

evidence for lack of carcinogenicity." [International Agency for Research on Cancer, Preamble to the *IARC Monographs*, <http://monographs.iarc.fr>]

Dr. Guttenplan

214. When epidemiological data is used in performing a risk assessment, the latency period is extremely important. Usually a latent period of 20 years is taken for cancer, but this varies with the carcinogen. It is indeed necessary to determine incidence or prevalence at different times after the onset of exposure. Attempting to perform a risk assessment based on epidemiological data obtained too soon after the onset of exposure can seriously underestimate risk. With respect to hormones in meat, it appears they have now been consumed for a sufficient number of years to observe strong or moderate increases in risk. However, if the risk increase is small, a large enough identifiable long-term exposed population may not be available.

Lagiou P. Trichopoulou A. Trichopoulos D. Nutritional epidemiology of cancer: accomplishments and prospects. [Lectures] Proceedings of the Nutrition Society. 61(2):217-22, 2002, para. 149 of EC Rebuttal Submission (US Case), para. 143 of EC Rebuttal Submission (Canada case).

24. To what extent is it possible to identify possible co-founding factors causing cancer and attribute them to identified sources? What are the implications of these factors for the conduct of a risk assessment evaluating the adverse effects caused by residues of growth promoting hormones in meat? Would your reply have been different at the time of adoption of the EC Directive in September 2003? If so, why?

Dr. Boisseau

215. Generally, it is very difficult to identify possible co-founding factors causing cancer and attribute them to identified sources. The reply given to the question No 23 applies also to this question No 24.

216. My reply would not have been different at the time of adoption of the EC Directive in September 2003.

Dr. Boobis

Confounding factors in cancer attribution

217. Although the causes of many cancers remain to be identified (though they are likely to be multi-factorial), strong risk factors have been identified for a number of cancers. The main hormonally-related cancers are breast, ovarian and endometrium in women, testes and prostate in men (*IARC, 1999*). In females, genetic factors, particularly *BRCA1* and *2*, have a strong influence on a small number of breast cancers (*Wooster and Weber, 2003*). Breast and ovarian cancer are affected by a number of lifestyle factors, which in general influence circulating oestrogen levels (*Amant et al, 2005; Henderson and Feigelson, 2000; Vogel and Taioli, 2006*). These include parity (number of children), age at first birth, age at menarche, menopausal state. In addition, there are associations with diet, such as fat and meat consumption, for male and female hormonally-related cancers (*Colli and Colli, 2006; Gonzalez, 2006; Kushi and Giovannucci, 2002; Rieck and Fiander, 2006; Shirai et al, 2002; Wakai et al, 2005*). Exogenous exposure to high levels of hormones, such as oestradiol, can cause cancer (*JECFA, 2000b; IARC, 1999*). However, though HRT (hormone replacement therapy) and oral contraceptive steroids (OCS) represent exposures orders of magnitude greater than those encountered in consuming meat from treated animals, the relative risk is still relatively modest (RR of 1.3 for oestrogen-only HRT (*Beral, 2003*); RR of 1.24 in women taking combined OCS

(*Collaborative Group on Hormonal Factors in Breast Cancer, 2006*). These risks were detectable only because of the very large populations involved.

218. A number of such factors could confound a study of the effects of growth promoting hormones in meat (they should not affect the risk assessment, but the interpretation of the data used in the risk assessment). For example, socioeconomic and demographic differences in lifestyle choices and diet may utterly confound exposure comparisons of growth promoting hormones in meat. Meat consumption, regardless of whether it is from animals treated with growth promoting hormones, is an independent risk factor for a number of hormonally-related cancers, including breast in females and prostate in males (*Colli and Colli, 2006; Gonzalez, 2006*). The average age at first pregnancy, a lifestyle choice, is appreciably greater in some countries than in others (*Beets, 1999; United Nations Economic Commission for Europe, 2003*), as is socioeconomic status (*Robert et al, 2004*). Thus any apparent effect of growth hormone exposure from meat of treated animals may be confounded by other known risk factors. Whilst it is sometimes possible to correct for confounding, when the risk from the confounder is appreciably greater than the risk from the exposure of interest, which is likely to be the situation here, it is very difficult to account for all of the confounding (for example, see *Toledano et al, 2005*). Residual confounding may still bias the result, obscuring a null difference.

219. My reply would not have been different at the time of adoption of the EC directive in September 2003.

Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E and Vergote I (2005). Endometrial cancer. Lancet, 366:491-505

Beets G (1999). Education and age at first birth. DEMOS, 15 (Special Issue)

Beral V (2003). Million Women Study Collaborators. Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet, 362:419-427. Erratum in: Lancet, 2003, 362:1160

Collaborative Group on Hormonal Factors in Breast Cancer (1996). Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. Lancet, 347:1713-1727

Colli JL and Colli A (2006). International comparisons of prostate cancer mortality rates with dietary practices and sunlight levels. Urol Oncol, 24:184-194

Gonzalez CA (2006). The European Prospective Investigation into Cancer and Nutrition (EPIC). Public Health Nutr, 9:124-126.

Henderson BE and Feigelson HS (2000). Hormonal carcinogenesis. Carcinogenesis, 21:427-433

JECFA (2000b). Toxicological Evaluation of Certain Veterinary Drug Residues in Food: Who Food Additives Series 43, WHO, Geneva, Switzerland

Kushi L and Giovannucci E (2002). Dietary fat and cancer. Am J Med, 113, Suppl 9B:63S-70S

Rieck G and Fiander A (2006). The effect of lifestyle factors on gynaecological cancer. Best Pract Res Clin Obstet Gynaecol, 20:227-251

Robert SA, Strombom I, Trentham-Dietz A, Hampton JM, McElroy JA, Newcomb PA and Remington PL (2004). *Socioeconomic risk factors for breast cancer: distinguishing individual- and community-level effects. Epidemiology, 15:442-450*

Shirai T, Asamoto M, Takahashi S and Imaida K (2002). *Diet and prostate cancer. Toxicology, 181-182:89-94.*

Toledano MB, Nieuwenhuijsen MJ, Best N, Whitaker H, Hambly P, de Hoogh C, Fawell J, Jarup L and Elliott P (2005). *Relation of trihalomethane concentrations in public water supplies to stillbirth and birth weight in three water regions in England. Environ Health Perspect, 113:225-232*

United Nations Economic Commission for Europe (2003). *Trends in Europe and North America*

Vogel VG and Taioli E (2006). *Have we found the ultimate risk factor for breast cancer? J Clin Oncol, 24:1791-1794*

Wakai K, Tamakoshi K, Date C, Fukui M, Suzuki S, Lin Y, Niwa Y, Nishio K, Yatsuya H, Kondo T, Tokudome S, Yamamoto A, Toyoshima H and Tamakoshi A; JACC Study Group (2005). *Dietary intakes of fat and fatty acids and risk of breast cancer: a prospective study in Japan. Cancer Sci, 96:590-599*

Wooster R and Weber BL (2003), *Breast and ovarian cancer, N Engl J Med 348:2339–2347*

Dr. Cogliano

220. It is generally possible to identify confounding factors in epidemiological studies. [Please note that the question mentions "co-founding factors."] It is often difficult, however, to determine whether the observed tumours can be attributed to the agent under study or to a confounding factor. When a causal interpretation is credible but confounding factors cannot be ruled out, IARC considers this to provide *limited evidence of carcinogenicity*.

Dr. Guttenplan

221. Although the question mentions co-founding, from the documents submitted, what is probably meant is "confounding" factors. These are factors other than the one investigated which may also correlate with the disease endpoint. For instance, if meat eaters are also obese, the observed effects may result from obesity and not meat-eating (although the two may be related to each other). It would be very difficult to identify all of the confounders with meat eating and cancer, although there are many models for the effects of dietary agents on cancer incidence. One would want a perfectly matched group of consumers of hormone-treated meat with consumers of non-hormone-treated meat, where both groups have the same "lifestyle". This is probably difficult to achieve, since individuals consuming non-hormone-treated meat are probably very health conscious or from a different geographical region. A number of confounders can indeed be identified (e.g., age, race, sex, medications), but one is never sure if all or even most have been identified. These are important considerations for risk assessment of adverse affects caused by residues of growth promoting hormones in meat, as the effects of the hormones (if any) are likely to be small and might be obscured by confounders. The reply would not be different in 2003.

Potter JD. *Colorectal cancer: molecules and populations. [Review] Journal of the National Cancer Institute. 91(11):916-32, 1999*

25. **To what extent do the three recent studies referred to by the European Communities confirm a risk to human health from the consumption of meat from cattle treated with growth promoting hormones? Please also comment on the EC statement that one of the studies "was carried out after the introduction of the ban on the use of hormones for growth promotion in Europe, which means that the subjects should have been exposed to hormone-free meat in their diet. This may further imply that it cannot be excluded that the risk of cancer may be further increased if meat treated with hormones for animal growth promotion were to be consumed". [see paras. 145-148 of EC Rebuttal Submission (US case) and paras. 139-142 of EC Rebuttal Submission (Canada case), footnote 97 in para. 147 of EC Rebuttal Submission (US case), and Exhibits EC-71, 72, 73]**

Dr. Boisseau

222. My comment concerning zeranol in the reply given to question 13 can apply to the first study about zeranol reported in this question 25.

223. My reply to question 23 can be used as the requested comment regarding the second study about colorectal cancer risk reported in this question 25.

224. Comments to be made on the EC statement that one of the studies " was carried out ... to be consumed" can be derived from the very careful conclusions drawn by SCVPH in its 1999 report which say that " the link, if any, with consumption of hormone-treated meat cannot, at present, be confirmed nor refuted ...there is moderately consistent evidence that higher meat consumption ... is associated with higher risk of breast cancer ... it was therefore concluded that diets high in meat possibly increase the risk of prostate cancer ... there is a weakly consistent evidence that total meat consumption is associated with the risk of prostate cancer. For red meat, the evidence is moderately consistent". In conclusion, this EC statement just expresses a concern but does not provide any scientific evidence supporting this concern.

225. The third study about the treatment of postmenopausal women reported in this question No 25 is out of scope.

Dr. Boobis

The three recently published studies referred to by the EC (note that the relevant EC exhibits are EC-062, EC-071 and EC-072, respectively)

- (a) Liu S and Lin YC (2004). Transformation of MCF-10A human breast epithelial cells by zeranol and oestradiol-17beta. *Breast J*, **10**:514-521.

226. This paper reports the effects of oestradiol-17 β and zeranol on the breast cancer derived cell line MCF-10A. This cell line is devoid of ER α and has little or no ER β . The data show that both compounds produced changes in the cells following multiple exposures that were characteristic of malignant transformation. However, it should be noted that the malignancy of the cells was not tested by inoculation into animals, which is the final evidence that complete transformation has occurred. The doses of the compounds used were high, particularly for the effects observed in MCF-7 (ER α positive) cells, in which the EC50 for proliferation is around 2 pM (cf LOEC of 15 nM in present study). There was no change in the response with concentration in the MCF-10A cells. There was no demonstration by the authors or by others of the metabolic capacity of the cells. The two compounds were equi-potent in both the ER α negative (MCF-10A) and ER α positive (MCF7) cells. This is somewhat surprising given the known difference in both oestrogenic (*Le Guevel and Pakdel, 2001*) and genotoxic (*Metzler and Pfeiffer, 2001; Stopper et al, 2003*) potency of the two compounds.

227. Nevertheless, the two compounds do appear to produce a positive response in the cells, which is consistent with previous studies in this cell line (*Russo et al, 2002*). However, it is well established that oestrogens can be genotoxic in certain in vitro test systems, most likely a consequence of redox cycling with generation of reactive oxygen species. Evidence for this mechanism has been reported recently by *Cuendet et al (2004)*. As indicated above, genotoxicity by this mechanism should exhibit a threshold and is also militated against in vivo by antioxidant defence systems and efficient repair of oxidant-damaged DNA. Hence, the study by Liu and Lin (2004) does not confirm a risk to human health from the consumption of meat from cattle treated with growth promoting hormones.

Cuendet M, Liu X, Pisha E, Li Y, Yao J, Yu L and Bolton JL (2004). Equine estrogen metabolite 4-hydroxyequilenin induces anchorage-independent growth of human mammary epithelial MCF-10A cells: differential gene expression. Mutat Res, 550:109-121

Le Guevel R and Pakdel F (2001). Assessment of oestrogenic potency of chemicals used as growth promoter by in-vitro methods. Hum Reprod, 16:1030-1036

Russo J, Lareef MH, Tahin Q, Hu YF, Slater C, Ao X and Russo IH(2002). 17Beta-estradiol is carcinogenic in human breast epithelial cells. J Steroid Biochem Mol Biol, 80):149-16.

- (b) Norat T, Bingham S, Ferrari P, Slimani N, Jenab M, Mazuir M, Overvad K, Olsen A, Tjonneland A, Clavel F, Boutron-Ruault MC, Kesse E, Boeing H, Bergmann MM, Nieters A, Linseisen J, Trichopoulou A, Trichopoulos D, Tountas Y, Berrino F, Palli D, Panico S, Tumino R, Vineis P, Bueno-de-Mesquita HB, Peeters PH, Engeset D, Lund E, Skeie G, Ardanaz E, Gonzalez C, Navarro C, Quiros JR, Sanchez MJ, Berglund G, Mattisson I, Hallmans G, Palmqvist R, Day NE, Khaw KT, Key TJ, San Joaquin M, Hemon B, Saracci R, Kaaks R and Riboli E (2005). Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition. *J Natl Cancer Inst, 97:906-916.*

228. This paper reports the results of a prospective epidemiological study into the relationship between diet and colorectal cancer. The study, in a large number of subjects, confirms previous reports that there is a statistically significant association between the consumption of red meat and the risk of developing colorectal cancer. This association has been known for some time (for example see *Modan, 1977*). Moreover, it is a relatively consistent observation, regardless of geographical area (subject to allowance for any confounding by known risk factors) (*Marques-Vidal et al, 2006*). A number of possible explanations have been proposed for this association, including the formation of mutagens during the cooking of meat (*Sinha et al, 2005*) and the generation of nitroso compounds in the colon through the effects of haem iron from meat (*Cross et al, 2003*). Although the geographical variation in risk is consistent with a role of meat consumption in colorectal cancer, it provides little support for a contribution from hormones present in meat from their use as growth promoters. This is because the association is just as strong in regions where hormones are not used as where they are used. Age standardized rates for colorectal cancer in males and females are 48.2 and 36.9 in Australasia, 44.4 and 32.8 in North America, 37.5 and 26.4 in Northern Europe and 35.9 and 23.5 in Southern Europe (*IARC, GLOBOCAN 2002*). In comparison, meat consumption as protein is as follows: Australia 40.3 g/day, USA 40.2 g/day and Europe 25.2 g/day (FAO, 2003).

229. Hence, the study by Norat et al (2005) does not confirm a risk to human health from the consumption of meat from cattle treated with growth promoting hormones.

Cross AJ, Pollock JR, Bingham SA (2003). Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat. Cancer Res, 63:2358-2360

FAO (2003). FAOSTAT (<http://faostat.fao.org/>)

IARC (2002). GLOBOCAN (<http://www-dep.iarc.fr/>)

Marques-Vidal P, Ravasco P and Ermelinda Camilo M (2006). Foodstuffs and colorectal cancer risk: a review. *Clin Nutr*, 25:14-36

Modan B (1977). Role of diet in cancer etiology. *Cancer*, 40 (4 Suppl):1887-1891

Sinha R, Peters U, Cross AJ, Kulldorff M, Weissfeld JL, Pinsky PF, Rothman N and Hayes RB (2005). Meat, meat cooking methods and preservation, and risk for colorectal adenoma. *Cancer Res*, 65:8034-8041

- (c) Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM and Ockene J; Writing Group for the Women's Health Initiative Investigators (2002). Risks and benefits of oestrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA*, 288:321-333.

230. This paper reports the results of a randomised controlled trial on the use of a combination of oestrogen and progestin as hormone replacement therapy. The hazard ratio in the group receiving the hormones for breast cancer was 1.26 (95% CI: 1.00-1.59). There was a reduction in the risk of colorectal cancer (0.63; 0.43-0.92) and no change in the risk of endometrial cancer (0.83; 0.47-1.47). This study confirms a number of previous reports, that exposure of postmenopausal women to an oestrogen-progestin combination increases the risk of breast cancer (see above; *Beral, 2003*). This was recognised by JECFA in its risk assessment of these hormones (*JECFA, 2000b*). However, whether the finding is relevant to the risk from residues of the hormones in meat due to their use as growth promoters depends upon the conclusions of the risk assessment. Hence, as explained above, the weight of evidence is such that the hormones cause cancer by a mechanism exhibiting a threshold. As long as exposure does not consistently exceed the ADI, there should be no appreciable risk to human health. Related to this, the doses to which the women receiving hormone replacement therapy were exposed in this study were many times those to which consumers would be exposed from meat from cattle treated with growth promoting hormones.

231. Hence, the study by Rossouw et al (2002) does not confirm a risk to human health from the consumption of meat from cattle treated with growth promoting hormones.

232. With respect to the EC statement on the significance of the fact that one of the studies (*Norat et al, 2005*) was performed after a ban on the use of hormones for growth promotion in Europe, this will depend on the interpretation of the risk assessment. If there is no risk from the consumption of meat from animals treated with the hormones, as was the view of JECFA, then it would be immaterial whether the study was conducted before or after the ban, the risk would have been the same. If the risk is non-zero, as suggested by the EC, then certainly there might be an incrementally greater risk if such meat were consumed. However, as indicated elsewhere in my responses, the evidence is against an increased risk from such exposures. In addition, the EC statement is not scientifically defensible. The risk of cancer when eating meat from treated animals has not been measured. Hence, it is impossible to infer anything from the risk in the absence of such exposure. The EC could have made such a statement in the absence of any study as it is based entirely on conjecture.

Dr. Cogliano

233. The study by Norat et al (2005) indicates a risk to human health from the consumption of meat. The other two studies suggest a risk to human health (the term "suggest" is used rather than "indicate" because the exposure levels in these studies are higher than those found in meat residues). When a dietary study includes exposures to hormone-free meat, this would reduce the observed level of risk. As a result, the risk from exposure to meat containing hormones would likely be higher than what was indicated in the study.

Dr. Guttenplan

234. The first of the studies suggests a risk from zeranol. That observation was not previously reported. However, the results were obtained in cultured cells and the relevance to human exposure to hormone-treated cannot be extrapolated from this study because of a myriad of uncertainties in such extrapolation. The study does suggest that additional tests of zeranol should be carried out. There is also some evidence that a metabolite of zeranol (zearalenone) induces oxidative damage in cultured cells. This is a possible genotoxic effect, but again it cannot be extrapolated to meat consumption. The other two studies do not confirm a risk from hormone-treated meat. The statement that one of the studies was carried out after the introduction of the ban on the use of hormones for growth promotion in Europe, negates any relevance to the possible connection of hormone-treated meat consumption and cancer.

26. Does the scientific evidence referred to by the European Communities, in particular any epidemiological studies, identify a relationship between cancer and residues of hormonal growth promoters? In its risk assessment of 1999, the European Communities makes reference to the higher rates of breast and prostate cancer observed in the United States as compared to the European Communities. Can a link be established between these statistics and the consumption of meat from animals treated with the hormones at issue? Would your reply have been different at the time of adoption of the EC Directive in September 2003? If so, why? [see pages 17-19 of 1999 Opinion of the SCVPH and related Tables A4-A5 on pages 83-91]

Dr. Boisseau

235. My replies to questions 23 and 25 apply also to this question 26.

236. My reply would not have been different at the time of adoption of the directive in September 2003.

Dr. Boobis

Significance of epidemiological studies for risk from the hormones

237. Information relevant to this issue can be found in my responses to questions 24 and 25 above.

238. There is no scientific evidence demonstrating any association between consumption of meat from animals treated with growth promoting hormones and the risk of cancer in humans. There are some studies that are consistent with such an association, but there are several other possible explanations for the findings, some of which are more plausible than hormones in meat as being causal. In addition, there is some lack of consistency in the epidemiological studies between the associations observed and use of the growth promoting hormones.

239. There are an appreciable number of studies showing an association between the risk of certain cancer types, including breast and prostate and the consumption of meat (*Colli and Colli, 2006; Norat et al, 2005; see also SCVPH Opinion, 1999*). For breast, the incidence is similar in developed countries such as Western Europe, North America and Australasia. The correlation is strongest with meat consumption and shows little relationship with whether the meat is from animals treated with growth promoting hormones or not. For example rates in Iceland (87.2 per 100,000), where such hormones are not used, are not dissimilar to those in the USA (101.1 per 100,000), where they are used. Prostate cancer rates are 124.8/100,000 in the USA and 90.9 per 100,000 in Sweden (*IARC, 2002*). For comparison, average daily consumption of meat (as protein) in 2000 was as follows: USA 40.2 g/day; Iceland 29.5 g/day; Sweden 24.8 g/day (*FAO, 2003*). Hence, there is a much better association with meat consumption and risk of breast or prostate cancer than there is with the use of growth promoting hormones to treat cattle. It is also important not to infer too much from geographical differences in cancer incidence rates with respect to causation. This is because of what is known as the ecological fallacy. This has been defined as the inference that a correlation between variables derived from data grouped in social or other aggregates (ecological units) will hold between persons (individual units) (*Society for Risk Assessment, 2004*). The difficulty is that many factors will vary between populations, including ethnicity, genetics, health and socioeconomic status, diet, lifestyle and environment. Without considering the possibility of confounding, such ecological data is really only of value in generating hypotheses (*Morgenstern, 1995*). These would need to be evaluated in more structured investigations, with better control of confounding variables.

Morgenstern H (1995). Ecologic studies in epidemiology: concepts, principles, and methods. Annu Rev Public Health, 16:61-81

Society for Risk Assessment (2004). Glossary of Risk Analysis Terms. (http://www.sra.org/resources_glossary.php)

240. My reply to this question would have been the same at the time of the adoption of EC Directive in September, 2003. Although some of the studies cited above were not published at that time, there was still sufficient information to identify the clear trend between meat consumption and the risk of breast and prostate cancer, independent of the pattern of use of the growth promoting hormones.

