



**Food and Agriculture Organization
of the United Nations**

**World Health
Organization**

***Joint FAO/WHO Expert Committee on Food Additives
Fifty-second meeting
Rome, 2-11 February 1999***

SUMMARY AND CONCLUSIONS

A meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was held in Rome, Italy, from 2 to 11 February 1999. The purpose of the meeting was to evaluate certain residues of veterinary drugs in food.

Dr J. Boisseau, Director, National Agency for Veterinary Medicine, Fougères, France, served as chairman and Professor J.G. McLean, South Melbourne, Victoria, Australia, served as vice-chairman.

Mr J. Weatherwax, Food Quality and Standards Service, Food and Nutrition Division, Food and Agriculture Organization of the United Nations, served as FAO Joint Secretary. Dr J.L. Herrman, International Programme on Chemical Safety, World Health Organization, served as WHO Joint Secretary. Professor A.R. Boobis, section on Pharmacology, Division of Medicine, Imperial College School of Medicine, London, England, and Dr R. L. Ellis, Food Safety and Inspection Service, Department of Agriculture, Washington, DC, USA, served as Joint Rapporteurs.

The present meeting was the fifty-second in a series of such meetings and was the twelfth meeting of JECFA convened to deal exclusively with residues of veterinary drugs in food. The primary tasks before the Committee were to further elaborate principles for evaluating the safety of residues of veterinary drugs in food and for establishing acceptable daily intakes (ADIs) and maximum residue limits (MRLs) for certain drugs when they are administered in food-producing animals in accordance with good practice in the use of veterinary drugs.

The report of the meeting will appear in the WHO Technical Report Series (TRS). Its presentation will be similar to that of previous reports, namely, general considerations, specific comments on substances on the agenda, and recommendations. The report will include an annex containing a detailed table (similar to Table 1 in this report) summarizing the conclusions reached by the Committee after its evaluations of the substances on the agenda.

Toxicological monographs summarizing the data that were considered by the Committee in assessing the safety of the substances on the agenda will be published in *WHO Food Additives Series* No. 43. Residues monographs summarizing the data that were considered by the Committee in establishing MRLs will be published in *FAO Food and Nutrition Paper* series No. 41/12.

TABLE 1. RECOMMENDATIONS ON COMPOUNDS ON THE AGENDA

β-Adrenoceptor-blocking agent

Carazolol

Acceptable daily intake: 0 – 0.1 µg/kg bw (established at the forty-third meeting of the Committee (WHO TRS 855, 1995))
Acute RfD: 0 – 0.1 µg/kg bw
Residue definition: Carazolol

Recommended maximum residue limits (MRLs)¹

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)
Pigs	5	25	25	5 ²

¹Recommended at the forty-third meeting of the Committee (WHO TRS 855, 1995). Residues of carazolol at the injection site two hours after treatment may result in an intake that exceeds the acute RfD. Therefore, unless appropriate measures can be taken to ensure that residues at the injection site do not exceed the acute RfD, the use of carazolol during the transport of animals to slaughter is inconsistent with safe use of the drug.

²Fat/skin

Anthelmintic agent

Doramectin

Acceptable daily intake: 0 – 0.5 µg/kg bw (established at the forty-fifth meeting of the Committee (WHO TRS 864, 1996))
Residue definition: Doramectin

Recommended maximum residue limits (MRLs)¹

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)
Cattle ²	10	100	30	150
Pigs	5	100	30	150

¹The Committee noted the high concentrations of residues at the injections sites.

²Recommended at the forty-fifth meeting of the Committee (WHO TRS 864, 1996)

Antimicrobial agents

Dihydrostreptomycin/streptomycin

Acceptable daily intake: 0 – 50 µg/kg bw (established at the forty-eighth meeting of the Committee (WHO TRS 879, 1998))
Residue definition: Sum of the concentrations of dihydrostreptomycin and streptomycin

Recommended maximum residue limits (MRLs)

Species	Muscle ¹ (µg/kg)	Liver ¹ (µg/kg)	Kidney ¹ (µg/kg)	Fat ¹ (µg/kg)	Milk (µg/kg)
Cattle	600	600	1000	600	200 ²
Pigs	600	600	1000	600	
Sheep	600	600	1000	600	
Chickens	600	600	1000	600	

¹The Committee was aware of more sensitive analytical methods for dihydrostreptomycin and streptomycin in edible tissue and requested that additional analysis methods be made available to the Committee for evaluation in 2001.

²Temporary. The following information is required for evaluation in 2001:

1. A validated analytical method that will quantitate both compounds in milk at a low level.

Neomycin

Acceptable daily intake: 0 – 60 µg/kg bw (established at the forty-seventh meeting of the Committee (WHO TRS 876, 1998))

Residue definition: Neomycin

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk (µg/kg)	Eggs (µg/kg)
Cattle	500 ¹	15 000	20 000	500 ¹	500 ¹	
Pigs	500 ¹	500 ¹	10 000 ²	500 ¹		
Sheep	500 ¹	500 ¹	10 000 ²	500 ¹		
Goats	500 ¹	500 ¹	10 000 ²	500 ¹		
Turkeys	500 ¹	500 ¹	10 000 ²	500 ¹		
Ducks	500 ¹	500 ¹	10 000 ²	500 ¹		
Chickens	500 ¹	500 ¹	10 000 ²	500 ¹		500 ¹

¹Recommended at the forty-third meeting of the Committee (WHO TRS 855, 1995)

²Recommended at the forty-seventh meeting of the Committee (WHO TRS 876, 1998)

Thiamphenicol

Acceptable daily intake: 0 – 5 µg/kg bw

Residue definition: Sum of thiamphenicol and thiamphenicol conjugates, measured as thiamphenicol

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)
Cattle ¹	withdrawn	Withdrawn	Withdrawn	withdrawn
Pigs ²	50	100	500	50
Chickens ¹	withdrawn	withdrawn	withdrawn	withdrawn
Fish ²	50			

¹The previous temporary MRLs for cattle and chickens were withdrawn as the data required by the forty-seventh meeting of the Committee (WHO TRS 876, 1998) were