Dr. Cogliano

241. The difference between the US and the EC in rates of breast cancer and prostate cancer almost certainly has multiple causes. It is possible that differences in exposure to exogenous hormones can be one cause, but the data are not sufficiently specific to establish a link between these observations.

Dr. Guttenplan

242. The epidemiological studies do not identify a relationship between cancer and residues of hormonal growth promoters. The references to the higher rates of breast and prostate cancer observed in the United States as compared to the European Communities are not very convincing as there is considerable variation in rates in different geographical locations. Also, the differences in rates of breast and prostate cancer observed in the United States as compared to the European Communities are relatively small. There is no way to definitely establish a link between these statistics and the consumption of meat from animals treated with the hormones at issue as there are many possible confounders, and the differences in cancer rates are small. However, the results are at least consistent with a possible effect of hormones on breast and prostate cancer. My reply would not have been different in 2003.

Pages 17-19 of 1999 Opinion of the SCVPH and related Tables A4-A5 on pages 83-91.

(b) Residue analysis

27. How do the residues in meat from cattle treated with the three synthetic growth promoting hormones differ from residues in meat from cattle treated with the three natural growth promoting hormones at issue?

Dr. Boisseau

243. Residues in meat from cattle treated with the three synthetic growth promoting hormones differ from residues in meat from cattle treated with the three natural growth promoting hormones at issue because the parent substances being different chemical entities associated with specific toxicological and physiological properties, the residues deriving from these different substances will be also different chemical entities associated with specific toxicological and physiological properties.

Dr. De Brabander

244. The residues of the three synthetic growth promoting hormones are substances which are exogenous: they do not occur in the body of a healthy human being or animal.

245. The structures of the synthetic hormones are different from those of the natural hormones. Melengestrol and trenbolone could be considered as being derived from respectively progesterone and testosterone while zeranol has a totally other structure.

28. How do the hormones naturally present in animals, meat, or human beings differ from the residues in meat of the three natural hormones used for growth promotion purposes?

Dr. Boisseau

246. The definition of residues encompasses both the parent substance and all the metabolites derived from this parent substance. Therefore, in the case of the part of residues of the natural hormones which consists of parent substances, there is no difference between hormones naturally present in food producing animals, meat or human beings. Metabolites of these natural hormones existing in cattle and meat are, obviously, the same. To my knowledge, there is no scientific evidence showing that the main metabolites of the three natural hormones existing in cattle and humans are not similar.

Dr. De Brabander

247. At first sight there are no differences between residues in meat of the three natural hormones used for growth promotion purposes and the hormones naturally present in animals, meat and human beings. However ...

- The use of the three natural hormones used for growth promotion purposes will trigger a mechanism of reactions in the body of animals and human beings which may lead to the presence of other substances which **are not** naturally present. The conversion of testosterone to boldenone is an example. Boldenone is a very potent hormone used by "body-builders"
- The "natural" hormones used for growth promotion purposes are synthesised (prepared) from plant material. In plant material the ¹³C/¹²C ratio is different from the

$^{13}\text{C}/^{12}\text{C}$ ratio of animals. Research is ongoing which demonstrates that the pattern of hormones changes by the application of the "natural" hormones used for growth promotion purposes. (see further question 32)

- The residues of the natural hormones in cattle are in the 17α form (inactive) while the use of "natural" hormones used for growth promotion purposes may lead to residues in the β form (active form)
- The residues of (the esters of) the natural hormones are incorporated in hair of the animals.

29. To what extent do the SCVPH Opinions evaluate evidence on the actual residue levels of the synthetic hormones found in meat in their assessment of the risks from such residues? Are specific references provided as to how the evidence on residues relates to the observance of good veterinary practices or lack thereof? How do they compare with the MRLs set by Codex? [see para. 165-176 of EC Rebuttal Submission (US case); pages 55-68 of the Opinion of the SCVPH of 30 April 1999 in US Exhibit 4, para. 144 of US First Submission, Exhibits US-6 and 7, footnote 46 of US Rebuttal Submission]

Dr. Boisseau

248. Levels of residues are taken into consideration at the third step, exposure assessment, of the risk assessment procedure after an ADI has been established at the end of the second step, hazard characterisation, of this procedure. As, in its 1999 report, SCVPH concluded "that no threshold level and, therefore, no ADI can be established for any of the six hormones" (including the three synthetic ones), there was no need for SCVPH to conduct a quantitative assessment of the exposure of consumers to the residues of hormonal growth promoters including the determination of the levels of residues in food from treated animals, the impact of the non observance of good veterinary practices on these levels and the comparison between these levels and the MRLs set up by Codex.

Dr. De Brabander

249. In the case of the 3 synthetic hormones the assessment of risk as evaluated by the SCVPH is in terms of actual residue levels is less complex than in the case of the natural hormones: since these hormones does not occur naturally the endogenous levels in humans and in the environment are to be considered as zero.

250. Each administration of these 3 hormones will increase the levels of these hormones in human causing a number of effects, cited by the SCVPH and leaving the doubt about a number of still unknown effects. Moreover, for the risk assessment some "old" data for residue concentrations are used.

251. In my experience, extreme care should be taken regarding publications of concentrations of residues. These concentrations cited (e.g. Table 8 : residue levels of alpha and beta trenbolone in tissues of treated cattle ; p 56 ; exhibit US-4) are extremely low (e.g. 10 ng/kg (= 10 ppt)) and serious doubts about their accuracy can be made. At the time they were produced (1987) there were no analytical methods available to quantify these residues at that concentration level in a correct way (methods as GC-MS-MS or LC-MS-MS). The detection capability and way of validation of analytical method have changed a lot in the last 20 years. The concentrations may seriously be underestimated making the risk assessment even more risky.

252. Moreover, all studies are too much focused on the direct effect on human health **only** (NOEL, ADI, MRL). As demonstrated in several documents a major part of the hormones used are excreted

through the faeces (for MGA ca. 75 %) from which they enter into the environment causing a number of uncontrollable effects. In the case of the 3 synthetic hormones there are not enough data on their metabolites, possible transition products and effects on the large number of aquatic life in our environment.

253. The MRLs set by the codex are high in relation to modern analytical limits (normally $\leq 1 \mu\text{g/kg}$)

Trenbolone and zeranol	Cattle - Muscle -	2 $\mu\text{g/kg}$
	Cattle - Liver -	10 $\mu\text{g/kg}$
Melengestrol acetate	Cattle-liver	5 $\mu\text{g/kg}$
	Cattle-fat	8 $\mu\text{g/kg}$

254. Both from their relation to human health in all of its aspects and from an analytical point of view these MRLs are not acceptable.

30. To what extent do the SCVPH Opinions evaluate evidence on the actual residue levels of the three natural hormones in meat in their assessment of the risks from such residues? Is it possible to compare these to the ADIs recommended by JECFA in 1999? Are specific references provided as to how the evidence on residues relates to the observance of good veterinary practices or lack thereof? [see paras. 120-123 and 155-164 of EC Rebuttal Submission (US case), pages 33-54 of 1999 Opinion in Exhibit US-4, para. 144 of US First Submission, and 52nd JECFA Report in Exhibit US-5]

Dr. Boisseau

255. My reply to question 29 applies also to this question.

Dr. Boobis

Exposure assessment in the SCVPH Opinions

256. In the SCVPH Opinions of 1999 and 2002, the Committee did not itself evaluate evidence on the incurred residue levels of the three natural hormones in meat in their assessment of risks from such residues. In their 1999 Opinion, the Committee cited the data tabulated by JECFA but then calculated an exposure based on the US tolerances, which resulted in higher estimates of exposure than the theoretical maximum daily intakes (TMDIs) calculated on the basis of incurred residue levels by JECFA in 1999. In this Opinion, there is also some confusion between ADIs and tolerances (which are equivalent to MRLs). Tolerances are set to enable compliance to be determined. However, exposure at the tolerance level cannot be equated with risk to health. It is the ADI that determines whether there is a health risk at a given exposure. In their 2002 Opinion, the Committee did not revisit exposure following use according to GVP. Rather, the Committee considered potential exposure following several inappropriate use scenarios. This was based on a series of experimental studies, to determine the consequences of a number of defined misuses on hormone levels in meat. However, whilst of potential value in any risk assessment, these data are limited in the absence of any information on the frequency of occurrence of such misuse in the use of the products in question in normal veterinary practice. It would have been possible to compare the SCVPHs estimates of exposure with the ADIs derived by the JECFA but this was not done. The ADI would have exceeded the exposure estimates for the three hormones. In both Opinions, comparison was with estimated intake at the US tolerances.

257. References are provided to a series of studies on the effect of various misuse scenarios on residue levels. However, no references are provided that the effects GVP or the lack thereof has on residue levels in normal veterinary practice.

SCVPH (2002). Opinion of the Scientific Committee on Veterinary Measures 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 (http://ec.europa.eu/food/fs/sc/scv/out50_en.pdf)

Dr. De Brabander

258. In the case of the three natural hormones the assessment of risk as evaluated by the SCVPH is in terms of actual residue levels and is more complicated than in the case of the synthetic hormones. Different data of concentrations of endogenous levels of the 3 hormones in humans and in farm animals could be found in literature.

259. The argument used in favour of the allowance of natural hormones is often that the contribution of the residues of these hormones in meat is only a small part of the naturally produced hormones in the body of a human being (JECFA,1987). Again, for the risk assessment some "old" data for residue concentrations are used and their accuracy could be doubted.

260. As for the three synthetic hormones, all studies are focused too much on the direct effect on human health only (as measured with a NOEL, ADI, MRL etc.). As demonstrated in several studies a major part of the hormones used are excreted through urine and faeces and the administration of natural hormones to a herd increases the concentration of these hormones in the environment. Recently it was demonstrated in our laboratory that maggots of flies were able to convert high concentrations of natural hormones to strongly anabolic agents as boldenone and boldione

261. Moreover it should not be overlooked that these hormones may act as pheromones. The best known example is androstenone (the pheromone of the boar). But on Thursday, 10 February, 2005 BBC news mentioned that: a spray that helps increase women's enjoyment of sex has undergone successful trials. The spray, developed by Australian company Acrux, contains the male sex hormone testosterone. It was initially designed with post-menopausal women in mind, but has also been shown to work for young women with a low libido. The spray was tested over four months in three doses on 261 women with a low sex drive and low testosterone levels.

262. The tests with this type of spray illustrated the impact of hormones on human behaviour. Dr Geoff Hackett, of the British Society for Sexual Medicine, said it was important that the spray was only given to women who had been thoroughly assessed, and shown to have low testosterone. Dr Hackett also warned that raising testosterone levels too high was linked to side effects such as beard growth, hair loss, greasy skin and acne.

263. In addition to the above there is also ZMA (sold by a well known lab in the US and on the internet). ZMA is a scientifically designed anabolic mineral formula. It contains Zinc Monomethionine Aspartate plus Magnesium Aspartate and vitamin B-6, and is an all-natural product that has been clinically proven to significantly increase anabolic hormone levels and muscle strength in trained athletes. In tests the ZMA group had 30% increases in free and total testosterone levels compared to 10 percent decreases in the placebo group. The ZMA group also had a slight *increase* in insulin-like growth factor-1 (IGF-1) levels compared to a 20 percent *decrease* in the placebo group.

264. In all studies on residue levels of natural hormones I have found no indication of the influence of such ZMA formulations (and synergism with implantation). However, elements such as Zn and Mg are known to play an important role in enzymatic reactions.

31. Please comment on the US statement that "concentrations of oestradiol-17 β in meat from treated cattle do not vary significantly from concentrations in untreated cattle, i.e., residue levels in meat from hormone-treated cattle are well within the physiological range of residue levels in untreated cattle. While tissue concentrations of oestradiol-17 β in treated cattle may be slightly higher than those in untreated cattle, this increase is much smaller than the large variations observed in (reproductively) cycling and pregnant cattle and is thus well within the range of naturally observed levels." In your reply please take into account the US 11th Carcinogenesis Report where it is stated that "Meat and milk may contain estrogens. Veterinary use of steroidal estrogens (to promote growth and treat illness) can increase estrogens in tissues of food-producing animals to above their normal levels" and the statement by the European Communities that "meat consumption from pregnant heifers is exceptional as usually these animals are not slaughtered. [see para. 51 and 144 of US First Submission and Exhibits US-6 and 7, para. 98 of EC Replies to Panel Questions, Exhibit EC-101, and para. 2.3.2.3 of the 1999 Report of SCVPH]

Dr. Boisseau

265. The US statement that " concentrations ...naturally observed levels " is certainly right if one considers included among the physiological range of oestradiol-17 β and of progesterone in cattle the levels of these hormones occurring during pregnancy. It is also true that meat and milk from non treated cattle contain residues of these two natural hormones. The comment of the European Communities that "meat consumption from pregnant heifers is exceptional as usually these animals are not slaughtered " is also true. Even if, accepting the substance of the EC comment, it is possible to limit the physiological range of oestradiol-17 β and of progesterone in cattle, it has nevertheless to be recognized that (1) consumers are exposed to these two natural hormones through their consumption of meat and milk from the different non treated food producing animals and, mainly as least for women, through their endogenous production, (2) this exposure cannot be avoided. Therefore, the use of the concept of threshold in the risk assessment of the natural hormone residues is legitimate and the additional intake of residues of these natural hormones from the meat from treated cattle has to be considered in this context and not according to a theoretical " no additional intake of residues is acceptable".

Dr. De Brabander

266. The US statement that "concentrations of oestradiol-17 β in meat from treated cattle do not vary significantly from concentrations in untreated cattle, i.e., residue levels in meat from hormone-treated cattle are well within the physiological range of residue levels in untreated cattle" may at first sight be correct. However, pregnant animals are normally not slaughtered and even if one is, the consumption of meat from those animals is small. On the contrary, if all animals are treated with estradiol-17 β there could be a significant increase of estradiol in human food. Meat and milk may contain estrogens (as also other foods which may contain estrogens but which don't pose problems at a normal food consumption level) and therefore there is no need to add more by artificial ways.

267. Moreover, the use of hormones is not only questioned from the point of view of the risk of food:

- There is also the influence on animal welfare, which is an important item. There are a lot of publications indicating that the use of hormones will influence behaviour in animals (and humans). Animals treated with hormones may become more aggressive or feel uncomfortable. Nowadays this is not tolerated.

- There is the influence on the environment. All natural hormones as well as the synthetic ones end up in the environment. If the load of hormones is too high this may influence the life and behaviour of some fish and invertebrates and changing the normal pathway of life. There are a lot of investigations in that area
- Finally, most consumers in Europe don't want that the meat they eat is derived from animals treated with hormones (and want to also keep the level of veterinary drugs used as low as possible). This tendency of the consumer, increasing over the years is taken over by distribution chains who finally are deciding what is coming into the market.

32. Please comment on the conclusions of the EC risk assessment (Opinion of the SCVPH of April 2002) that ultra sensitive methods to detect residues of hormones in animal tissues have become available but need further validation. What is the significance of this with regard to identifying whether the natural hormones in meat are endogenously produced or are residues of hormones used for growth promotion purposes?

Dr. Boisseau

268. It is a general requirement that any analytical method, used to detect residues among others, be validated before being used. This validation must be carried out in compliance with well defined and internationally accepted criteria. As these ultrasensitive methods, referred to by the European Communities, are not yet properly validated, there is a need for additional work in this domain. Nevertheless, it has to be reminded that, when MRLs have been established for a given substance, there is not any more a need for highly sensitive analytical methods but for a validated analytical method the sensitivity of which must be consistent with the values of the established MRLs. In addition, if it is true that ultrasensitive analytical methods remain useful to control the use of forbidden veterinary drugs, such as for example growth promoters in EU, they are less useful in the case of the three natural hormones, which are endogenously produced by food producing animals.

Dr. De Brabander

269. Methods for the determination of hormones are improving constantly and it can be foreseen that they will do so for some time. When I started -as a chemist- in the faculty of veterinary medicine (1973) the method of choice was TLC (Thin Layer Chromatography) with fluorescence detection. That method has been used (with succes) for some time untill GC-MS (gas chromatography with mass spectrometric detection) was coming up (in practice end of the 80's). Later MS-MS and even MSⁿ could be used as a very selective detector. In the middle of the 90's affordable LC-MS systems came on the market.

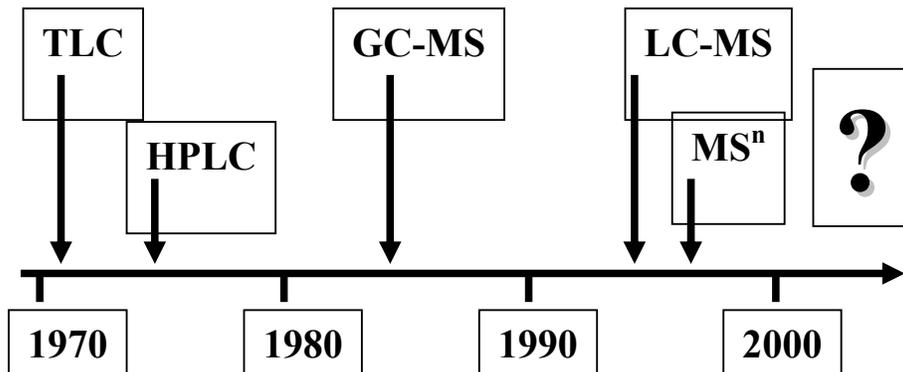


Fig 32.1: Evolution of the availability of analytical techniques in function of time.

270. LC-MS is still strongly in evolution. As an illustration Fig 32.2 shows the evolution of limits of detection of GC and LC-MS. As can be seen the LOD's has decreased considerably in the last years.

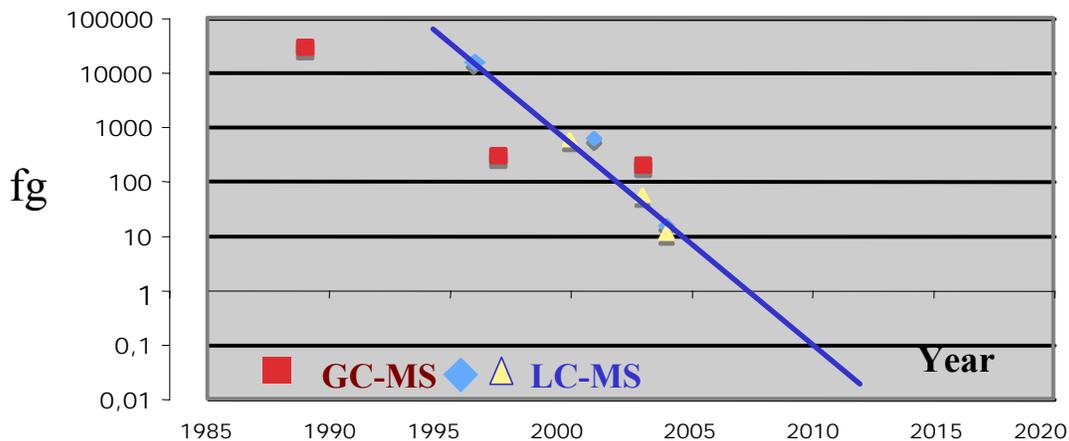


Fig 32.2: Evolution of the limits of detection in function of time

271. The improvement of limits of detection is more pronounced in LC than in GC-MS and will continue with the introduction of new methods of separation and detection. GC and LC-MS should also be used complementary. An illustration with an example: the main metabolite of stanozolol (a synthetic anabolic steroid) in cattle is 16-hydroxystanozolol. This was only observed when samples are also analysed with LC-MS next to GC-MS. Indeed, according to its structure the determination of 16-hydroxystanozolol with GC-MS is very difficult or nearly impossible.

Multi-laboratory study of the analysis and kinetics of stanozolol and metabolites in treated calves. H.F. De Brabander, K. De Wasch, L.A. van Ginkel, S.S. Sterk, M.H. Blokland, Ph. Delahaut, X. Taillieu, M. Dubois, C.J.M. Arts, M.J. van Baak, L. G. Gramberg, R. Schilt, E.O. van Bennekom, D. Courtheyn, J. Vercammen, R.F. Witkamp The Analyst, 123, 12 (1998) 2599-2604.

272. Consequently all data on stanozolol metabolism before the use of LC-MS (1998) have little value because the major metabolite was not detected. There are a number of analogous cases where "older" analytical data should be used with caution. Moreover, when apparatus allowing better separation and/or lower limits of detection become available it is not always possible to repeat expensive animal experiments to update the data.

273. Here is a strong difference between Europa and the US. A difference which can be clearly measured in the citation of literature. Since little researchers in the US work on the determination of residues of banned substances (since they are not banned) publications on these matters (the development of methods) are not cited very much in the US and this could be measured on the citation index.

274. This question refers also to the use of GC-C-IRMS (gas chromatography combustion isotope ratio mass spectrometry). As mentioned in my reply to question 28 the "natural" hormones used for growth promotion purposes are synthesised (prepared) from plant material. In plant material the $^{13}\text{C}/^{12}\text{C}$ ratio is different from the $^{13}\text{C}/^{12}\text{C}$ ratio of animals. There are now new data available demonstrating that the pattern change of hormones by the application of the "natural" hormones used for growth promotion purposes.

275. The methods are still in strong evolution –as well in doping control laboratories as in residue analysis. In practice new methods and apparatus need some time to improve technical details in order to improve the robustness of the apparatus. Therefore validation of the methods take considerable time.

33. What were the reasons for the re-evaluation by JECFA of the three natural hormones in 1999? Were the residues data used for the three natural hormones in 1999 the same as those used in 1988? What additional information was used for the JECFA evaluation in 1999 of the three natural hormones which was not available in 1988? How did the conclusions differ? What led JECFA to establish ADIs for the three natural hormones? What are the implications of establishing ADIs? Why were JECFA's more recent recommendations not considered by the Codex Committee on Residues of Veterinary Drugs in Food? What is the status of these recommendations? [see paras. 96-97 of EC Rebuttal Submission (US case), para. 79-80 of EC Rebuttal Submission (Canada case)]

Dr. Boisseau

276. As already mentioned in my reply to the question 29, it is useless to establish MRLs in the absence of ADIs. As, in its 1988 session, JECFA concluded that, given the outcome of its risk assessment for the three natural hormones, there was no need to establish ADIs in order to protect human health, it was therefore useless to establish MRLs (cf the reply given to the question No 18 which applies also to this question No 33). For the reasons explained in this reply to the question No 18, JECFA decided in 1999 to establish ADIs for these three natural hormones. Usually, establishing ADIs leads to establishing MRLs. In the case of these three natural hormones, the outcome of the assessment of the exposure of consumers by JECFA in 1999 having shown in the reply to question 18 that the highest estimated intake of residue was so low compared with the values of the corresponding ADIs, that there was no need to establish MRLs to protect human health.

277. The residues data considered in 1999 for assessing the exposure were those already used in its thirty second session.

278. If the wording of the conclusions adopted by JECFA has been formally different, the substance of these conclusions remained unchanged.

279. Establishing such ADIs had no specific implications as no MRLs have been established.

280. These new recommendations have not been considered by CCRVDF because CCRVDF did not request JECFA to reassess these hormones and because the new proposals of JECFA did not change the substance of the previous ones.

Dr. Boobis

Reasons for JECFA re-evaluation of the natural hormones

281. The hormones were re-evaluated by the 52nd JECFA on the suggestion of the JECFA Secretariat, due to the availability of new information that had appeared in the published literature since the last evaluation (*FAO, 2000*). This was endorsed by the 11th session of the Codex CCRVDF in their revision of the priority list of substances for review (*CCRVDF, 1998*).

CCRVDF (1998). Report of the Eleventh Session of the Codex Committee on Residues of Veterinary Drugs in Foods, 15-18 September, 1998, Washington, DC: Alinorm 99/31 (<http://www.fao.org/docrep/meeting/005/X0203E/x0203e00.htm#Contents>)

282. I am unable to comment on whether the residues data were the same as evaluated in 1988.

283. There were a number of additional studies on the toxicology and human (including epidemiological) evaluation of therapeutic exposures to the hormones (e.g. in the form of oral contraception or for hormone replacement therapy) that were not available in 1988.

284. The conclusion of the 1999 JECFA was to establish ADIs for the hormones evaluated whereas in 1988 it was considered unnecessary to allocate ADIs or indeed to prepare toxicological monographs on the hormones. This was presumably because at the time, the view was that it was unnecessary to conduct a detailed evaluation of the toxicology of substances produced endogenously. However, in the intervening time from the first to the second evaluation, it became clear that exposure to the natural hormones, albeit at levels appreciable higher than found in meat from treated cattle, could have adverse effects in humans. Hence, the implicit conclusion was that it was necessary to establish ADIs, to serve as health based guidance values. These could then be used as a benchmark for comparison with exposure via the diet. In JECFA's opinion, exposure at levels up to the ADI daily over a lifetime would be without appreciable risk (IPCS definition of ADI; this is also the CVMP definition of the ADI for a veterinary drug residue (EMA, 2005)).

285. "Acceptable daily intake (ADI): the estimate of the residue, expressed in terms of micrograms or milligrams per kilogram of bodyweight, that can be ingested daily over a lifetime without any appreciable health risk."

EMA (2005). Volume 8: Notice to applicants and Guidance: Veterinary medicinal products. Establishment of maximum residue limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin (http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/vol-8/pdf/vol8_10-2005_.pdf)

286. The data used to establish ADIs for oestradiol and for progesterone in 1999 were from studies in humans that had been undertaken since 1988.

287. According to their report, the CCRVDF did not consider these recommendations because they had not requested the review and found no basis to change their previous decision that MRLs need not be specified for the natural hormones.

"Recognizing that this Committee had not requested the re-evaluation of these substances and that the new MRLs recommended by the 52nd JECFA did not differ significantly from the current MRLs, the Committee decided not to consider these new recommendations" (*CCRVDF, 2000*)

*CCRVDF (2000). Report of the Twelfth Session of the CCRVDF (ALINORM 01/31)
Washington, D.C., 28-31 March 2000
(http://www.codexalimentarius.net/download/report/217/A101_31e.pdf)*

288. JECFA establishes ADIs but recommends MRLs. CCRVDF endorsed the recommendation that MRLs for the natural hormones did not need to be specified.

289. The consequence of this decision is that the current status of the Codex MRLs for the three natural hormones is that they are listed as "unnecessary" for tissues from cattle (the species in which the hormones are used).

Dr. De Brabander

290. The driving force after the re-evaluation by the JECFA of the 3 natural hormones can only be guessed (by me). Obviously scientific thinking has evolved in such a way that in 1988 the JECFA found it "unnecessary" to establish ADI's and MRL's for the 3 natural hormones while in 1999 this has changed.

291. The residue data used by the JECFA in 1999 were according to my knowledge the same as those in 1998. Data on residues were generated with radioimmunoassays, which are according to the 2002/657/EC only permitted as screening methods and the validation procedure of the methods can be doubted. The additional information should be from tests on experimental animals and human beings. However, no references to the open literature are given in the 52th JECFA report.

292. The conclusions are different in the fact that ADIs were established: this is recognition of the danger of hormones to human health and welfare in all of his aspects.

293. The last question: Why were JECFA's more recent recommendations not considered by the Codex Committee on Residues of Veterinary Drugs in Food? What is the status of these recommendations? It is out of my power to answer.

34. Please comment on the EC argument that the 1999 JECFA report based its findings on (a) outdated residues data and (b) not on evidence from residues in meat but on studies with experimental animals and on general studies of IARC. If the data were not new, did JECFA take this into account in its evaluation? What are the implications of using such data for the purpose of conducting a risk assessment? How reliable are extrapolations from animal studies to possible adverse effects on humans? How does this compare with the kind of data and studies used with respect to other veterinary drugs? [see para. 120 of EC Rebuttal Submission (US case), para. 102 of EC Rebuttal (Canada case)]

Dr. Boisseau

294. As mentioned in my reply to question 33, it is true that (1) the residues data considered by JECFA in 1999 for assessing the exposure were those already used in its thirty second session held in 1987, (2) some of them have not been published in peer-reviewed scientific journals. It is just a banality to say that JECFA is provided with new data when it is requested to assess veterinary drugs recently placed on the market and older data in the case of veterinary drugs already marketed since a

long time ago. Anyway, the quality and the number of the available data are more important than the dates at which these data have been produced. In order to carry out the assessment of the residue exposure and to establish the appropriate MRLs for the substances under review, JECFA has to consider the available data regarding residues. If it considers that the quality and the number of these data, the validation of the analytical methods used to provide these data are satisfactory, JECFA assesses the exposure and establishes MRLs. If it think that it is not the case, it does not establish MRLs and ask for additional data. In the case of hormones, JECFA has considered that the quality and the number of the available residue data were satisfactory and therefore the fact that these data were not new had no specific impact on its evaluation. In addition, the very wide margin of safety between the exposure of consumers to hormones residues and the value of the established ADIs did not raise an acute problem in this case (see my reply to question 18).

295. I don't really understand the meaning of the second EC comment stating that "the toxicological findings in the 1999 JECFA report ... are not based on evidence from residues in meat from animals treated with these hormones for growth promotion purposes". As already mentioned in my reply to question 13, toxicological studies are, practically, always carried out with amounts of substances always larger than those corresponding to residues and it is assumed that residues have the same toxicological potential like the tested substances. In the case where there is, among these residues, a metabolite associated with a specific toxicological potential which is of concern with regard to public health, JECFA can base its risk assessment on this metabolite if appropriate and technically possible. Moreover, it is the normal way for assessing the toxicological potential of a substance to take into consideration in vivo studies with experimental animals, in vitro studies and also reports already published by internationally recognized scientific organisations such as IARC.

296. Extrapolation from animals to humans regarding the toxicological potential of the substances under review is not avoidable as it is not possible to carried out experiments in humans. It is the responsibility of JECFA to assess to what extent this extrapolation is meaningful for the products under review.

297. For assessing the growth promoters, JECFA has used the same procedure it has used for all the other veterinary drugs.

Dr. De Brabander

298. I think that the EC argument that the JECFA report is based on outdated residue data is correct. I was unable to find in the open scientific literature much modern residue data. Moreover, the laboratories which were able to produce such data and publish them are scarce. Because hormones are banned in Europe and animal experiments on farm animals as cattle are more and more complicated by all kinds of regulations the frequency of these experiments faded out since the 1980's. In the US the intensity of recent data on residue analysis is even more scarce. Only a few US researcher participate in the leading residue conferences which are held in Europe regularly.

299. I haven't seen any impact of studies of the IARC in the JECFA report. However, there are such studies available: a.o. on the website of IARC a recent press release (No 167 of 29 July 2005) could be found: *IARC monographs programme finds combined estrogen-progestogen contraceptives and menopausal therapy are carcinogenic to humans*. This press release illustrates the danger of hormones and also the problematic of synergism: less studies involve combinations of hormones as they are used as production aids (e.g the combination of estradiol and progesterone).

300. Hereunder a citation of the IARC press release

"Breast cancer and endometrial cancer are increased. Epidemiological studies consistently demonstrate an increased risk of breast cancer in women who used

combined menopausal therapy. Largely confined to current or recent users, the risk increases with duration of use and exceeds that in women taking estrogen-only therapy. Endometrial cancer risks depend on the number of days that progestogens are included in the combined therapy. When progestogens are taken fewer than 10 days per month, the risk of endometrial cancer is increased, but when progestogens are taken daily, the risk is similar to that in women who never used hormonal therapy. There was not sufficient evidence to conclude that hormonal therapy has a protective effect at any cancer site.

Overall risks and benefits should be weighed carefully. Both beneficial and adverse effects other than cancer have been established for combined estrogen-progestogen menopausal therapy. As for oral contraceptives, a rigorous risk/benefit analysis would be useful to put the different effects in perspective and assess the overall consequences for public health."

301. The implications of not using such (modern) data are that the results of the risk assessment are biased in favour of the "allowance" of hormones. Giving an answer on the reliability of extrapolations from animal studies to possible adverse effects on humans is and remains difficult. Therefore a safety factor is used.

302. For the comparison with other veterinary drugs (MRLs) the following reasoning could be held. Veterinary (and human) drugs are not used on each animal (as hormones as production aids are) but only in case that they are needed (disease). This is a totally different situation.

35. Please comment on the European Communities claim that nearly all the studies referred to in the 2000 JECFA report on MGA date from the 1960s and 70s. Is this correct? Have subsequent reports of JECFA, prior or subsequent to the adoption of the Directive, also relied on the same studies? [see para. 171 of EC Rebuttal Submission (US case), para. 161 of EC Rebuttal Submission (Canada case), para. 55, including footnote 60 of US First Submission and Exhibits CDA-20, 33, 34, and 35]

Dr. Boisseau

303. It is correct to say that nearly all the studies referred to in the 2000 JECFA report on MGA date from the 1960s and 70s. The comment to be made on this issue is similar to the comment already made in the reply to the question 34. JECFA considered a wide series of toxicological studies in its assessment, used as end point a non hormonal effect dose by far more conservative than a NOAEL based on tumorigenic effect and adopted a 200 safety factor to derive an ADI from this NOAEL.

Dr. De Brabander

304. I think that the claim of the European Communities that nearly all the studies referred to in the 2000 JECFA report on MGA date from the 1960s and 70s is correct. I scanned the literature on "melengestrol acetate" on the web of science and found only 257 hits. Of these 213 are published in 1980 and later.

305. Of these publications only a few refer to the use of melengestrol acetate as a growth promotor in cattle and therefrom only a few originate from US authors.

Hereunder a selection could be found:

Tepfer AJ, McFee RM, Bott RC, et al.

Feeding melengestrol acetate (MGA) to bulls during the peri or pre-pubertal period induces differences in endocrine profiles which may lead to alterations in testis size.
BIOLOGY OF REPRODUCTION: 225-225 Sp. Iss. SI 2005

Merritt DA, Wilson EM, Martin RA, et al.
Metabolism of melengestrol acetate (MGA) in the bovine: Biological activity assessment of tissue residues and implications for human food safety.
ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY 228: U102-U102
118-AGRO Part I AUG 22 2004

Schiffer B, Totsche KU, Jann S, et al.
Mobility of the growth promoters trenbolone and melengestrol acetate in agricultural soil: column studies
SCIENCE OF THE TOTAL ENVIRONMENT 326 (1-3): 225-237 JUN 29 2004

Lange IG, Daxenberger A, Hageleit M, et al.
Non-invasive screening for treatment of heifers with the anabolic steroid melengestrol acetate (MGA) by feces analysis
JOURNAL OF IMMUNOASSAY & IMMUNOCHEMISTRY 24 (3): 265-272 AUG 2003

Schiffer B, Daxenberger A, Meyer K, et al.
The fate of trenbolone acetate and melengestrol acetate after application as growth promoters in cattle: Environmental studies
ENVIRONMENTAL HEALTH PERSPECTIVES 109 (11): 1145-1151 NOV 2001

Hageleit M, Daxenberger A, Meyer HHD
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 APR 2001

Daxenberger A, Meyer K, Hageleit M, et al.
Detection of melengestrol acetate residues in plasma and edible tissues of heifers
VETERINARY QUARTERLY 21 (4): 154-158 OCT 1999

Karg H, Meyer HHD
Update evaluation of trenbolone acetate, zeranol and melengestrol acetate as growth promoters (considerations concerning the "hormone issues" between EU and USA at the WTO)
ARCHIV FUR LEBENSMITTELHYGIENE 50 (2): 28-37 MAR-APR 1999

Henricks DM, Brandt RT, Titgemeyer EC, et al.
Serum concentrations of trenbolone-17 beta and estradiol-17 beta and performance of heifers treated with trenbolone acetate, melengestrol acetate, or estradiol-17 beta
JOURNAL OF ANIMAL SCIENCE 75 (10): 2627-2633 OCT 1997

CAMPBELL HM, SAUVE F
LIQUID-CHROMATOGRAPHIC DETERMINATION OF MELENGESTROL ACETATE IN FEEDS
JOURNAL OF AOAC INTERNATIONAL 76 (6): 1163-1167 NOV-DEC 1993

NEIDERT EE, GEDIR RG, MILWARD LJ, et al.
DETERMINATION AND QUALITATIVE CONFIRMATION OF MELENGESTROL ACETATE RESIDUES IN BEEF FAT BY ELECTRON-CAPTURE GAS-

CHROMATOGRAPHY AND GAS-CHROMATOGRAPHIC CHEMICAL IONIZATION MASS-SPECTROMETRY
JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY 38 (4): 979-981 APR 1990

CHICHILA TMP, EDLUND PO, HENION JD, et al.
DETERMINATION OF MELENGESTROL ACETATE IN BOVINE-TISSUES BY AUTOMATED COUPLED-COLUMN NORMAL-PHASE HIGH-PERFORMANCE LIQUID-CHROMATOGRAPHY
JOURNAL OF CHROMATOGRAPHY-BIOMEDICAL APPLICATIONS 488 (2): 389-406
MAR 24 1989

KRZEMINSKI LF, COX BL, GOSLINE RE
FATE OF RADIOACTIVE MELENGESTROL ACETATE IN THE BOVINE
JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY 29 (2): 387-391 1981

306. Also: If you look at the "International Portal on Food Safety, Animal & Plant Health" <http://www.ipfsaph.org/servlet/CDSServlet?status=ND1jdGh0dHB3d3dmYW9vcmdhb3NpcGZzYXBoaW5mb3JtYXRpb25zb3VyY2VqZWNmYS5KRUNGQUVWQUxtZWxlbmdlc3Ryb2xhY2V0YXRlJjY9ZW4mMzM9Zm9ybWFsX3RleHQmMzc9aW5mbw~~> the three pdf files relating melengestrol acetate, which can be downloaded contain only "old" references (before 1980).

(c) Dose-response relationship

36. **How would you describe a dose-response assessment? Is it, as suggested by Canada in para. 78 of its Rebuttal Submission, "widely, if not universally, accepted that adverse effects arising from hormonal activities are dose-dependent"? Is dose-response assessment a necessary component of hazard characterization? Or, is there an alternative approach which can replace the dose-response assessment. Is a dose-response assessment feasible/necessary for substances that are found to be genotoxic or to have genotoxic potential? [see para. 153 of EC Replies to Panel Questions, para. 200 of EC Rebuttal Submission (US case); paras. 143, 154, and 156 of US First Submission, paras. 70-74 of US Replies to Panel Questions, and paras. 34 and 37-40 of US Rebuttal Submission; paras. 76-82 of Canada Rebuttal Submission]**

Dr. Boisseau

307. The comment made by Canada regarding the dose response relationship is correct. Dose-response assessment is a necessary component of hazard characterisation and there is, to my knowledge, no alternative approach which can replace this dose- response assessment. A dose-response assessment is not feasible for substances that are found to be genotoxic or to have genotoxic potential if, as it has been already said in the reply to the question No 19, these substances are xenobiotics and if it is thought that this genotoxic potential can be expressed in in vivo conditions.

Dr. Boobis

308. Dose-response assessment is analysis of the relationship between the total amount of an agent administered to, taken up or absorbed by an organism, system or (sub)population and the changes developed in that organism, system or (sub)population in reaction to that agent, and inferences derived from such an analysis with respect to the entire population (*IPCS, 2004*). In a dose-response assessment, information that is sought includes whether there is evidence for a compound-dependent effect, if so, the dose range over which these effects occur, the quantitative relationship between dose and the magnitude or incidence of the effect, the steepness of the dose-response curve, the severity or incidence of the maximum effect observed, whether there is a threshold and if so its location and the

spacing between the no observable adverse effect level (NOAEL) and the lowest observable adverse effect level LOAEL).

309. It is generally accepted that adverse effects arising from hormonal activities are dose-dependent (*IUPAC, 2003*). The caveat is that this is when the response is mediated by the physiological mechanisms of the hormone, via receptor occupancy. However, even should this not be the case because of a genotoxic mode of action, the response would depend on dose. The difference would be in the shape of the dose response curve. In the former, there would be a threshold, a dose below which there would be no effect (NOAEL). In the latter, there would not necessarily be a such a threshold. Hence, although risk would diminish as dose decreases, it may never reach zero.

IUPAC(2003). Special Topic Issue on the Implications of Endocrine Active Substances for Humans and Wildlife, Pure Appl. Chem., Vol. 75, Nos. 11–12

310. In risk assessment, dose-response assessment is an essential part of hazard characterization, as it forms the basis for deriving health-based guidance values such as the ADI, with which dietary intake can be compared. The only exception would be a hazard-based approach, that is recommendations as to potential safety based on intrinsic capacity to cause harm rather than on the probability of harm occurring. The former can be based on hazard identification whilst the latter requires hazard characterization and exposure assessment. The most widely used instance of hazard-based safety guidance is that for substances that are genotoxic or that have genotoxic potential. However, this needs to be qualified, in that not all such substances would be treated in this way. This is discussed in my reply to question 19 above. There may be kinetic (e.g. phenol following oral exposure, *European Chemicals Bureau, 2006*) or dynamic (e.g. spindle inhibitors such as thiophanate-methyl, *EC, 2005a*) reasons why a genotoxic compound exhibits a threshold in its dose-response relationship. Similarly, compounds that are genotoxic via the formation of reactive oxygen species also exhibit a threshold in their dose-response curve (*Brusick, 2005*). Such effects would be treated as would non-genotoxic endpoints, i.e. an ADI would be established using the threshold dose (NOAEL) as a starting point, and appropriate uncertainty factors. Where the mechanism of genotoxicity is not known, DNA reactivity is assumed. However, for compounds that are known or assumed to be genotoxic via DNA reactivity, genotoxic potential would normally have to be confirmed in vivo before this endpoint would be used as the basis for a risk assessment (*CVMP, 2004*). For such compounds, unless there is good evidence to the contrary, it is assumed that there is no threshold for the dose-response curve.

311. The approach for such compounds that are known or assumed to exhibit no threshold in their dose-response curve, varies from one region to another. In Europe and generally within JECFA, once a compound is identified as an in vivo DNA-reactive mutagen, or as causing a carcinogenic response via a genotoxic mode of action, no exposure is considered without risk, and hence a recommendation of maintaining intake as low as reasonable practicable (ALARP) may be made, without any consideration of the dose-response relationship. In the case of a veterinary drug residue the simplest way to achieve ALARP would be not to use the drug in veterinary practice. In other regions, such as the USA, appropriate dose-response assessment will be undertaken, using an approach that assumes no threshold (linear, low dose extrapolation). In this approach, if intake is below that associated with a very low risk (often an incidence of 1 in 10^6 of the population), exposure may be considered acceptable (*US EPA, 2005; US FDA, 2005*).

US FDA, Guideline No. 3, 2005. General Principles for Evaluating the Safety of Compounds Used in Food-Producing Animals (<http://www.fda.gov/cvm/Guidance/GFI1003.htm>)

US EPA, 2005. Guidelines for Carcinogen Risk Assessment (http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=439797)

Dr. Cogliano

312. Dose-response assessment is a quantitative characterization of the relationship between the dose of an agent and the occurrence of adverse effects. In my view, it is widely accepted that adverse effects arising from hormonal activities depend on the dose; that is, the level of effect depends on the level of exposure. Dose-response assessment is not, however, a necessary component of hazard characterization. Without a dose-response assessment, it is possible to conclude that an agent can alter the risk of one or more adverse effects. With a dose-response assessment, it may also be possible to estimate how much the risk may be altered for a given level of exposure.

37. Do JECFA or Codex materials confirm Canada's statement in para. 80 of its Rebuttal Submission that "...while international risk assessment techniques suggest that a dose-response assessment is optional for biological or physical agents when the data cannot be obtained, a dose-response assessment should always be conducted for chemical agents..."? [see Exhibit CDA-25]

Dr. Boisseau

313. JECFA has always established ADIs for veterinary drugs on the basis of a dose-response assessment. As already said in the reply to the question No 7, JECFA did not establish any ADI when it was not possible to carry out a dose-response assessment such as with xenobiotic compounds such as chloramphenicol and nitroimidazoles.

Dr. Boobis

Is dose-response assessment optional?

314. Codex and JECFA materials certainly require that a dose-response assessment should always be conducted as part of the risk assessment of a chemical agent (*CAC, 2005; IPCS: EHC 70, 1987 and EHC 104, 1990; IPCS, 2005; WHO, 1996 and 2001*). JECFA would be unable to recommend MRLs (or conclude that they need not be specified) unless it had quantitative information on the levels of exposure that were considered not to cause harm (from either observations in humans and/or studies in experimental animals) and an estimate of actual human exposure. If JECFA concluded that the toxicity of a compound was without threshold because it was a genotoxic carcinogen of potential relevance to humans (the default assumption), it may well conclude that it would be inappropriate to recommend MRLs, and hence in this specific circumstance a dose-response assessment might be considered unnecessary. However, this is a very unlikely occurrence for a veterinary drug because, in general, producers tend to screen out genotoxic compounds during the development process. This is not to say that such drugs will never be encountered, but simply to point out that there are procedures in place that are such that they will substantially reduce the probability of this occurring.

(d) Sensitive populations

38. Please describe the range of physiological (or background) levels of the sex hormones in humans and identify the variations in these levels on the basis of age, sex group, and physiological stages.

Dr. Boisseau

315. According to several scientific publications reported by the SCVPH in its 1999 Opinion, the endogenous steroid hormone levels in human females and males are the following:

Hormone	Women Prepubertal	Women Follicular-Luteal	Women Postmenopausal	Men Prepubertal	Men Adult
Oestradiol pg/ml	8-23	10-375	0-28	5-14	6-44
Estrone pg/ml	19	15-250	15-55	16	15-65
Progesterone ng/ml	0.1-0.4	0.2624		0.1-0.3	0.3-0.5
Testosterone ng/ml	0.1-0.2	0.4-0.8	0.4-0.8	0.1-0.2	3-9

316. According to the data reported by JECFA, in its 32nd session held in 1987, and by the SCVPH in its 1999 Opinion, the daily production rates for hormones in human females and males are the following:

Hormone	Women Prepubertal	Women Follicular	Women Pregnancy	Men Prepubertal	Men Adult
Oestradiol µg/day		445	37.800	6,5	48
Progesterone µg/day		418	94.000	150	416
Testosterone µg/day	32-65	140-240		32-65	6.500

317. According to the SCVPH in its 1999 Opinion, new ultrasensitive bioassays, 100 fold more sensitive than RIA methods used to provide the data reported in the above tables, would lead to lower values, respectively 0.6 pg/ml and 0.08 pg/ml of oestradiol-17β for prepubertal girls and boys. It would be important to know whether these new bioassays have been properly validated as this SCVPH Opinion says nothing about that and whether the data obtained with these methods for both men and women are also totally different from those obtained with the RIA methods.

39. Please comment on the SCVPH opinion stating that "any excess exposure towards oestradiol-17β and its metabolites resulting from the consumption of meat and meat products presents a potential risk to public health in particular to those groups of the populations which have been identified as particularly sensitive such as prepubertal children" [see para. 147 of the EC Replies to Panel Questions]

Dr. Boisseau

318. As already said in my reply to question 31, the comment of SCVPH needs to integrate a quantitative assessment of the risk associated with this excess exposure for the groups of populations which have been identified as particularly sensitive such as prepubertal children. This excess exposure of these sensitive populations needs to be assessed and compared with the exposure resulting from the daily consumption of meat from cattle which have not been treated by growth promoters, from other food and products of animal origin and from their own production of hormones.

Dr. Sippell²

319. The SCVPH opinion that "any excess exposure towards estradiol-17β ... prepubertal children" is supported by increasing evidence from more recent scientific data in the international literature, both from Europe and from America. Due to the almost 100 times lower estradiol-17β (E₂) serum levels found by modern ultrasensitive assay techniques (Klein et al 1994, Larmore et al 2002) in prepubertal children as compared to conventional E₂ assays, the resulting potential E₂ exposure risk from consumption of meat and meat products has greatly increased by a factor of at least 160 times, if one compares the maximum acceptable daily intake (ADI) estimates of E₂ of 65 ng/day (old) and 0.4 ng/day (new) in prepubertal boys (Andersson & Skakkebaek, 1999). This revised ADI threshold would be reached already after ingestion of as little as 10 g of E₂ –treated meat from cattle

² A full list of references cited in responses from Dr. Sippell can be found in Attachment 2.

(Daxenberger et al 2001). Moreover, in this comparison only E₂ but not its numerous estrogenic metabolites, glucosidic conjugates and fatty acid esters (Maume et al 2001) were taken into account.

320. It has been shown in numerous scientific publications in vitro, in vivo and in the human that infants and prepubertal children are highly sensitive to increased E₂-levels, resulting in premature breast development (Schmidt et al 2002), growth acceleration (Lampit et al 2002), earlier sexual maturation in girls, in particular in the USA (Sun et al 2002, WU et al, 2002) and less in Europe (Muinck-keizer & Mul 2001), and the well known significantly higher incidence of precocious puberty in girls than in boys (Teilmann et al 2005). Accidental exposure of prepubertal boys to estrogens has resulted in gynecomastia and advanced bone maturation (Felner & White 2000).

321. Late effects: There is now increasing epidemiological evidence that exposure to elevated estrogen levels during early life (pre- and postnatally) carries an increased risk of breast cancer in adult life (Ekbom et al 1997, Swerdlow et al 1997, Weiss et al 1997, Halakivi-Clarke et al 2000), whereas conditions with low E₂ levels, such as preeclampsia, seem to have a protective effect (Innes & Byers 1999). Moreover, indirect evidence suggests that male reproductive disorders such as testicular cancer, cryptorchidism, hypospadias and poor sperm quality may also have their origin in hormonal disturbances induced by E₂ and/or estrogenic substances during fetal life (Skakkebaek et al 2001) and also during childhood (Higuchi et al 2003, Ramaswamy 2005).

Andersson AM & Skakkebaek NE (1999) Exposure to exogenous estrogens in food: possible impact on human development and health. Eur. J. Endocrinol. 140, 477-485.

Daxenberger A, Ibarreta D & Meyer HH (2001) Possible health impact of animal oestrogens in food. Hum. Reprod. Update. 7, 340-355.

Ekbom A, Hsieh CC, Lipworth L, Adami HQ & Trichopoulos D (1997) Intrauterine environment and breast cancer risk in women: a population-based study. J. Natl. Cancer Inst. 89, 71-76.

Felner EI & White PC (2000) Prepubertal gynecomastia: indirect exposure to estrogen cream. Pediatrics 105, E55.

Halakivi-Clarke L, Cho E, Onojafe I, Liao DJ & Clarke R (2000) Maternal exposure to tamoxifen during pregnancy increases carcinogen-induced mammary tumorigenesis among female rat offspring. Clin. Cancer Res. 6, 305-308.

Higuchi TT, Palmer JS, Gray LE, Jr. & Veeramachaneni DN (2003) Effects of dibutyl phthalate in male rabbits following in utero, adolescent, or postpubertal exposure. Toxicol. Sci. 72, 301-313.

Innes KE & Byers TE (1999) Preeclampsia and breast cancer risk. Epidemiology 10, 722-732.

Klein KO, Baron J, Colli MJ, McDonnell DP & Cutler GB, Jr. (1994) Estrogen levels in childhood determined by an ultrasensitive recombinant cell bioassay. J. Clin. Invest 94, 2475-2480.

Lampit M, Golander A, Guttman H & Hochberg Z (2002) Estrogen mini-dose replacement during GnRH agonist therapy in central precocious puberty: a pilot study. J. Clin. Endocrinol. Metab 87, 687-690.

Larmore KA, O'Connor D, Sherman TI, Funanage VL, Hassink SG & Klein KO (2002) Leptin and estradiol as related to change in pubertal status and body weight. Med. Sci. Monit. 8, CR206-CR210.

Maume D, Deceuninck Y, Pouponneau K, Paris A, Le Bizec B & Andre F (2001) Assessment of estradiol and its metabolites in meat. APMIS 109, 32-38.

Muinck-Keizer SM & Mul D (2001) Trends in pubertal development in Europe. Hum. Reprod. Update. 7, 287-291.

Ramaswamy S (2005) Pubertal augmentation in juvenile rhesus monkey testosterone production induced by invariant gonadotropin stimulation is inhibited by estrogen. J. Clin. Endocrinol. Metab 90, 5866-5875.

Schmidt IM, Chellakooty M, Haavisto AM, Boisen KA, Damgaard IN, Steendahl U, Toppari J, Skakkebaek NE & Main KM (2002) Gender difference in breast tissue size in infancy: correlation with serum estradiol. Pediatr. Res. 52, 682-686.

Skakkebaek NE, Rajpert-De Meyts E & Main KM (2001) Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum. Reprod. 16, 972-978.

Teilmann G, Pedersen CB, Jensen TK, Skakkebaek NE & Juul A (2005) Prevalence and incidence of precocious pubertal development in Denmark: an epidemiologic study based on national registries. Pediatrics 116, 1323-1328.

40. The European Communities states that "the levels of endogenous production of the hormones by prepubertal children is much lower than previously thought and this finding, which is subsequent to the 1999 JECFA report, casts serious doubts about the validity of JECFA's findings on the dose-response relationship..." Please comment on the methodology used by the SCVPH to support the conclusion that hormone levels are lower than previously thought, and in particular comment on the validity of these methodologies and their conclusions. Would your conclusions have been the same at the time of adoption of the Directive in September 2003?

Dr. Boisseau

322. My comment given at the end of question 38 replies to this question.

323. My reply would not have been different at the time of adoption of the EC Directive in September 2003.

Dr. Boobis

Hormone production in prepubertal children

324. There is certainly some evidence that endogenous levels of hormones in children are lower than previously thought. However, the suggestion that this is by orders of magnitude is not substantiated by the data. One group has reported very low levels of oestradiol in male children, 0.08 pg/ml (*Klein et al, 1994*), but in a later study (*Klein et al, 1998*), the same group reported mean levels somewhat higher, at 0.27 pg/ml. The reliability of the Klein et al assay has yet to be determined. The assay is particularly sensitive to oestradiol, but there is no obvious explanation for this, as it relies upon affinity for the oestrogen receptor. Diethylstilbestrol is a potent oestrogen yet is

much less sensitive than oestradiol in the assay. *Klein et al (1994)* have reported that there are unidentified factors in plasma and in blood collection tubes that can interfere in the assay. In contrast, using a similar yeast-based assay, *Coldham et al (1997)* found that oestradiol and DES had similar potency, and other have found that, if anything, DES is more potent than oestradiol in such assays (*Folmer et al, 2002*). At the very least, this shows that results with the yeast reporter assay are not consistent, and use of such data in risk assessment requires that the assay be adequately validated.

Coldham NG, Dave M, Sivapathasundaram S, McDonnell DP, Connor C and Sauer MJ (1997). Evaluation of a recombinant yeast cell estrogen screening assay. Environ Health Perspect, 105:734-742

Folmar LC, Hemmer MJ, Denslow ND, Kroll K, Chen J, Cheek A, Richman H, Meredith H and Grau EG (2002). A comparison of the estrogenic potencies of estradiol, ethynylestradiol, diethylstilbestrol, nonylphenol and methoxychlor in vivo and in vitro. Aquat Toxicol, 60:101-110

Klein KO, Baron J, Colli MJ, McDonnell DP and Cutler GB Jr (1994). Estrogen levels in childhood determined by an ultrasensitive recombinant cell bioassay. J Clin Invest, 94:2475-2480

Klein KO, Baron J, Barnes KM, Pescovitz OH and Cutler GB Jr (1998). Use of an ultrasensitive recombinant cell bioassay to determine estrogen levels in girls with precocious puberty treated with a luteinizing hormone-releasing hormone agonist. J Clin Endocrinol Metab, 83:2387-2389

325. However, there are studies from two other groups using more specific methods than the original radioimmunoassay, reporting that levels were somewhat higher than this. *Ikegami et al (2001)* used a very sensitive, 2-stage immunoassay technique. This was shown to be specific and sensitive. In this assay, mean levels of oestradiol in prepubertal males were 1.85 pg/ml (6.8 pmol/ml). *Paris et al (2002)* used a recombinant oestrogen receptor assay in a mammalian cell line, a similar principle to the assay of Klein et al. In this study, estrogenic levels in prepubertal males were found to be 1.44 pg/ml. There are many issues affecting such measurements. These include the presence of binding proteins, relative specificity and sensitivity. None of the assays is entirely specific for oestradiol. Both the oestrogen receptor and the antibodies used could cross-react with structurally related compounds. Depending on how the assay is performed, protein binding could reduce the concentration of hormone detectable in the assay by sequestering hormone from the assay target. However, it should be noted that whilst binding to protein in plasma may reduce clearance it will also reduce the biologically active dose. In general, it is the free concentration that determines biological activity (*Teeguarden and Barton, 2004*). Hence, if SHBG is elevated in children this would tend to reduce the effect of an equivalent total plasma concentration by reducing the free concentration.

326. The advantage of the recombinant assays is that they measure biologically active material, whereas the immunoassays may include cross-reacting less or inactive metabolites. Whilst the recombinant assays may include hormonally active material other than the specific analyte, this does provide an indication of to what the body is exposed in vivo. Hence, on balance, the data of *Paris et al (2002)* may be the most meaningful to date. This presumably reflects circulating total active oestrogenic material, but not that bound to proteins.

Ikegami S, Moriwake T, Tanaka H, Inoue M, Kubo T, Suzuki S, Kanzakili S and Seino Y (2001). An ultrasensitive assay revealed age-related changes in serum oestradiol at low concentrations in both sexes from infancy to puberty. Clin Endocrinol (Oxf), 55:789-795

Paris F, Servant N, Terouanne B, Balaguer P, Nicolas JC and Sultan C (2002). A new recombinant cell bioassay for ultrasensitive determination of serum estrogenic bioactivity in children. J Clin Endocrinol Metab, 87:791-797

Teeguarden JG and Barton HA (2004). Computational modeling of serum-binding proteins and clearance in extrapolations across life stages and species for endocrine active compounds. Risk Anal, 24:751-770

327. Assuming a plasma concentration of 1.44 pg/ml (*Paris et al, 2002*), this would be equivalent to a daily oestradiol production of 2 µg/day. These data suggest that exposure to oestradiol at levels near the JECFA ADI of 50 ng/kg, equivalent to 1.3 µg/day (assuming a body weight of 26 kg) could result in intakes close to the daily production of oestradiol in prepubertal males, the group suggested to be most at risk. However, this exposure is via the oral route, and bioavailability by this route is very low (<5%) (*Fortherby, 1996*). In addition, very little of the absorbed hormone will be free, over 95% being bound to plasma proteins such as SHBG. Such binding reduces the biological activity of the hormone (*Teeguarden and Barton, 2004*). Hence, the JECFA ADI would appear to be appropriate for all groups of the population. This conclusion would have been the same at the time of adoption of the Directive by the EC in September 2003.

Dr. Sippell

328. There is no doubt that the development of an ultrasensitive recombinant cell bioassay (RCBA) of E₂ by Karen Klein, Gordon Cutler and co-workers at the N.I.H. in Bethesda, USA (Klein et al 1994) represented a quantum leap in E₂ assay methodology. It opened a new door on our understanding of basic physiological phenomena, e.g. why normal puberty starts so much earlier in girls than in boys or why bone maturation in children differs so much between the sexes. The validity of the N.I.H.-RCBA has now been confirmed by another RCBA of E₂ which was developed by Charles Sultan's group at the University of Montpellier, France (*Paris et al 2002*). Unfortunately, the complexity of the RCBA so far prevents its wider use for routine measurements in small serum samples from infants and prepubertal children.

329. Since pediatric endocrinologists and other researchers in the field had already been hearing and discussing these breakthrough findings since 1993/94, and again in 2001/02, I would certainly have come to the same conclusions in September 2003.

Klein KO, Baron J, Colli MJ, McDonnell DP & Cutler GB, Jr. (1994) Estrogen levels in childhood determined by an ultrasensitive recombinant cell bioassay. J. Clin. Invest 94, 2475-2480.

Paris F, Servant N, Terouanne B, Balaguer P, Nicolas JC & Sultan C (2002) A new recombinant cell bioassay for ultrasensitive determination of serum estrogenic bioactivity in children. J. Clin. Endocrinol. Metab 87, 791-797.

41. Why would individuals with the lowest endogenous hormone levels be at greatest risk? How would the risks for these individuals arising from hormones naturally present in meat differ from the risks arising from the residues of hormone growth promoters?

Dr. Boisseau

330. Individuals with the lowest endogenous hormone levels have been considered as being at greater risk by JECFA because the rationale followed by JECFA was, in the case of natural hormones, to avoid that any excess intake of hormonally active residues be significant with regard to the rate of their daily production of hormones in order to protect them from any physiological disturbance. In this respect, the target was that the hormonal residue intake does not account for more than 1% of the daily production rate of any group of the human population. Therefore, the lower is the endogenous production of hormones in a given human group, the lower must be the excess intake of hormonally active residues for the individuals of this group.

331. From a qualitative point of view, the risks for these individuals arising from residues resulting from the use of hormones growth promoters in cattle does not differ from the risks arising from the residues of hormones naturally present in meat. The potential problem which may exist is only a quantitative one.

Dr. Boobis

Relevance of endogenous hormone levels to risk

332. The reason that those with the lowest levels of hormone production are considered at the greatest risk is because for a given exposure this group will experience the greatest percentage change in their circulating hormone levels. There is evidence that the levels are normally low in such subjects to ensure a biological effect that is no greater than that appropriate to their gender and age. Hence, the extent of the biological response is likely to reflect the percentage increase in hormone levels. In addition, prior to puberty, some biological systems are more sensitive to hormonal perturbation than after puberty (*Caruso-Nicoletti et al, 1985; Miyamoto and Burger, 2003*).

Caruso-Nicoletti M, Cassorla F, Skerda M, Ross JL, Loriaux DL and Cutler GB Jr (1985). Short term, low dose estradiol accelerates ulnar growth in boys. J Clin Endocrinol Metab, 61:896-898

333. There is no basis to think that the effect of hormone growth promoters would be different in any way whatsoever from hormones naturally present in meat, at equivalent internal exposure levels.

Dr. Sippell

334. This question relating to the specific vulnerability of young children having the lowest levels of endogenous hormones (estradiol, testosterone, progesterone and their metabolites) has largely been answered above (see my reply to question no. 39).

335. The risk to children arising from hormones which are naturally present in meat as compared to that from residues of hormonal growth promoters has, to my knowledge, been estimated for E₂ only and only in beef (Daxenberger et al 2001). The average E₂ content of 500 g of meat (standard daily consumption of 300 g muscle, 100 g liver, 50 g kidney and 50 g fat according to JECFA) was 4.3 ng and 20 ng of E₂ in untreated and E₂-treated cattle, respectively. The new threshold of 0.4 ng E₂ /day would thus be reached after the ingestion of 47 g of untreated meat and of as little as 10 g of hormone treated meat. These authors also estimated that eating meat from E₂-treated cattle increased the daily intake of E₂ from food by 38% compared to non-treated meat. This percentage, and thus the potential health risk, will be considerably higher if the food intake from pork, poultry, eggs and dairy products derived from E₂-treated farm animals are taken into account.

336. Synthetic hormone growth promoters such as Zeranone and its metabolites have been shown to be as potent as E₂ and diethylstilbestrol (DES) in increasing the expression of estrogen-related genes in human breast cancer cells (Leffers et al 2001). On the other hand, the synthetic androgen Trenbolone and the gestagen Melengestrol bind with high affinity to the human androgen and progesterone receptors, respectively (Bauer et al 2000). Exposure during pregnancy might result in severe transplacental virilisation of a female fetus.

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

Daxenberger A, Ibarreta D & Meyer HH (2001) Possible health impact of animal oestrogens in food. Hum. Reprod. Update. 7, 340-355.

Leffers H, Naesby M, Vendelbo B, Skakkebaek NE & Jorgensen M (2001) Oestrogenic potencies of Zeranol, oestradiol, diethylstilboestrol, Bisphenol-A and genistein: implications for exposure assessment of potential endocrine disrupters. Hum. Reprod. 16, 1037-1045.

- 42. To what extent, in your view, has JECFA taken into account the particular situation of sensitive populations, in particular prepubertal children, in its risk assessments with respect to oestradiol-17 β ? Please compare the original data concerning endogenous production of natural hormones by prepubertal children upon which JECFA based its assessment and those used by the European Communities in its risk assessment. In your view, does the scientific material referred to by the European Communities require a revision of the Codex recommendation with respect to oestradiol-17 β ?**

Dr. Boisseau

337. Individuals with the lowest endogenous hormone levels have been considered as being at greater risk by JECFA because the rationale followed by JECFA was, in the case of natural hormones, to avoid that any excess intake of hormonally active residues be significant with regard to the rate of their daily production of hormones in order to protect them from any physiological disturbance. In this respect, the target was that the hormonal residue intake does not account for more than 1% of the daily production rate of any group of the human population. Therefore, the lower is the endogenous production of hormones in a given human group, the lower must be the excess intake of hormonally active residues for the individuals of this group.

338. From a qualitative point of view, the risks for these individuals arising from residues resulting from the use of hormones growth promoters in cattle does not differ from the risks arising from the residues of hormones naturally present in meat. The potential problem which may exist is only a quantitative one.

Dr. Boobis

Consideration of sensitive populations by JECFA

339. In keeping with its risk assessment principles, the ADI established by JECFA would have been designed to protect all segments of the population, including prepubertal children (*IPCS: EHC 70, 1987 and EHC 104, 1990; IPCS, 2005; WHO, 1996 and 2001*). For this reason, in establishing the ADI from the NOAEL in a human study, JECFA used a 10-fold safety factor to protect sensitive populations, in addition to a 10-fold factor to allow for interindividual variation within the adult human population. For an evaluation of the impact of differences in assumed endogenous production of hormones by JECFA and the EC on the risk assessment see my reply to question 40 above.

340. In my view, there is no requirement for any revision in the Codex recommendation with respect to oestradiol-17 β on the basis of the material referred to by the EC.

Dr. Sippell

341. To my knowledge, the particular situation of sensitive populations, in particular infants and prepubertal children, has not been adequately taken into account by JECFA in respect of E₂.

342. The original data on prepubertal children used by JECFA have been questioned in a number of more recent publications (Andersson & Skakkebaek 1999, Maume et al 2001, Partsch & Sippell

2001) in view of the new ADI based on the ultrasensitive RCBA determination of E₂ in children. The data have already been compared in the answer to questions 39 and 41.

343. In my view, the scientific material referred to by the European Communities definitely requires a revision of the codex recommendation with respect to E₂, as outlined above.

Andersson AM & Skakkebaek NE (1999) Exposure to exogenous estrogens in food: possible impact on human development and health. Eur. J. Endocrinol. 140, 477-485.

Maume D, Deceuminck Y, Pouponneau K, Paris A, Le Bizec B & Andre F (2001) Assessment of estradiol and its metabolites in meat. APMIS 109, 32-38.

Partsch CJ & Dr. Sippell WG (2001) Pathogenesis and epidemiology of precocious puberty. Effects of exogenous oestrogens. Hum. Reprod. Update. 7, 292-302.

[For the questions in this section, see paras. 121-122 of EC Rebuttal Submission (US case), paras. 103-104 of EC Rebuttal Submission (Canada case), Exhibits EC-88, 99, paras. 42-45 of US Rebuttal Submission, paras. 84 and 159 of US First Submission, and for JECFA's work Exhibits CDA 11, 16, 17, 18, 39]

(e) Bioavailability

43. Please define bioavailability, comment on the significance of bioavailability to assessments of risk, and on the degree of bioavailability of the residues of the hormones at issue when consumed in meat, taking into account parties' differing views on this matter. [see paras. 123-124 of EC Rebuttal Submission (US case), para. 105-106 of EC Rebuttal Submission (Canada case), paras. 100, 155-159 of the EC Replies to Panel Questions, paras. 32 and 41-42 of US Rebuttal Submission, paras. 69, 71, 88-89 and 146 of US First Submission, and para. 134 of Canada Rebuttal Submission]

Dr. Boisseau

344. Bioavailability is the capacity of a substance to enter the general blood circulation and to diffuse into the whole body of the animal or the human being administered this substance. The physico-chemical characteristics of substances and their route of administration to humans and animals are of a paramount importance regarding the rate at which they are bioavailable. Oral route, which is the route for the ingestion of residues, is not the most efficient. It is even very poor for the three natural hormones.

345. The bioavailability of residues has to be taken into consideration in the risk assessment, in particular at the third step regarding the exposure assessment of residues. Residues which are not bioavailable are not of concern for the public health if the toxicological potential of the substance is a systemic one. The residues, the bioavailability of which has not been determined, are considered as totally bioavailable. Those which have been recognised as non bioavailable can be discarded from the exposure assessment.

346. Natural hormones are known to be poorly bioavailable in humans : oestradiol-17 β is inactive orally, the bioavailability of progesterone and of testosterone are respectively less than 10% and around 4%.

347. The bioavailability of melengestrol, trenbolone and zeranol residues have not been determined. Therefore all their residues have been considered as being totally bioavailable.

Dr. Boobis

348. Bioavailability can be defined as that fraction of a dose that is available to the systemic circulation. It can take values from 0, no systemic availability, to 1, 100% availability. It is normally estimated by comparing the dose-corrected area under the plasma concentration time curve (AUC) after dosing by the route of interest, for example orally, with the dose-corrected AUC after intravenous administration (assumed to be completely available) (*Rowland and Tozer, 1995*). The bioavailability reflects both the extent of absorption and any presystemic metabolism that occurs. In the case of the oral route, this could be in the intestinal tract and/or the liver

Rowland M and Tozer T (1995). Clinical Pharmacokinetics: Concepts and Applications, Lippincott Williams and Wilkins, London

349. In general, only that fraction of the dose that is bioavailable is toxicologically relevant. The exceptions would be when some or all of the non-bioavailable dose is in the form of a bioavailable metabolite that is biologically active, or when the effects of concern occur presystemically, for example local effects on the gastrointestinal mucosa. In the case of the natural hormones, low bioavailability is due to presystemic metabolism to products with substantially reduced hormonal activity and their bioavailability is <5-10% (*Christiaens et al, 2005; Hoogenboom et al, 2001; JECFA, 2000; Jockenhövel, 2002; Kuhnz et al, 1993; Stanczyk, 2003*). The effects of concern are systemic. Hence, the toxicological significance of the low bioavailability of hormones is that the risk is less than from the equivalent dose produced endogenously or administered by some other route with higher bioavailability (e.g. subcutaneous implant).

Christiaens V, Berckmans P, Haelens A, Witters H and Claessens F (2005). Comparison of different androgen bioassays in the screening for environmental (anti)androgenic activity. Environ Toxicol Chem, 24:2646-2656

Hoogenboom LAP, de Haan L, Hooijerink D, Bor G, Murk AJ and Brouwer A (2001). Estrogenic activity of estradiol and its metabolites in the ER-CALUX assay with human T47D breast cells. APMIS, 109:101-107

Jockenhövel F (2002). Practical aspects of testosterone substitution. Aging Male, 5 (Suppl 1):21-46

Stanczyk FZ (2003). All progestins are not created equal. Steroids, 68:879-890

350. However, low bioavailability does not necessarily increase the margin of safety (the ratio of ADI to actual exposure). This is because the effects of concern are usually determined following exposure by the route of interest, in this case oral. Hence, the ADI represents a "bioavailability adjusted" dose, just as the TMDI does. The consequence of this is that anything that increases bioavailability will reduce the margin of safety whilst anything that reduces bioavailability will increase the margin of safety. In the case of the natural hormones, changes in bioavailability are likely to be a consequence of changes in the enzymes of metabolism in the liver and/or small intestine.

351. The bioavailability of the non-natural hormones (melengestrol acetate, trenbolone acetate and zeranol) in humans is, to my knowledge, not known. Whilst it is likely that it will be less than 100% there is no specific information available. However, it should be noted that in the risk assessment of these hormones by JECFA, the risk characterization involved comparison of the theoretical maximum daily intake with the ADI. No correction was made for bioavailability. Hence, the situation is likely to be similar to that for the natural hormones, in that changes in bioavailability from the normal value would change the margin of safety.

Dr. Guttenplan

352. Bioavailability is defined as the fraction of a chemical that enters the general circulation by oral administration compared with that entering the circulation by iv. Injection.

353. Presumably, only the bioavailable chemical can produce adverse (or any) effects, thus in terms of risk assessment, only the portion of the dose of chemical that is bioavailable is significant. (*Toxicokinetics in the National Toxicology Program. NIDA Research Monograph. 173:273-304, 1997.*)

354. The US and Canada maintain that orally ingested estradiol (the major potentially harmful hormone) is essentially inactive, because of poor bioavailability.

355. The US states (para. 41, US rebut. Sub.) "The EC also asserts that the U.S. argument that estradiol 17 β is generally inactive when given orally, while "well known", is "still controversial and not consensually accepted by the scientific community." To the contrary, estradiol's low oral bioavailability has found international support in Codex and JECFA ("[i]n general, estradiol 17 β is inactive when given orally because it is inactivated in the gastrointestinal tract and liver"), as well as support within the EC from the CVMP, which noted that "the *bioavailability of 17 β -oestradiol* esters after oral administration *is low (3% as unchanged oestradiol)*, but might be higher if estrone, an estrogenic metabolite, is included." Also, included is a reference that in a cell culture model (immortalized human intestinal cells (Caco-2 cells)) estrogen does not enter the cell although the estradiol convertible metabolite was detected in the cells. The US states that this supports the hypothesis that estradiol cannot cross the intestinal wall. This latter finding seems paradoxical, because somehow estradiol was converted to estrone, so it must have entered the cell.

356. On the other hand the EC maintains (EC Rebut, para 123) that "recent developments put in doubt the findings of the 1999 JECFA report concerns the bioavailability of residues of these hormones. The 1999 and 2002 SCVPH reports have found that data on which JECFA based its findings are incorrect or insufficient." For instance, "Metabolic studies of orally administered 17 β -oestradiol indicate that as much as 20 percent of a 2 mg dose of micronized E2 is absorbed, with a serum half-life in the range of 2 to 16 hours (Zimmermann et al., 1998; Vree and Timmer, 1988; Ginsburg et al., 1998) and in it is stated that the above reports indicate, the bioavailability of fine-particle 17 β -oestradiol administered orally was determined to be 5% compared to a dose administered intravenously. It is also pointed out (EC Rebut, para 124) lipoidal esters (metabolites of estradiol) have high bioavailability and may accumulate in adipose tissue. No data accompany the accumulation hypothesis, although it seems reasonable.

357. It appears that the bioavailability of estrogen is low but not insignificant (probably between 5 and 20%, if estrone is also taken into account. (Estrone is readily inter-convertible with estrogen). Calculations are presented in the above reference that suggest that even with low percentages of bioavailability of estrogen, the levels in meat could result in bioavailable estrogen approaching the daily production rate of oestradiol in pre-pubertal children (EC Rebut, para 122). This would represent a risk factor (EC Rebut, para 122).

(f) Good veterinary practice (GVP)

44. Please define "good veterinary practice" (GVP) and/or "good practice in the use of veterinary drugs" (GPVD). What are the relevant Codex standards, guidelines or recommendations relating to GVP/GPVD? Please comment on the statement by the European Communities that the definition of the GPVD is "circular and hence problematic." [see para. 88 of the EC Replies to Panel Questions]

Dr. Boisseau

358. There are several definitions for the good veterinary practice (GVP) and the following one adopted by Codex is satisfactory : " GVP in the use of veterinary drugs is the officially recommended or authorized usage, including withdrawal periods, approved by national authorities, of veterinary drugs under practical conditions "

359. Nevertheless, to my knowledge, the Codex Commission has only adopted, in July 2005, a guideline on GVP intended to minimize and put under control the microbial resistance. It did not adopt any guideline on GVP aimed at minimizing the occurrence of veterinary drug residues in animal derived food.

360. The comment of the European Communities about GVP is not clear. I suppose that the European Communities means that the conditions of used of veterinary drugs, even officially approved, may differ in a very significant way from one country to another one.

Dr. De Brabander

361. Most probably, several definitions could be found for defining GVP. Hereunder I give one of the Veterinarians of Europe:

Federation of Veterinarians of Europe: Code of good veterinary practice

Veterinarians play an important role in protecting animal welfare, animal health, public health as well as the environment and provide a wide range of services.

This Code of Good Veterinary Practice is a standard specifying the European veterinary ethics and principles of conduct as well as the requirements relating to the quality management system within a veterinary organisation, when the latter:

1. *Wishes to improve its ability to give services in conformity with:*

- *The legislation in force,*
- *The Professional Code of Conduct in force,*
- *The requirements of the clients,*
- *The ethics principles relating to the services provided and/or the animals under its care.*

2. *Must demonstrate its ability to deliver services, which are constantly in line with customer requirements and the legislation in force.*

362. A selection of paragraphs on Public Health and the environment is also given below:

2. *G Veterinarians and Public Health*

- *Veterinarians shall seek to ensure the best protection of public health.*
- *Veterinarians shall, whenever appropriate, advise their customers about measures to minimise the risk of exposure to zoonotic agents, food borne pathogens, residues, contaminants (biological and chemical agents) and antimicrobial resistance.*
- *Veterinarians shall make animal owners aware of their responsibilities to the public.*

2. *H Veterinarians and the Environment*

- *Veterinarians shall attempt to reduce pollution of the environment by waste avoidance, recycling, using re-usable articles when appropriate, and correct disposal of waste.*
- *Veterinarians shall endeavour to reduce environmental pollution by careful and appropriate use of disinfectants, medicinal products and other chemicals.*
- *Veterinarians shall aim to be environmentally responsible by the economical use of energy and water.*
- *Veterinarians shall organise facilities for separate collection of different types of waste so that they can be sent to the appropriate recycling points.*
- *Veterinarians shall encourage customers to dispose of veterinary waste in a safe manner.*

363. This selection illustrates the importance of the veterinarians in reduction of pollution of the environment and the link between residue analysis and environmental sciences. This applies also to the use of hormones

364. Good practice in the use of veterinary drugs (GPVD), *as defined by the CCRVDF, is the official recommended or authorized usage including withdrawal periods, approved by national authorities, of veterinary drugs under practical conditions (document: Recommended international code of practice for control of the use of veterinary drugs cac/rcp 38-1993 ; cxp_038e).*

365. The statement by the European Communities that the definition of the GPVD is "**somewhat circular and hence problematic**" refers to the fact that the national authorities have a large impact on this so-called international standard and can influence the application of it.

366. Neither GVP neither GPVD will reduce the risk of "using hormones" for several reasons. Also education plays an important role. Two years ago we had some American students in veterinary medicine in an exchange program: their knowledge of "hormones" their use in the USA and the risks involved was almost zero.

45. In conducting a risk assessment of specific veterinary drugs, what assumptions are made concerning GVP, if any? How, if at all, are risks that might arise from the failure to follow good veterinary practice in the administration of veterinary drugs addressed?

Dr. Boisseau

367. Residue data considered by all the committees such as JECFA, CVMP etc... conducting risk assessments of veterinary drugs are always obtained from studies in which veterinary drugs under review have been administered to the target food producing animals according to the officially approved conditions of use of these veterinary drugs. Therefore, MRLs adopted by Codex are only meaningful in countries where GVP are effectively implemented. In addition, it would not be appropriate for the risk assessors to address the case where the veterinary drugs under review could not be used in practice according to the GVPs. It would not be appropriate because it would not be possible for the risk assessors to identify all the possible misuses/abuses and to get the residue data derived from these misuses/abuses. It would not be appropriate also because it would not be ethical for the case where such data, being available, would lead to the conclusion of the risk assessment that,

given a possibly wide margin of safety for a veterinary drug under review, the excess intake of residues associated with these misuses/abuses does not raise any problem of public health. That would encourage all these misuses/abuses. Therefore, this issue of GVP, misuses/abuses must be taken into consideration not by the risk assessors but by the risk managers.

Dr. De Brabander

368. In conducting a risk assessment of specific veterinary drugs, it is assumed that GVP is followed. However can this be guaranteed?

369. In "A Primer on Beef Hormones" Date : 02/26/99 text released by the U.S. Interagency Task Force on Beef Hormones. the following could be read:

"Furthermore, the prescribed dosage is the level which produces the maximum economic response in the animal -- the law of diminishing returns -- so that there is no economic incentive for a farmer to use additional implants. A U.S. control system ensures that animals taken to slaughter have normal hormone levels. Thus, farmers have no incentive, economic or otherwise, to misuse the implants."

370. However, around the same date studies could be found on the use of Zilpaterol (a powerful beta-agonist of the 3th generation) on top of revalor (both control animals and test animals are implanted with revalor). This illustrates that farmers (and vets) have indeed economic incentives to misuse growth promoting substances (implants or others).

371. I am not aware of any study on public health of the combination of zilpaterol with the substances in revalor. Are the animals of this experiment destroyed?

372. Proceedings, Western Section, American Society of Animal Science Vol. 50, 1999
INFLUENCE OF THE beta-AGONIST, ZILPATEROL, ON GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF FEEDLOT STEERS

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ABSTRACT: One hundred forty crossbred steers (373 kg) were used in a randomized complete block design experiment (14 pens, 10 steers/pen) to evaluate the influence of supplementation of a steam-rolled wheat-based finishing diet with 6 mg/kg (as-fed basis) zilpaterol during the final 6 weeks of the finishing period on growth performance and carcass characteristics. Supplemental zilpaterol did not influence ($P > .20$) DM intake (8.55 vs 8.45 kg/d), but enhanced ($P < .01$) ADG (27%, 1.42 vs 1.94 kg/d), and feed efficiency (28%, 6.08 vs 4.37). Based on observed NE intake, ADG of the non-supplemented steers was 99% of expected. In contrast, with zilpaterol supplemented steers ADG was 29% greater ($P < .01$)

Experimental Procedure:

One hundred and forty crossbreed yearlings steers (373 kg) were used in a 42-d finishing trial. Steers were blocked by weight and randomly assigned, within weight groupings, to 14 pens (10 steers/pen). Pens were 510 m with 64 m overhead shade, automatic waterers, and 17 m fence-line feed bunks. The trial was initiated July 22, 1997. Treatments consisted of a steam-flaked wheat-based finishing diet (Table 1) supplemented (as fed basis) with 0 or 6 mg/kg zilpaterol (Zilmax, Hoechst Roussel Vet, D.F., Mexico). Steers were implanted with Revalor (Hoechst Roussel Vet, D.F., Mexico) upon initiation of the trial. Steers were allowed ad libitum access to experimental diets.

46. To what extent were risks from misuse or abuse assessed by JECFA in its evaluation of the hormones at issue? In terms of the three synthetic hormones at issue, how is GVP relevant to the establishment of MRLs by JECFA?

Dr. Boisseau

373. My reply to question 45 above applies also to this question. It covers also the three synthetic hormones which do not raise any specific problem in this domain.

Dr. Boobis

Assessment of risks from misuse and abuse by JECFA

374. Where the conclusion of a risk assessment is such that it is considered appropriate to establish an ADI to protect health (i.e. the critical effects exhibit a threshold), no consideration is given to the likely levels of exposure. The ADI is driven entirely by the toxicology and other relevant biological effects of the substance, which determine the point of departure (usually the NOAEL), and an appropriate uncertainty (or safety) factor. The uncertainty factor used has to account for any inter-species extrapolation, interindividual differences, sensitive sub-populations, absence of a NOAEL and any non-critical gaps in the database (see my reply to question 8 above). In establishing MRLs (relevant here only for the three synthetic hormones), the appropriate residues studies are those obtained after the normal use of the hormones, i.e. in accordance with GVP. This is the policy of all agencies and organisations involved in such activities (*EEC, 1990; EMEA, 2005; FAO, 2006*) (see response to question 62 below under "Multiple implanting, multiple dosing"). The point at which misuse and abuse are relevant in the risk assessment is at the risk characterisation stage, when potential exposure is compared with the ADI. Hence, whilst the TMDI is estimated following use of the hormones according to GVP, it would also be possible to consider other exposure scenarios, in which the hormones were misused or abused. Where exposure exceeded the ADI, the toxicological implications of this would depend on a number of factors (see response to question 62 below). These are as follows:

- The likelihood of violations or off-label use
- The residue levels occurring after such misuse or abuse
- The extent to which exposure to such residues will result in an exceedance of the ADI
- The likely frequency or period over which the ADI will be exceeded
- The acute consequences of exceeding the ADI
- The severity of the endpoint upon which the ADI is based
- The steepness of the dose-response curve for the endpoint upon which the ADI is based

375. JECFA did consider misuse of zeranol when it was evaluated in 1988 (*JECFA, 1988b*). JECFA gave some consideration of the effects of doses of up to 20 times those approved for the use of MGA on residue levels (*JECFA, 2000a*). JECFA does not appear to have explicitly considered misuse or abuse of trenbolone. The implications of misuse and abuse of these hormones for human health are considered below (question 62). There would have been no implications for the MRLs recommended. It would have been a decision of Codex as to how it would deal with any exceedances of the ADIs as a consequence of misuse or abuse, as this involves risk management decisions.

EEC (1990). Council Regulation (EEC) No 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. OJL 224, 18.8.1990, p. 1-8

FAO (2006). Updating the Principles and Methods of Risk Assessment: MRLs for Pesticides and Veterinary Drugs, Rome Italy
(http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/bilthoven_2005.pdf)

JECFA (1988b). Residues of some veterinary drugs in foods and animals, Vol. 41/1, FAO, Rome, Italy

Dr. De Brabander

376. Up to my knowledge, risks from misuse or abuse are not assessed (even denied) by JECFA in its evaluation of the hormones at issue. As was illustrated by the example of the experiments with Zilpaterol (question 45), GVP is very relevant to the establishment of MRLs by JECFA. If other substances (like zilpaterol or ZMA etc...) or incorrect use of implants are used the principle of the establishment of MRLs by JECFA is certainly invalid.

47. How significant are any differences in GVP in the European Communities, the United States, and Canada? Does the EC risk assessment take into account relevant control mechanisms with respect to GVP in place in the United States and/or Canada? If so, what are their conclusions?

Dr. Boisseau

377. In my e-mail of 26/04/06, I have indicated that I did not think that I am in the position to answer this question. Nevertheless, I think that, as far as growth promoters are concerned, the main problem for the European Communities is that these products are, in the USA and Canada, sold over the counter without any veterinary prescription.

378. As, as it has already been said, the European Communities did not conduct any quantitative risk assessment for growth promoters, it is not possible to say that the European Communities took into account relevant control mechanisms with respect to GVPs in place in the USA and/or Canada.

Dr. De Brabander

379. The interpretation of the rules of GVP can be different from country to country. Concerning the dispute on hormones THE difference is of course that in Europe the use of hormones as production aids is not allowed (and thus not included in GVP).

380. As to the relevant control mechanisms with respect to GVP in place in the United States and/or Canada: any control mechanism, that is only based on audits and paper work will not prevent farmers to use either uncorrect use of legal production aids either the use of other illegal growth promoters which are readily available in the US and Canada through the internet.

381. An example: on 2006-17 April 21, 2006 (thus very recently) Health Canada advises consumers not to use unauthorized products containing anabolic steroids. http://www.hc-sc.gc.ca/ahc-asc/media/advisories-avis/2006/2006_17_e.html

382. OTTAWA - Health Canada is advising consumers not to use five products containing illegal anabolic steroids, as they can potentially cause serious health issues such as liver disorders and heart problems. The five products are: Anabolic Xtreme Superdrol, Methyl-1-P, Ergomax LMG, Prostanazol and FiniGenX Magnum Liquid. They are not authorized for sale in Canada as either drugs or natural health products. Canadians using any of these products or any other supplements containing anabolic steroids are advised to stop taking these products immediately and consult with a health care professional.

383. All these products are available through the Internet, maybe mostly for use in bodybuilding but they can also be used in cattle fattening.

384. Are there methods and laboratories to control the abuse of these substances in the US and Canada?

385. Note that "Health Canada" (correctly) recognises the danger of these substances.

48. To what extent does the scientific evidence referred to by the European Communities assess risks to human health from residues of misplaced implants or improper administration, i.e. when administered differently than indicated on the label of the manufacturer or contrary to GVP, of any of the six hormones? Would your reply have been different at the time of adoption of the EC Directive in September 2003? What are the potential hazards, if any, to human health of the use of large quantities, or doses higher than recommended, of any of the six hormones in dispute?

Dr. Boisseau

386. Once again, the reply given to the question No 45 applies also to this question. The outcome of a risk assessment of veterinary drug residues, including the six hormones at issue, cannot apply in the case of misuse/abuse such as use of dosages higher than those approved, use of not approved combinations, repeated administrations, deep intramuscular injections instead of implantation of pellets etc...In this respect, the European Communities is right to state that, in case of these different misuses/abuses, the exposure of consumers may be totally different. Once again, this situation is not specific to hormones as it applies also to all the veterinary drugs already assessed by JECFA, EU, USA or anywhere else in the world.

387. Having said that, as the European Communities did not conduct any quantitative risk assessment for growth promoters, it is not possible to say that the scientific evidence referred to by the European Communities assesses the risk to human health from residues resulting from these misuses/abuses.

388. My reply would not have been different at the time of adoption of the EC directive in September 2003.

389. ADIs are established in order to prevent the toxicological/physiological effects, associated with the corresponding NOAEL, to occur. In case of misuses/abuses, the exposure of consumers to residues may increase to such an extent that the intake of these residues may exceed the established ADI and the toxicological/physiological effects intended to be avoided may occur.

Dr. Boobis

Assessment of risk to human health from misuse or abuse of the hormones in the scientific evidence referred to by the EC

390. The evidence itself is discussed in detail under question 62 below. There was no attempt to evaluate the risks from the resultant exposures on misuse or abuse, either in the papers cited or by the SCVPH (2002) in their evaluation of these studies. Indeed, the SCVPH (2002) simply noted that "Therefore, these data have to be considered in any quantitative exposure assessment exercise", without undertaking such an exercise. However, it should perhaps be pointed out that the EC had previously taken the view that there was no threshold for some of the critical effects of the hormones and that it was therefore not appropriate to conduct a quantitative risk assessment (*SCVPH, 1999*). The SCVPH did not change their view on this in their 2002 evaluation of the new information.

391. My reply would not have been different at the time of adoption of the EC directive in September, 2003.

392. In my view, the potential hazards from the use of large quantities of the six hormones in dispute are those dependent on their endocrine activity, including cancer in hormonally responsive tissues. However, I should stress that this is their potential hazard. The potential risk, i.e. the probability that effects would occur, would depend on a number of factors. These include the magnitude of the exposure, the duration of the exposure and the life stage of the exposed individual. From the range of exposures likely from anticipated misuse or abuse the risks are likely to be very low (see question 62).

Dr. De Brabander

393. The answer to this question is already given in question 47. Improper administration of implants or misplaced implants create potential hazards to human health. Moreover, as already mentioned the potential hazards to human health are not the only factor in the debate of the use of hormones as production aids. There are also:

- animal welfare: how do animals feel at improper administration of implants or misplaced implants?
- the environment: excretion of an excess of hormones at improper administration of implants or misplaced implants disturbs the hormonal balance in surface water.
- transformation of hormones: enzymatic reactions are equilibrium reactions (the enzyme is just a catalyst for the reaction); excess of hormones can drive a enzymatic reaction in another direction (e.g. formation of boldenone out of testosterone). Little is still known about this phenomenon.

394. If my reply would have be different at the time of adoption of the EC Directive in September 2003 is a difficult question. Fact is that my reply now is certainly different: more and more scientific data sustain the ban on the use of hormones: the economical profits resulting from using hormones do not balance the potential danger in all of its aspects.

49. What analytical methods, or other technical means, for residue detection in tissues exist to control the use of the six hormones in dispute for growth promotion purposes in accordance with good animal husbandry practice and/or good veterinary practice? What tools are available to control the use by farmers of the six hormones in dispute for growth promotion purposes in accordance with good animal husbandry practice and/or good veterinary practice?

Dr. De Brabander

395. There are a large number of analytical methods available to control the use of the six hormones in dispute for growth promotion purposes. New methods are regularly presented in international conferences and in the open literature. In Europe a system of community reference (CRL) and national reference laboratories (NRL) is installed so that the analysis carried out by the field laboratories are kept up to the standards of the moment. If necessary I can provide the panel with a large number of methods but I don't think that is the purpose.

396. To control the use by farmers of the six hormones in dispute for growth promotion purposes a number of tools can be used:

- Analysis for legal and illegal hormones in urine, faeces, hair, animal feed and drinking water.
- Audit of the farms on the presence of legal and illegal substances in all kind of formulations.
- Control of the weight gain of the animals in the farm in comparison with normal weight gains.
- Inspection of the herd on certain symptoms (e.g. hypothyreosis).
- Inspection of the animals at slaughter (e.g. for injection sites).

50. Are there other measures available to the European Communities (other than a complete ban) which could address risks arising from misuse and failure to follow good veterinary practice with respect to the use of the hormones at issue for growth promotion purposes? Would your reply have been different at the time of adoption of the EC Directive in September 2003? If so, why?

Dr. Boisseau

397. In my e-mail of 26/04/06, I have indicated that I did not think that I am in the position to answer this question.

398. Nevertheless, I would like to say that, in a given country, the implementation of GVPs and the elimination of misuses/abuses of veterinary drugs must be the responsibility of the official authorities of this country which should be able to demonstrate that (1) veterinary drugs are effectively used in compliance with GVP, (2) veterinary drugs associated with sensitive public health issues are used under veterinary control, (3) the official controls, including surveillance of residues, of the implementation of these GVPs are efficient. In the case where it can be established that an exporting country is not in the situation to guarantee that veterinary drugs are effectively used in compliance with GVP, any importing country should have the freedom to take any appropriate measure likely to protect the health of its population. Ban is the last possible measure if all the other options have failed or have been proved ineffective. In the case of hormonal growth promoters, due to the temptation for the farmers to use these products in a way different from the approved ones in order to expect some more economical profit, an agreement should be made between an exporting and an importing country about the content of GVP regarding the use of these products, including a possible involvement of veterinary supervision/prescription and an appropriate scheme of residue surveillance. In addition, if legally authorised and technically feasible, an appropriate information of the consumers of the importing country through a clear labelling could be considered as there is nowadays an increasing demand from consumers about the tracking of food with an informative labelling even concerning legally approved food additives.

399. My reply would not have been different at the time of adoption of the EC directive in September 2003.

Dr. De Brabander

400. To my knowledge, there are no other measures possible to the European Communities, other than a complete ban, which could address risks arising from misuse and failure to follow good veterinary practice with respect to the use of the hormones at issue for growth promotion purposes.

401. My reply now is even stricter than it would have been at the time of adoption of the EC Directive in September 2003.

51. Does the material put forth by the European Communities regarding misuse or abuse of the hormones at issue in the United States and Canada call into question the potential

applicability of Codex standards with regard to imports of meat from cattle treated with hormones from the United States and Canada?

Dr. Boisseau

402. The replies given to the questions No 45 and 48 apply also to this question.

Dr. De Brabander

403. The material put forth by the European Communities regarding misuse or abuse of the hormones at issue in the United States and Canada calls indeed into question the potential applicability of Codex standards with regard to imports of meat from cattle treated with hormones from the United States and Canada. Since the "older" experiments on which the MRLs (for the 3 synthetic hormones) and the ADIs (for the 3 natural hormones) are based, the scientific knowledge on residues, their link with animal welfare and the impact on the environment has increased considerably. These items are already discussed in the answers on other questions and show clearly that the economical profit of using hormones as production aids doesn't balance the present and potential hazards. Moreover, most consumers aren't prepared to take this risk.

404. Regularly new findings on the residue domain are published. As example, the findings in our own laboratory with maggots of *Lucilia Sericata*, a blowfly. We were able to demonstrate that maggots of *Lucilia Sericata*, when exposed to the hormone are able to convert testosterone into boldenone (2.2 %), boldione (or ADD, 1 %) and AED (15 %).

Boldenone formation by maggots of Lucilia Sericata K. Verheyden, H. Noppe, J. Vercruyss, E. Claerebout, V. Mortier, C.R. Janssen, H.F. De Brabander Anal. Chim Acta 2006, submitted

405. This is only one example of a number of still unknown reactions and illustrates that it is dangerous to introduce substances which may disturb the equilibrium of enzymatic reactions in the body of an animal or a human being (in this case equilibrium of hormones). I have serious doubts that in all the "old" studies which have lead to the establishment of ADI's and MRL's for these substances, these and analogous reactions are taken into account and for instance boldenone en ADD are measured.

[For questions on GVP see the SCVPH Opinions in Exhibits US-1,4, and 17, para.125-127 of EC Rebuttal Submission (US case), paras. 107-109 of EC Rebuttal Submission (Canada case), para. 154 of EC Replies to Panel Questions, Exhibits EC-12, 67, 68, 69, 70, 73, 96, 102, 103, paras. 32 and 54-65 of US Rebuttal Submission, para. 75 of US First Submission, paras. 107-111 of Canada Rebuttal Submission, page 40 of Exhibit CDA-27]

(g) Other

52. **Do the risk assessment of the European Communities or any other scientific materials referred to by the European Communities demonstrate that a potential for adverse effects on human health arises from the consumption of meat from cattle treated with any of the six hormones in dispute for growth-promotion purposes? If yes, why? If not, what kind of evidence would be required to demonstrate such potential adverse affects? Would your response have been different at the time of adoption of the Directive in September 2003?**

Dr. Boisseau

406. As already said in the reply to the question No 16, the European Communities did not carry out, strictly speaking, a risk assessment but provided scientific data and hypothesis supporting its worries regarding the safety of these six hormones for human health. Therefore, the European Communities concluded that, given the genotoxic potential of oestradiol-17 β , it is not possible to accept any excess intake of residues of this hormone as they are likely to raise a problem of health for consumers and that the available data for the five other hormones were not sufficient to carried out a risk assssment. For the three natural hormones, the European Communities should have integrated in its risk assessment the exposure of consumers to these hormones resulting from the consumption of hormone residues from animals which have not been treated by hormonal growth promoters and the from the daily production of these hormones by humans. So, to my view, the European Communities did not demonstrate that a potential for adverse effects on human health arises from the consumption of meat from cattle treated with any of the six hormones in dispute for growth promotion purposes. The kind of evidence required to demonstrate such potential adverse effects should be (1) toxicological data indicating that the values of the ADIs established by JECFA are not conservative enough, (2) data on residues in treated/non treated cattle and on daily production of hormones in sensitive individuals indicating that the hormonal residue intake associated with the consumption of meat from treated cattle is such that the established ADIs would be exceeded in the case of use of growth promoters.

407. My reply would not have been different at the time of adoption of the EC directive in September 2003.

Dr. Boobis

Risk to humans from consumption of meat from animals treated with growth promoting hormones

408. This is a complex question to address. A risk assessment involves some interpretation of data. For example, whether an effect is compound-related, whether an effect is adverse, the location of any threshold, whether effects seen in vitro are apparent in vivo, whether associations reported in epidemiological studies might be subject to bias or confounding (*IPCS: EHC 70, 1987 and EHC 104, 1990; WHO, 1996 and 2001*). Hence, whether a risk assessment demonstrates that a potential for adverse effects on human health arises from consumption of meat from cattle treated with any of the six hormones in dispute depends on those conducting the risk assessment. However, whether the scientific information on which this risk assessment was based, or any other materials referred to by the EC demonstrate such potential is another question. In my view, none of information provided by the EC demonstrates the potential for adverse effects in humans of any of the six hormones in meat from cattle in which they are used for growth promotion purposes at the levels to which those consuming such meat would be exposed. The studies on genotoxicity provide no convincing evidence of potential for harm in consumers. The weight of evidence is that the hormones are not genotoxic in vivo even at doses well above those that would be present in meat from treated cattle. The carcinogenic effects observed are entirely consistent with a hormonal mode of action that exhibits a threshold that would be well above the intake arising from consumption of meat from treated cattle. Other effects of the hormones that have been observed either in experimental animals or in exposed subjects occur at doses much higher than those to which consumers would be exposed via meat from treated cattle. As such, there would be no risk of such effects in humans from such exposures. There is much debate about the possible endocrine effects of low dose exposures to hormones such as oestradiol. However, all of the major reviews in this topic have concluded that whilst there are data gaps, there is no evidence that low level exposure is causing harmful effects in humans.

409. "Analysis of the human data by itself, while generating concerns, has so far failed to provide firm evidence of direct causal associations between low-level (i.e., levels measured in the general population) exposure to chemicals with EDCs and adverse health outcomes" (*Damstra et al, 2002*)

Damstra, T., Barlow, S., Bergman, A., Kavlock, R., and Van der Kraak, G. (2002). Global Assessment of the State-of-the-Science of Endocrine Disruptors. WHO publication no. WHO/PCS/EDC/02.2. World Health Organization, Geneva, Switzerland.

410. "However, it is somewhat reassuring that after substantial research in the past decade, there have been no conclusive findings of low-level environmental exposures to EASs [endocrine active substances] causing human disease" (*Miyamoto and Burger, 2003*).

411. The question on what sort of evidence would be required to demonstrate such potential adverse effects presupposes that they are demonstrable. In order to demonstrate whether or not such effects occur it would be necessary to conduct studies of human systemic exposure from consumption of meat from treated cattle. Such studies would require very sensitive analytical methods, capable of establishing whether there is any change in circulating hormone levels in the first place. Studies would need to be carried out in relevant sub-populations, such as prepubertal males. Epidemiological studies, in which both exposure and outcome are carefully assessed would also be necessary. However, it should be emphasised that on the basis of the information available, I would rate the risk of adverse effects in humans consuming meat from treated cattle as minimal.

412. My response to this question would have been the same at the time of adoption of the EU Directive in September 2003.

Dr. Guttenplan

413. Calculations are presented (EC Rebut, para 122) that suggest that even with low percentages of bioavailability of estrogen, the levels in meat could result in bioavailable estrogen exceeding the daily production rate of oestradiol in pre-pubertal children (EC Rebut, para 122). Although the US and Canada question the accuracy of the assay originally employed for estrogens at the low levels found in children, recent reports (Wang, S., Paris, F., Sultan, C. S., Song, R. X., Demers, L. M., Sundaram, B., Settlage, J., Ohorodnik, S., and Santen, R. J. Recombinant cell ultrasensitive bioassay for measurement of estrogens in postmenopausal women. *J Clin Endocrinol Metab*, 90: 1407-1413, 2005 and references therein), indicate that more recently reported levels used by the EC are accurate. In addition, levels in post-menopausal women were also very low. However, other approximations are used in that calculation. For pre-pubertal children, even with the low bioavailability of estrogen along with and its low levels in meats, it appears possible that intake levels would be within an order of magnitude of those of the daily production rate. This is greater than FDA's ADI and suggests some risk to this population. If there genotoxic effects of estradiol in children, they may be reflected over a lifetime, as mutations arising from DNA damage are permanent. It seems the more accurate methods of analysis could now be used to measure the effect of eating hormone-treated beef on blood levels of estrogen in children and post-menopausal women. If practical, this experiment would be important in establishing or refuting the arguments of the EC.

414. My response would have been more uncertain in 2003, because the assay for serum levels of estrogens was less validated then.

53. Please comment on the statement by the European Communities that the natural hormones progesterone and testosterone are used only in combination with oestradiol-17 β or other oestrogenic compounds in commercial preparations? Would the systematic use of these and the synthetic hormones in combination have any implications on how the scientific experiments and the risk assessments are to be carried

out? If so, have the scientific materials referred to by the European Communities or relevant JECFA reports taken into account the possible synergistic effects of such combinations on human health? [see sections 4.2-4.3 of the Opinion of the SCVPH of 2002 in US Exhibit 1]

Dr. Boisseau

415. It is true, as it is written in the section 4.3. of the 2002 Opinion of SCVPH, that the data about genotoxicity of hormones have obtained from tests conducted only with individual substances as it has been always the case for all the toxicity studies considered everywhere in the risk assessment of veterinary drug residues. Considering that it has been established that progesterone and testosterone are not genotoxic, it is not likely that the testing of combinations of progesterone or testosterone with oestradiol-17 β would have led to synergistic effects compared with those obtained from these individual substances.

Dr. Guttenplan

416. From the data presented, it is true that progesterone and testosterone are used only in combination with oestradiol-17 β or other oestrogenic compounds in commercial preparations (sections 4.2-4.3 of the Opinion of the SCVPH of 2002).

417. In principle the use of mixtures should complicate risk assessments/scientific experiments, as they would have to evaluate/investigate each component alone and in combination. This is a major undertaking as effects of individual agents may be additive, inhibitory, and synergistic or there may no effect. It appears from the evidence submitted that, by far, estrogen is the major agent of risk and because the concentrations of all of the hormones in beef are so low, that they would be unlikely to affect the potency of estrogen. However, it appears that no experiments on effects of combinations were performed, so some uncertainty exists there.

54. What is the acceptable level of risk reflected in the Codex standards for the five hormones at issue? How does this compare to the European Communities' stated objective of "no risk from exposure to unnecessary additional residues in meat of animals treated with hormones for growth promotion". [see para. 149 of EC Rebuttal Submission (US case)]

Dr. Boisseau

418. The acceptable level of risk reflected in the Codex standards for the five hormones at issue is, as for all the other substances already assessed, expressed by the ADIs established for these substances. The spirit of the risk assessment procedure adopted by the Codex and implemented by JECFA is that the amount of residues expressed by an ADI represents the quantity of these residues which can be ingested daily by consumers over life time without causing any problem of health. This approach is obviously different from the European Communities' stated objective of "no risk from exposure to unnecessary additional residues in meat of animals treated with hormones for growth promotion" which implies, to be reached, that these five hormones should not be used. The idea supporting this statement is probably that growth promoters raise a specific problem regarding the benefit/risk assessment associated with their use. In the case of veterinary drugs used for therapy, the risk is expressed in term of human health and the benefit in terms of animal health and that can be considered as ethical. For the growth promoters, the benefit is "only" economical and for this reason, the European Communities may not accept any risk, even theoretical, resulting from the use of these growth promoters only intended to increase economical profits.

Dr. Boobis

Acceptable level of risk

419. The Codex standards for the hormones at issue represent "no appreciable risk with daily exposure over a lifetime" (Definition of ADI by WHO, 1996, 2001 and CVMP, 2005). This is based on the JECFA conclusion that all of the potentially adverse effects of the hormones have thresholds. By using a NOAEL or surrogate if necessary, such as the LOAEL, and appropriate uncertainty (or safety) factors, a level of exposure is determined up to which the risk is considered minimal, i.e. the ADI (see my replies to questions 7-12 above). The level of any residual risk has never been quantified but is considered to be acceptable to society. The Codex standard is equivalent to the EC's stated objective of "no risk from exposure to unnecessary additional residues in meat of animals treated with hormones for growth promotion", and indeed is the same as the CVMPs (2005) definition of ADI. This is because the EC uses the same risk assessment paradigm as JECFA for establishing ADIs and hence if the data are interpreted in the same way there should be no difference in the level of risk identified in the risk assessment. From a scientific perspective, the difference arises from differences in the way that the data are interpreted and in particular whether or not it is concluded that there is a threshold for the effects of concern.

420. However, the distinction between risk assessment and risk management must be stressed (see my reply to question 5 above). Whether to invoke the precautionary principle is a risk management decision. It is beyond the scope of my responses to go into this in detail. One input to risk management decisions is the output of the risk assessment, part of which is an evaluation of the uncertainty associated with that output. Risk management has to weigh the risk assessment and a number of other factors in reaching a conclusion. Hence, the issue is in part not so much the level of risk that is acceptable, but the level of concern should the risk estimates be incorrect.

Dr. Guttenplan

421. Codex has set ADI's for the hormones (except MGA, which, I couldn't locate) but states that MRL's are not necessary as meat from animals maintained with good animal husbandry practices would not be likely to pose a threat to human health (CDA 22, Codex list of standards). This is not in accord with the EC's stated objective. The EC maintains that for estrogen and possibly the other hormones, some potential risk exists. The question of what level of risk has not been addressed by the EC.

55. Do the Opinions of the European Communities or other scientific materials referred to by the European Communities evaluate the extent to which residues of growth promoting hormones in meat contribute to what the European Communities calls "additive risks arising from the cumulative exposures of humans to multiple hazards, in addition to the endogenous production of some of these hormones by animals and human beings"? Would your reply have been different at the time of adoption of the EC Directive in September 2003? If so, why? [see para. 151 of EC Replies to Panel Questions, paras. 43-44 of US Rebuttal Submission, paras. 83-85 of Canada's Rebuttal Submission]

Dr. Boisseau

422. The European Communities did not assess quantitatively the extent to which residues of growth promoting hormones in meat contribute to "additive risks arising from the cumulative exposures of humans to multiple hazards, in addition to the endogenous production of some of these hormones by animals and human beings". Besides the European Communities recognizes that in saying, in the para 151 of its reply to Panel questions that " it is not so much necessary to compare (if

it is only possible) the two situations (residues of hormones in meat from cattle not treated with growth-promoting hormones and residues in meat from cattle treated with growth-promoting hormones) and then try to quantify how much one is more risky than the other one". Therefore, it can be thought that the position of the European Communities is a position of principle. This position is influenced by risk management considerations and by the implementation of the so called precautionary principle.

423. My reply would not have been different at the time of adoption of the EC Directive in September 2003.

Dr. Boobis

Additive risks

424. Two different issues are represented here. One is so-called aggregate risk. This has been defined as "the likelihood of the occurrence of an adverse health effect resulting from all routes of exposure to a single substance" (*US EPA, 2001*). Hence, at issue is the extent to which exposure to the natural hormones present in meat from treated cattle aggregates with endogenous levels of the same hormone, and exposure to that hormone from any other source, such as in the form of a therapeutic agent.

425. The second is cumulative risk. This has been defined as "the likelihood of the occurrence of an adverse health effect resulting from all routes of exposure to a group of substance sharing a common mechanism of toxicity" (*US EPA, 2001*). Here, the issue is the extent to which compounds with similar effects should be cumulated, with each other and with other similar substances of either exogenous or endogenous origin.

US EPA Office of Pesticide Programs (2001). General Principles For Performing Aggregate Exposure And Risk Assessments (<http://www.epa.gov/pesticides/trac/science/aggregate.pdf>)

426. The EC Opinions and other materials referred to by the EC do not quantify the extent to which residues of the hormones contribute to aggregate exposures or cumulative exposures to multiple hazards. Aggregate risk where one of the major exposures is from a substance found endogenously, is most common for essential minerals and vitamins. However, here the exposures of concern are mainly exogenous, as for most vitamins and minerals there is no endogenous production (*see SCF, 2000*). However, there are a few exceptions, such as vitamin D. In this case, the *SCF (2002)* took into account the endogenous production rate in estimating a tolerable upper intake level. An important consideration was the extent to which exogenous exposure changed circulating levels of the active vitamin. This is somewhat analogous to the approach taken by *JECFA (2000b)* for the natural hormones, in that exposure was calibrated against doses that did or did not cause any change in circulating levels of the hormone.

SCF (Scientific Committee on Food) (2000). Guidelines of the for the development of tolerable upper intake levels for vitamins and minerals. SCF/CS/NUT/UPPLEV/11 Final (http://ec.europa.eu/food/fs/sc/scf/out80a_en.pdf)

SCF (Scientific Committee on Food) (2002). Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Vitamin D. SCF/CS/NUT/UPPLEV/38 Final (http://ec.europa.eu/food/fs/sc/scf/out157_en.pdf)

427. The issue of cumulative risk is more complex. Indeed, such considerations are currently applied in only very limited circumstances, for example dioxins and organophosphates. Within the EU, no such cumulative risk assessments have been undertaken routinely for any residues of

pesticides or veterinary drugs in food. Indeed, there is currently no agreement as to the appropriate methodology to use (see *EC, 2005b*) and such assessments do not appear to be foreseen in the near future for residues of veterinary drugs. Hence, risk assessments are performed on the individual compounds. However, it should be emphasized that any risk assessment group would deal with substances on a case by case basis, and the absence of agreed methodology would not necessarily preclude some consideration of cumulative risk, if this was deemed to be a major and immediate concern.

EC (2005b). Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC
(http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/oj/2005/l_070/l_07020050316en00010016.pdf)

428. The importance of both aggregate and cumulative risk would depend critically on whether or not there was a threshold for the dose-response relationship. One of the arguments of the EC is that hormones can cause cancer by a genotoxic mechanism which would have no threshold. If this were true, it is certainly correct that any additional exposure would have an incremental effect on risk, if one assume a linear no-threshold dose-response relationship. This would be the default under such circumstances. The incremental risk would depend on the extent to which the additional exposure from hormones in meat from treated cattle changed the overall exposure, with respect to the endogenous levels present. In contrast, for compounds with the same mechanism of action, if there were a threshold for all of the biological effects of concern, additional exposures would only be of concern when they resulted in a total potency-corrected exposure that was above the threshold (the ADI) (*Silva et al, 2002*).

429. My reply would have been the same at the time of adoption of the EC Directive in September 2003.

Silva E, Rajapakse N, Kortenkamp A (2002). Something from "nothing"--eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. Environ Sci Technol, 36:1751-1756

Dr. Guttenplan

430. In general the EC do not attempt to evaluate "the additive risks arising from the cumulative exposures of humans to multiple hazards, in addition to the endogenous production of some of these hormones by animals and human beings". However, as described in the answer to question 52, an estimated comparison has been presented of amount of estrogen contributed by consumption of meat from hormone-treated cattle and the amount normally produced in prepubescent children.

431. I am more comfortable with this estimation now, and then I would have been in 2003 because of improved analytical techniques.

56. Has JECFA/Codex considered in its risk assessment of the five hormones such "additive risks? Are there internationally recognized guidelines for conducting assessments of "additive risks"?"

Dr. Boisseau

432. JECFA/Codex considered in its risk assessment of the natural hormones such "additive risks" and concluded that, given the wide margin of safety between the maximum estimated intake of residues for the these hormones and the corresponding established ADIs, that there was no risk for consumers' health associated with the estimated ingestion of these residues. JECFA /Codex did not

consider such "additive risks" in its risk assessment of the synthetic hormonal growth promoters. To my knowledge, there is no internationally recognized guidelines for conducting assessment of "additive risks".

Dr. Boobis

Consideration of additive effects by JECFA/Codex

433. JECFA/Codex did consider aggregate risk from exposure to the natural hormones where present as residues in meat from treated cattle. Such exposures were considered to represent a trivial increase in overall exposure to hormonally-active material from other exogenous sources and in particular from endogenous sources (*JECFA, 2000*). JECFA/Codex did not use formal methodology to assess cumulative risk from exposure to the hormones. However, JECFA did consider that the dose-response curves for potential adverse effects from the hormones all exhibited thresholds and that there was a considerable margin of exposure for all of the hormones between the TMDI and the ADI. Hence, it was concluded that there would be no additional risk over background by exposure to any residues from meat from treated cattle. In a cumulative risk assessment consideration needs to be given to the exposure pattern. It is not appropriate to assume that exposure to each substance will be at the TMDI, as this would require chronic exposure to each hormone at the maximum possible level for all of them. To overcome such compounded conservatism in the evaluation, probabilistic approaches to exposure assessment have been used when performing a cumulative risk assessment (*US EPA, 2002*)

434. JECFA has developed specific methodology for performing a risk assessment of dioxins and related substances that share a common mechanism of action (*JECFA, 2002a*). *Ad hoc* approaches have been applied to a very limited extent to certain pesticide and veterinary drug combinations, from example when they share a common metabolite. However, there are no agreed international guidelines for conducting a full cumulative risk assessment. One of the difficulties is in obtaining representative consumption data for substances used in food production. Some of the issues involved, and the methodological approaches that have been developed have been reviewed by *Wilkinson et al (2000)*. However, it should be noted that these methodologies are applicable to compounds that share what has been defined as a common mechanism, i.e. there is a cumulative risk. There is no international agreement on how to undertake a combined risk assessment of compounds acting by the carcinogenic mechanisms suggested by the EC for the hormones, i.e. genotoxicity via direct or indirect interaction with DNA. If one were to assume no threshold for the effect, and apply ALARP, it would not be necessary to perform a cumulative risk assessment of such compounds.

JECFA (2002a). Safety Evaluation of Certain Food Additives and Contaminants: Polychlorinated Dibenzodioxins, Polychlorinated Dibenzofurans, and Coplanar Polychlorinated Biphenyls. WHO Food Additives Series: 48, WHO, Geneva

US EPA (2002). Organophosphate Pesticides: Revised Cumulative Risk Assessment (<http://www.epa.gov/pesticides/cumulative/rra-op/>)

Wilkinson CF, Christoph GR, Julien E, Kelley JM, Kronenberg J, McCarthy J and Reiss R (2000). Assessing the risks of exposures to multiple chemicals with a common mechanism of toxicity: how to cumulate? Regul Toxicol Pharmacol, 31:30-43

Dr. Guttenplan

435. I could find assessment of additive risks of the hormones in the documents, and I not aware of any internationally recognized guidelines.

57. **Canada comments that "one single molecule that the European Communities considers so dangerous from meat derived from animals treated with hormone growth promoters is suddenly not at all that dangerous when consumed from meat from animals treated for therapeutic or zootechnical purposes. The European Communities' concern about the genotoxic potential of oestradiol-17 β suddenly and inexplicably disappears." To what extent are hormone treatments of cattle for purposes other than growth promotion, such as for therapeutic or zootechnical purposes, taken into account by the European Communities, if at all, in its assessment of the cumulative effects from the consumption of meat containing residues of the hormones at issue? Would your reply have been different at the time of adoption of the EC Directive in September 2003? If so, why? [see para. 97 of Canada Rebuttal Submission; paras. 17-20 of US Opening Statement]**

Dr. Boisseau

436. The comment of Canada stating that "one single molecule ...inexplicably disappears" refers to the theoretical assumption that one single molecule of a genotoxic compound, coming in contact with the human genetic material, could be likely to damage it and therefore to induce a carcinogenic process. This worry supports to some extent the position of the European Communities regarding oestradiol-17 β . Therefore, starting from this theoretical and somewhat extreme assumption, Canada challenges the authorization, by the European Communities, of the use of oestradiol-17 β for therapeutic and zootechnical purpose. It has to be noted that, according to the directive 2003/74/EC, oestradiol-17 β can only be used for three precise therapeutic indications and only until 16/10/2006 for the induction of oestrus. The European Communities thinks that, given the conditions of these uses of oestradiol-17 β (limited number of treated animals, limited use in the life of these animals and very low probability to see these animals slaughtered after treatment), the exposure of consumers to oestradiol-17 β residues resulting from these uses can be considered as negligible. If this EC assumption can be accepted, it raises nevertheless a problem of principle as it represents an exception regarding the very strict position of EC stating that it is not possible to accept any increase of the exposure of consumers to oestradiol-17 β residues. As soon as the European Communities accepts to consider these residues resulting from these therapeutic and zootechnical use of oestradiol-17 β as negligible, it enters in a quantitative, or at least in a semi quantitative, exposure assessment procedure for these oestradiol-17 β residues and, starting from that, it has no good reason to object to consider a wider exposure assessment covering all the residues resulting from the different sources of oestradiol-17 β . This comment has already been made in my reply to question 31.

437. My reply would not have been different at the time of adoption of the EC directive in September 2003.

Dr. Boobis

Aggregate and cumulative exposure to hormones

438. As indicated above (in my reply to question 56), combined exposures to the same substance from more than one source has been described as aggregate exposure whilst exposure to more than one compound acting by the same mechanism has been described as cumulative exposure. To my knowledge no account is taken of hormone treatments of cattle for purposes other than growth promotion, such as for therapeutic purposes, by the EC in its assessment of the aggregate or cumulative effects of the hormones in meat from cattle treated for growth promotion. However, this reply needs some clarification. Firstly, the issue of aggregate and cumulative risk, as indicated above (in my reply to question 56), depends upon assumptions about the nature of the dose-response relationship. Second, though unlikely given the EC's views on the growth promoting hormones, the need for taking into account such other exposures would depend on how far exposure was considered

to be from any threshold, i.e. the margin of exposure. My reply would have been the same at the time of adoption of the EC Directive in September 2003.

Dr. Guttenplan

439. The EC does not really take into account hormone treatments of cattle for purposes other than growth promotion, such as for therapeutic or zootechnical purposes in their risk assessments. Their reasons are summarized in (EC rebut US sub. Para 114). "Exceptions to the ban on meat from hormone-treated cattle were made only for the use of certain of these substances for zootechnical and therapeutic purposes where no viable effective alternatives appeared to exist. This exception was based on the assessment that owing to the nature and limited duration of the treatments, the limited quantities administered and the strict conditions imposed to prevent misuse, that this use did not constitute a hazard for public health." This is a reasonable response. My response would not have been different in 2003.

58. Please comment on the EC statement in para. 94 of the EC Replies to Panel Questions that "the only rationale that can be inferred from the available scientific data is that the higher the exposure to residues from these hormones, the greater the risk is likely to be", taking into account para. 105 of Canada Rebuttal Submission.

Dr. Boisseau

440. My reply to question 55 applies to this question as well.

Dr. Boobis

Dependency of risk on dose

441. Again, this depends critically upon the conclusion regarding the nature of the dose-response relationship. JECFA has concluded that there was a threshold for all of the potential adverse effects of the hormones and that it was possible to establish ADIs. Hence, as indicated above, exposure below the ADI is considered to be without appreciable risk. Estimated exposure from consumption of meat from treated cattle would result in hormone intakes that were well below the respective ADI's, in the case of oestradiol its intake would represent only 1.5% of the ADI (*JECFA 2000a, b*). Hence, within quite broad limits, higher exposure would not result in any increase in risk. This would be the case until the ADI was exceeded. It should also be noted that for the critical endpoints of concern in the JECFA evaluation, including cancer, the risk would be significant only with prolonged exposure (*Coombs et al, 2005*). Hence, occasional exposure even above the ADI would be considered to pose no appreciable risk.

Dr. Guttenplan

442. This is indeed a very weak statement by the EC. However, the alternative would be to suggest a risk that might be wildly inaccurate, due to the limitations imposed by the lack of solid data on levels of hormones in meat. Perhaps a better approach would have been to suggest several scenarios. These could be validated or disproved by subsequent studies.

59. Does the scientific evidence referred to by the European Communities identify any adverse effects on the immune system from the consumption of meat from cattle treated with the growth promoting hormones at issue? Would your reply have been different at the time of adoption of the Directive in September 2003? If so, why? [see para. 132 of Canada Rebuttal Submission]

Dr. Boisseau

443. The scientific evidence referred to by the European Communities allows to identify adverse effects of hormonally active substances on the immune system. Nevertheless, as these data have not been used by the European Communities to conduct any quantitative risk assessment likely to establish, for these effects associated with the hormonal properties of growth promoters, thresholds and ADIs different from those proposed by JECFA, it is not possible to conclude that this scientific evidence allows to identify any adverse effects on the immune system associated with the consumption of meat from cattle treated with the growth promoters at issue.

444. My reply would not have been different at the time of adoption of the EC directive in September 2003.

Dr. Boobis

Effects on the immune system

445. The evidence on immune effects of hormones such as oestradiol referred to by the EC does not identify any adverse effects on the immune system from consumption of meat from treated cattle. In general, clear evidence for immune effects were observed only at high doses. There is no evidence that doses such as those resulting from consumption of meat from treated animals have any effect on the immune system (JECFA, 2000b; CVMP, 1999). It should also be noted, that in the case of immune effects, exposure relative to endogenous levels is a critical issue. Given the large margin of exposure on anticipated intake from residues in meat from treated animals, no effect on the immune system is anticipated, as immune modulation is dependent on dose and there are thresholds for such effects (Barton and Clewell, 2000; Kroes et al, 2004).

446. My reply would not have been different at the time of adoption of the EC Directive in September 2003.

Barton HA and Clewell HJ 3rd (2000). Evaluating noncancer effects of trichloroethylene: dosimetry, mode of action, and risk assessment. Environ Health Perspect, 108 (Suppl 2):323-334

Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, van Schothorst F, Vos JG and Wurtzen G; European branch of the International Life Sciences Institute (2004). Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. Food Chem Toxicol, 42:65-83

Dr. Guttenplan

447. The relationship between estrogen and autoimmune diseases has received considerable attention (Opinion SCVPH, April 30, 1999, section 2.4). There is evidence that estrogens can be involved in Lupus, rheumatoid arthritis, thyroiditis. In addition the development of allergies is thought to be at least partially related to estrogens. The studies in experimental animals also did not identify any immune-related effects, although it is not certain the types of possible effects in humans would be detected in experimental animals. No definitive studies have related intake of meat from hormone-treated animals to the above disorders.

448. My reply would not have been different in 2003.

60. Does the scientific evidence referred to by the European Communities identify and evaluate whether there is a difference in terms of potential adverse effects on human health from the consumption of meat from cattle treated with hormones for growth

promotion purposes when these hormones are administered as feed additives (MGA) or implanted? Are you aware of any differences?

Dr. Boisseau

449. I do not understand this question as MGA is only used as feed additive and the five other hormones are not used as feed additive. Regarding the exposure assessment, the risk is potentially higher with implanted growth promoters as there are more "options" in terms of misuses/abuses. Nevertheless, to this respect, the scientific evidence referred to by the European Communities does not identify and evaluate whether there is a difference in terms of potential adverse effects on human health from the consumption of meat from cattle treated with hormones for growth promotion purposes when these hormones are administered as feed additives or implanted.

Dr. Boobis

Relevance of method of use of hormones

450. The scientific evidence referred to by the EC does not identify any difference in terms of potential adverse effects on human health from the consumption of meat from cattle treated with hormones for growth promotion purposes and when they are administered as feed additives or implanted. I am not aware of any such differences. None would be expected, as in all cases maximum intake would be well below the ADI. However, there is a situation which, at least hypothetically, could give rise to a difference in effects. This would be the misuse or abuse of the compounds when used as growth promoters, giving rise to increased intake because of a misplaced implant or entry into the food chain of tissue containing the implant (i.e. cow's ear). However, whilst this would lead to increased exposures, it is still unlikely this would exceed the ADI, and certainly not for any period of time. It is also an unlikely occurrence in view of the way in which the hormones are used and controlled.

Dr. Guttenplan

451. This question appears not to be specifically addressed, but MGA is the only hormone which might be administered by both methods. The potential for excessive exposure to MGA exists by both routes (oral and implantation), but it cannot be stated and I am not aware of which route is more likely to contribute to high levels in meat (SCVPH, section 4.1.4).

61. In your view and in the light of information provided by the parties as well as the work undertaken at JECFA and Codex, did the the scientific evidence available to the European Communities at the time it adopted its Directive (September 2003) allow it to conduct an assessment (quantitatively or qualitatively) of the potential for adverse effects on human health arising from the consumption of meat from cattle treated with (a) progesterone; (b) testosterone; (c) trenbolone; (d) zeranol; and (e) melengestrol acetate? Would your response differ in light of the scientific evidence provided which is subsequent to the adoption of the Directive?

Dr. Boisseau

452. It is difficult to reply to this question as I don't really know what were the data available to the European Communities at the time it adopted its directive (September 2003). On the other hand, it is always possible to ask for more data in order to clarify more issues so that the willing to eliminate any scientific uncertainty could result in an endless assessment process.

453. My reply would not have differed in light of the scientific evidence provided which is subsequent to the adoption of the EC directive in September 2003.

Dr. Boobis

Availability of data for risk assessment prior to September 2003

454. In my view there was sufficient information available to the EC to have enabled it to have conducted an assessment of the risks to human health arising from consumption of meat from cattle treated with any of the six hormones at issue (see comments above for details of the basis of this response). My reply would not have been different at the time of adoption of the EC Directive in September 2003.

Dr. Guttenplan

455. The evidence does indicate that potential adverse effects exist for all of the hormones. However, the ability to make a risk assessment (qualitative or quantitative) does vary between compounds.

456. Progesterone, testosterone have been extensively investigated and the assessment seems sound and is based on the no effect level and a safety factor. (JECFA meeting 52, report-WHA TRS 893).

457. There is more limited evidence available for Trenbolone and Zeranol and most of it is *in vitro* (SCVPH 2002 Opinion) or not recent (e.g., JECFA meeting 34th report, 1989 and 32nd report, 1988). However, both appear to be potentially significantly estrogenic. Experimental and analytical methods have improved but it does not appear that accurate ADI's can be established at this point. Studies in experimental animals and studies on levels in beef are still needed. However, from the data available at the time of the Directive, the potential for adverse effects could not be ruled out.

458. Melengestrol acetate. The assessment for melengestrol acetate seems sound. Thorough metabolic and estrogenic studies have been carried out. Actual levels in beef were not provided. (JECFA 62 FNP 41/16).

459. My opinion would not have been different in 2003.

62. Does the scientific evidence relied upon by the European Communities support the EC contention that the new scientific studies that have been initiated since 1997 have identified new important gaps, insufficiencies and contradictions in the scientific information and knowledge now available on these hormones such that more scientific studies are necessary before the risk to human health from the consumption of meat from cattle treated with these hormones for growth promotion purposes can be assessed? Would your reply have been different at the time of adoption of the Directive in September 2003? If so, why?

Dr. Boisseau

460. The scientific evidence relied upon by the European Communities has certainly provided new interesting data potentially useful for conducting the risk assessment of growth promoter residues. For all that, these new data do not demonstrate any important gaps, insufficiencies and contradictions in the scientific information used by JECFA for conducting its risk assessments. In order to decide whether or not more scientific studies are necessary before the risk to human health from the consumption of meat from cattle treated with these hormones for growth promotion purposes

can be assessed, it should be necessary to conduct a temporary risk assessment with these new data in order to see to what extent the conclusions of this temporary risk assessment is significantly different from those already performed by JECFA. If it would be the case, it would be necessary, at this time, to identify the additional studies necessary to carry out in order to clarify all the outstanding issues and to complete the risk assessment of the residues of hormones used as growth promoters.

461. My reply would not have been different at the time of adoption of the EC directive in September 2003.

Dr. Boobis

Additional information provided by the studies initiated since 1997

Analytical techniques and bioassays for screening

462. The studies on developing improved analytical methods were considered by the SCVPH (2002) to have been inconclusive.

463. "Despite a number of positive analytical results in this study, the low number of samples does not allow a qualified validation of typical characteristics such as sensitivity, specificity, accuracy and reproducibility (study 1, study 8)." I agree with this conclusion.

464. "The obtained results suggest that the use of recombinant yeast and rainbow trout hepatocytes to detect oestrogenic compounds is not justified in view of their lack of sensitivity" (study 9). I agree with this conclusion. It is of note that despite the use of similar strategies, the limit of sensitivity of the yeast reporter assay used by Le Guevel and Pakdel (2001) in study 9 was substantial less than that of Klein et al (1994).

Bovine metabolism of oestradiol-17 and oestrogenic potency of residues

465. One of the potentially relevant observations was the finding of oestradiol fatty acid esters produced in cattle following treatment (study 3). The study of Hoogenboom et al (2001) showed that the intrinsic oestrogenicity of these esters was much lower than that of oestradiol, by 25-200 fold. Paris et al (2001), have shown that most likely due to kinetic differences, the oestrogenicity of the fatty acid esters in vivo is up to 10-fold greater than that of oestradiol. However, it is apparent that the difference in potency from the parent hormone is not very great or even apparent at low doses, where effects were minimal. Given these findings and that the esters are not the major residue in meat from treated animals, that in some tissues such as muscle, levels are much lower than those of oestradiol (Maume et al, 2001), and that total exposure will be very low (JECFA, 2000), particularly when the balance of the diet is considered, these findings do not raise additional concerns regarding the potential adverse health effects of the hormones when used to treat cattle. An additional point to note is that in all of the studies cited above on the fatty acid esters of oestradiol, concentrations were expressed in units of mass per litre or per kg. However, as only the oestradiol moiety is hormonally active (Hoogenboom et al, 2001), this tends to overestimate potency relative to that of oestradiol by a factor of 2-fold (due to the difference in molecular weight relative to that of oestradiol).

Maume D, Deceuninck Y, Pouponneau K, Paris A, Le Bizec B and Andre F (2001). Assessment of estradiol and its metabolites in meat. APMIS, 109:32-38.

Paris A, Goutal I, Richard J, Becret A and Gueraud F (2001). Uterotrophic effect of a saturated fatty acid 17-ester of estradiol-17beta administered orally to juvenile rats. APMIS, 109:365-375

Multiple implanting, multiple dosing

466. In study 5, the impact of misuse and multiple dosing on residual hormone levels in meat was determined. Dosing at up to 10 times the approved dose, resulted in an increase in the tissue concentrations of some hormones in some tissues to values above the MRL for those hormones for which Codex has established an MRL. For those hormones for which an MRL has not been specified, there were also increases in some tissues (Lange et al, 2001).

467. "Treatment with zeranol and testosterone propionate, even after multiple application, does not cause any problems, as far as infringement of threshold levels is concerned."

468. "Exceeding of the MRL was found in the liver in one out of two animals after 3-fold and in two out of two animals after 10-fold dose of the 200 mg trenbolone acetate-implant". No exceedances were seen in muscle, kidney or fat, even at 10-fold the approved dose.

469. For oestradiol, the maximum increase observed in any tissue was not greater than proportional to the dose applied. Hence, even at 10-fold the approved dose, intake would be well below the ADI. This would be offset by the fact that not all tissues had such elevated levels, and the probability of consuming such high residue levels of a regular basis is minimal. It should also be noted that Codex did not specify an MRL for oestradiol, as it was considered unnecessary.

Lange IG, Daxenberger A and Meyer HH (2001). Hormone contents in peripheral tissues after correct and off-label use of growth promoting hormones in cattle: effect of the implant preparations Filaplix-H, Raglo, Synovex-H and Synovex Plus. APMIS, 109:53-65

470. In the study on misplaced implantation sites (Daxenberger et al, 2000), substantial residual hormone was sometimes found at the implantation site when this was not as recommended. However, for these findings to have significance for the consumer a number of factors need to be considered. These include the likelihood of off-label use of the hormones, the failure to detect the implantation site, the use of the implantation site for food use, the contribution of the contaminated meat to the diet and the frequency of such contamination. No data have been presented on the prevalence of such significant contamination as a consequence of the veterinary use of the hormones. Indeed, no evidence is presented that such misuse does occur with the consequences suggested by the authors.

Daxenberger A, Lange IG, Meyer K, Meyer HH, Daxenberger A, Lange IG, Meyer K and Meyer HH (2000). Detection of anabolic residues in misplaced implantation sites in cattle. JAOAC Int, 83:809-819

471. In studies on MGA (Daxenberger et al, 1999) tissue levels increased with dose, most markedly in fat. Whilst in fat, there was a roughly proportional increase with dose, in other tissues (muscle, kidney, liver) the fold-increase was appreciably less than the fold-increase in dose. Using the values obtained in the study of Daxenberger et al (1999) at 10 times the maximum approved dose, consumption of all four tissues (liver, kidney, fat and muscle) at the JECFA levels (300 g muscle, 100 g liver, 50 g kidney and 50 g fat per day) would result in a slight exceedance of the ADI (2.5 µg cf 1.8 µg). However, it should be noted that this would require all of the tissues to be from animals treated with the high dose, and exposure would have to be over a prolonged period of time. The probability that this would occur is extremely low.

Daxenberger A, Meyer K, Hageleit M and Meyer HH (1999). Detection of melengestrol acetate residues in plasma and edible tissues of heifers. Vet Q, 21:154-158

472. In risk assessment amongst the objectives is to determine whether it is possible to set health based guidance values (e.g. ADI) and upper levels for exposure (reference values or MRLs). If

possible and necessary, such values should be established. MRLs are established following use of the drug according to Good Veterinary Practice (GVP; also Good Practice in the Use of Veterinary Drugs, GPVD). This is the policy of all agencies and organisations involved in such activities (*EEC, 1990; EC, 2005b; FAO, 2006*). An additional question that may be asked is the consequence of abuse or misuse. However, such inappropriate activity cannot be used as the basis for establishing MRLs. This is because whilst use according to GVP can be foreseen and regulated, it is not possible or appropriate to regulate any conceivable misuse or abuse, whether actual or hypothetical. Normally, the risk management strategy to deal with this is to ensure adequate surveillance of residues and to put in place a system of penalties for violation. This is the situation for veterinary drugs in all regions where they are subject to market authorisation, including the EU and the USA. In assessing the risks from abuse or misuse, from a human health perspective, the concern is whether the consequent residues would result in exposure that exceeded the ADI. Exceedance of the MRL or other nominal tolerance level for residues has implications for detecting abuse or misuse in surveillance programmes, but has no direct link with whether there is a health concern or not. For example, residue levels from the use of zeranol according to GVP would result in TMDIs below the ADI. MRLs were established accordingly, so that there is a margin by which the residues can exceed the MRLs, yet intake will still be no greater than the ADI (*JECFA, 1988a, b*).

473. In considering the consequences of abuse and misuse, the following aspects need to be considered:

- The likelihood of violations or off-label use
- The residue levels occurring after such misuse or abuse
- The extent to which exposure to such residues will result in an exceedance of the ADI
- The likely frequency or period over which the ADI will be exceeded
- The acute consequences of exceeding the ADI
- The severity of the endpoint upon which the ADI is based
- The steepness of the dose-response curve for the endpoint upon which the ADI is based

474. Taking account of all of these factors, the data generated by the EU research in question do not provide any indication that it is not possible to conduct a risk assessment of the hormones used as growth promoters. Nor do they provide any indication that even such misuse or abuse as investigated gives rise to undue risk from the resultant residues, as intake would only very rarely exceed the ADI and then only on a rare occasion.

Alteration of gene expression by oestrogenic compounds

475. The study referred to (study 17), reported in *Leffers et al (2001)*, showed that a number of oestrogenic compounds affected the expression of several genes in the ER α positive breast cancer cell line, MCF7. The responsiveness of this cell line to oestrogens is well established. It was of interest that all of the changes reported by *Leffers et al (2001)* were blocked by the selective ER α antagonist ICI82.780. The relevance of effects observed in a cultured cell line to the situation *in vivo*, where kinetic and metabolic factors will influence the magnitude of the response is not known, nor is the significance of changes in gene expression to the toxicity of the hormones known. Many of the changes will reflect the proliferative response to an oestrogenic stimulus. However, in general toxicogenomic data, in the absence on any information on the functional consequences, is not considered a sound basis for use in risk assessment (*IPCS, 2003*).

IPCS (2003). Toxicogenomics and the Risk Assessment of Chemicals for the Protection of Human Health (<http://www.who.int/entity/ipcs/methods/en/toxicogenomicssummaryreport.pdf>)

Leffers H, Naesby M, Vendelbo B, Skakkebaek NE and Jorgensen M (2001). Oestrogenic potencies of Zeranol, oestradiol, diethylstilboestrol, Bisphenol-A and genistein: implications for exposure assessment of potential endocrine disrupters. Hum Reprod, 16:1037-1045.

Recent findings on the mutagenicity and genotoxicity and of oestradiol-17 β

476. The recent reports (study 3 and study 8) on mutagenicity confirm that oestradiol can produce a genotoxic response in vitro in certain tests. Evidence was obtained that this was due, at least in part, to the formation of reactive oxygen species. Much of the work was undertaken with relatively high concentrations of metabolites added exogenously. For some of the genotoxic endpoints, oestradiol and the metabolites tested were negative. In one of the cited studies (*Chakravarti et al, 2001*) the effects of the 3,4-quinone metabolite were investigated in vivo. A relatively high dose was administered to mouse skin. Whilst mechanistic information of value can be obtained using a route other than the one of concern (i.e. oral for dietary residues), in this case there is concern that kinetic differences, particularly in the disposition of a quinone metabolite, make interpretation of the findings difficult. In addition, the mechanism for genotoxicity observed was not established, and the authors acknowledge that this could have been due to redox cycling. As indicated above, such a mechanism normally exhibits a threshold (see reply to question 19 above). It is known that compounds such as quinones can show marked route-dependent differences in their genotoxic effects. Indeed, several authorities, including the EC have accepted a threshold for the genotoxicity of some of these compounds in vivo following oral administration (*European Chemicals Bureau, 2006*). It was of interest that the mutations observed in vivo by *Chakravarti et al (2001)* in mouse skin involved adenine and not guanine. This is significant as it is the N7-guanine adducts that were "considered to play a crucial role in the initiation of oestrogen-dependent tumours" (*SCVPH, 2002*).

Chakravarti D, Mailander PC, Li KM, Higginbotham S, Zhang HL, Gross ML, Meza JL, Cavalieri EL and Rogan EG (2001). Evidence that a burst of DNA depurination in SENCAR mouse skin induces error-prone repair and forms mutations in the H-ras gene. Oncogene, 20:7945-7953

477. The genotoxicity and mutagenicity of oestradiol and, more particularly, its metabolites in vitro was already well established. No new evidence has been provided on the genotoxic potential of oestradiol in vivo. The study on the quinone metabolite on mouse skin does not further the risk assessment of the compound. No evidence was provided that oestradiol, or indeed any of its metabolites, is genotoxic in vivo following oral administration.

Recent findings on the biological effects of testosterone and progesterone

478. The SVCPH (2002) concluded in their report that on the basis of the most recently published papers "there is no evidence that progesterone or testosterone have genotoxic potential".

Recent findings on the biological effects of trenbolone and zeranol

Biotransformation

479. The metabolism of zeranol and trenbolone had been further investigated (study 4). These data do not appear to have been published in the peer reviewed literature to date.

480. The data on trenbolone show that the alpha enantiomer in liver slices from bovine is extensively conjugated and hence inactivated. There is some conversion of the alpha to the active beta isomer by human liver microsomes, but the kinetics of the reaction and the extent of conjugation have not been determined. No data were presented on levels of the alpha enantiomer in meat from treated cattle. However, these data do not affect the risk assessment of trenbolone acetate. This is because a) the toxicological studies were conducted in animals that would have been exposed to the

metabolites of concern, b) JECFA considered residues of both the alpha and the beta enantiomers in recommending MRLs for trenbolone acetate.

481. The study on zeranol suggested that some of the metabolites might undergo autooxidation. However, the extent of any such reaction in intact cells was not investigated, nor was the likely detoxication of the products formed. Perhaps more importantly, it is the toxicology of zeranol and its residues in meat that is at issue, and the evidence is such that it was possible to identify a threshold dose for all of the effects of concern.

Binding to sex hormone binding globulin

482. In this study (study 10), which does not appear to have been published in the peer reviewed literature, the interaction of growth promoting hormones with the binding of testosterone to plasma proteins was investigated. Some of the hormones, but not zeranol, were able to displace testosterone partially and only at concentrations very much higher than would ever be achieved from ingestion of meat from cattle treated even with high doses of the hormones for growth promotion. Hence, these findings have no significance for the risk assessment of the hormones. Although zeranol did not appear to bind to plasma proteins with high affinity, the ADI was established on the basis of the exogenous dose that had no effect. The fraction of protein binding should be the same for the ADI and the TMDI. Hence, the risk assessment of this hormone will not be affected by whether zeranol binds to plasma proteins or not.

Mutagenicity and genotoxicity

483. Study 4 reports recent observations on the genotoxicity and mutagenicity of zeranol and trenbolone. Both compounds were negative for tests of mutagenicity, i.e. induction of *lacI* mutations in *E coli* and induction of *hprt* mutations in V79 cells. Zeranol did not produce DNA adducts in rat hepatocytes whilst a low level of DNA adducts was observed with trenbolone. Both were very weakly positive in a micronucleus test, at high (potentially cytotoxic) concentrations. As indicated above (see my reply to question 21), micronuclei can arise via a non-genotoxic mechanism, particularly at concentrations that may have caused some toxicity. In addition, the 32P-post-labelling assay is not specific, and data cited above suggest that DNA adduction can arise by mechanisms other than direct interaction with DNA. These data are insufficient, given the number of well conducted studies in which the compounds were negative, to alter the conclusion that neither zeranol nor trenbolone acetate has genotoxic potential in vivo. Indeed, the *SVC PH (2002)* concluded that "both compounds exhibited only very weak effects" in those in vitro tests in which positive effects were observed.

Recent findings on the biological effects of MGA

Biotransformation

484. In study 4, unpublished preliminary findings on the in vitro metabolism of MGA were reported. This study provided some evidence for the formation of multiple metabolites of MGA by liver from human, rat and bovine. However, these findings do not affect the risk assessment of MGA because a) the toxicological studies were conducted in animals that would have been exposed to all of the metabolites of concern, b) JECFA assumed that all of the residues in meat from animals treated with MGA were as hormonally active as MGA when it proposed MRLs in 2002 (*JECFA, 2002b*). It was subsequently shown that this was a conservative decision, as not all of the residues were as active as MGA itself (*JECFA, 2006c*).

JECFA (2002b). Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper 41/14, Rome, Italy

JECFA (2006c). Residues of some veterinary drugs in animals and foods. FAO, Rome, Italy (in press)

MGA binding to sex hormone binding globulin

485. In study 10, preliminary and relatively poorly reported data on the interaction of MGA with the binding of testosterone to plasma proteins were presented. MGA, at concentrations very much higher than those that would arise from consumption of meat from animals treated with the hormone had some displacement activity against testosterone. The SCVPH (2002) commented on the absence of data on reproducibility in these studies and that in some experiments no concentration curves were developed. In conclusion, these findings do not alter the risk assessment of MGA.

Mutagenicity and genotoxicity of MGA

486. In study 4, MGA was negative in studies of the induction of *hprt* mutations in V79 cells, the induction of micronuclei in V79 cells and the induction of *lacI* mutations in *E coli*. Pure MGA had no effect on apoptosis, which could potentially confound interpretation of studies using V79 cells. Preliminary studies with rat liver slices, reported in an abstract but not yet published in the peer reviewed literature, suggested that MGA could produce unidentified adducts with DNA. As indicated above, there are mechanisms of adduct formation that do not involve direct interaction of the inducing compound with DNA. Overall, a report of putative covalent binding to DNA observed using 32P-post-labelling is not sufficient to over-ride the consistently negative results of MGA in a range of tests for mutagenicity. Hence, on the basis of the findings in study 5, there is no reasons to change the risk assessment or MGA.

Recent data on endocrine and developmental effects of the hormones

Experimental studies in rabbits

487. The EC commissioned a study on the effects of in utero exposure of rabbits to the three exogenous hormones, MGA, trenbolone acetate and zeranol (study 11). To date, only information on metabolism and disposition have been published (*Lange et al, 2002*). Given the time that has elapsed since this paper was published (submitted September 2001), it is somewhat surprising the data from the remainder of the study have not been published.

488. The Lange et paper (2002) demonstrates transplacental transfer of the three hormones. This is not surprising given the physicochemical properties of the compounds (lipid solubility, non-polar, molecular size) (*Syme et al, 2004*). In addition, endogenous hormones are known to cross the placenta. It is notable that in the study of Lange et al, fetal concentrations of the hormones and their metabolites were similar to or less than, sometimes much less than, those in corresponding maternal tissues, suggesting that there was no net accumulation of the compounds in fetal tissues. It is also noted that the number of animals studied was very small, a point commented on by the authors themselves.

Lange IG, Daxenberger A, Meyer HH, Rajpert-De Meyts E, Skakkebaek NE and Veeramachaneni DN (2002). Quantitative assessment of foetal exposure to trenbolone acetate, zeranol and melengestrol acetate, following maternal dosing in rabbits. Xenobiotica, 32:641-651

Syme MR, Paxton JW and Keelan JA (2004). Drug transfer and metabolism by the human placenta. Clin Pharmacokinet, 43:487-514

489. The unpublished component of this study was an investigation of the potential health consequences of in utero exposure of rabbits to the three hormones. From the information provided, low dose exposure in utero caused modest changes in some parameters, but was not associated with either cancer or adverse effects on reproductive capacity. There were no changes in sperm number. It is not clear whether the changes observed were consistent and hence compound-related as a only a single dose was used for each compound. Nor is it apparent whether the magnitude of all of changes discussed reached statistical significance (often the changes were described as slight and no measure of variance is provided). The doses used in this study would have provided much higher levels of exposure than those predicted to arise from residues in meat. In the case of trenbolone acetate and zeranol exposure was via the subcutaneous route, thus bypassing presystemic metabolism in the intestine and/or the liver. In the case of MGA the oral dose was over 16,500 times the ADI. Hence, even if the effects observed were of toxicological significance the ADI would provide a more than adequate margin of protection.

490. Overall, this study cannot be said to confirm a risk to human health from consumption of meat from animals treated with these hormones.

In utero exposure and breast cancer: a study in opposite sexed twins

491. This study (Kaijser *et al*, 2001; study 13) showed an association between birth weight and risk of breast cancer. This is consistent with in utero exposure to oestrogens as a risk factor in breast cancer. However, it does not establish such a relationship. The authors note that "Although statistically significant (P=0.03), these estimates were based on small numbers in the extreme categories. However, when the data were categorized in more equally sized groups, the associations were similar and retained statistical significance, albeit with lower point estimates." There is no specific consideration of exposure to hormones present in meat from treated animals. The risk of such exposure, with respect to the hypothesis proposed by Kaisjer *et al* (2001) would depend on the mode of action and the dose-response relationship. A recent study (de Assis *et al*, 2006) suggests that higher birth weight per se can increase the risk of breast cancer. However, the significance of this study in experimental animals to humans has yet to be determined. Given that exposure to oestradiol from meat of treated animals would be extremely low, particularly relative to endogenous hormone levels, which increase during pregnancy, (e.g. see Weiss, 2000) the findings of the Kaisjer *et al* study provide no evidence for risk from exposure to oestradiol residues in meat from treated animals.

De Assis S, Khan G and Hilakivi-Clarke L (2006). High birth weight increases mammary tumorigenesis in rats. Int J Cancer (in press)

Kaijser M, Lichtenstein P, Granath F, Erlandsson G, Cnattingius S and Ekblom A (2001). In utero exposures and breast cancer: a study of opposite-sexed twins. J Natl Cancer Inst, 93:60-62

Weiss G (2000). Endocrinology of parturition. J Clin Endocrinol Metab, 85:4421-4425

492. Overall, this study cannot be said to confirm a risk to human health from consumption of meat from animals treated with these hormones.

Retrospective study on long-term effects in children of following suspected exposure to oestrogen-contaminated meat

493. This paper (Chiumello *et al*, 2001; study 12) reviews the increased incidence of gynecomastia in male children observed over a certain interval in a school in Milan, Italy. As oestradiol is known to cause gynecomastia, the authors speculated on the possible role of oestrogens, perhaps from meat of treated animals, as causative agents in these effects. However, this is entirely speculative, as no data were obtained to suggest that there had been any such exposure. Further, it was not possible to

establish whether oestrogens were involved at all, as there are a number of other risk factors for gynocomastia. Indeed, the SCVPH (2002) concluded that "As the reason for this incident remains unknown, the relevance of these data remains unclear".

Chiumello G, Guarneri MP, Russo G, Stroppa L and Sgaramella P (2001). Accidental gynocomastia in children. APMIS 109 (Suppl 103): S203-S209

494. Overall, this study cannot be said to confirm a risk to human health from consumption of meat from animals treated with these hormones.

Conclusion on the evidence from the studies initiated since 1997

495. There is little information in the scientific studies initiated by the EC since 1997 that support the contention that they have identified important new gaps, insufficiencies and contradictions in the scientific information and knowledge on the hormones, and that additional studies are necessary before the risks to health of consumption of meat from treated animals can be assessed. Whilst additional information has been obtained on a number of aspects of the hormones in question, this was often not definitive, sometimes it was not relevant, in some instances it confirmed or expanded on previous knowledge. The evidence obtained did not indicate any additional concern regarding the risk from exposure to residues of the hormones in meat from cattle treated for growth promotion.

496. My reply would have been the same at the time of adoption of the Directive by the EC in September, 2003.

Dr. Guttenplan

497. Yes, I see several important gaps. For instance:

498. It is not known if eating beef from hormone-treated animals substantially elevates the blood levels of estrogen/estrone in prepubescent children. This can probably be investigated.

499. It is not known if eating beef from hormone-treated animals substantially elevates the level of estrogenic activity in blood.

- Experiments on the identification, quantification, bioavailability and accumulation of lipoidal esters of estrogen in humans and experimental animals should be conducted.
- Epidemiological studies comparing adverse effects in matched populations of children eating beef from hormone-treated and untreated animals have not been reported.

[Please see the following references for the two questions above:

- **paras. 58-94 and 125-129 of US First Submission, paras. 28-32 of US Rebuttal Submission**
- **paras. 116-124 of Canada First Submission, paras. 74, 130-135 of Canada Rebuttal Submission (Exhibit CDA-23)**
- **paras. 108, 147, 162-169 of EC Replies to Panel Questions, paras. 143-174 of EC Rebuttal Submission (US case), and paras. 148-166 of EC Rebuttal Submission (Canada case)**
- **Exhibit CDA-32 provides a detailed table outlining the chronology of JECFA's assessment of these hormones and the resulting documentation]**

ATTACHMENT 1

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(<http://www.absorption.com/Site/Glossary/Default.aspx>)
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ATTACHMENT 2

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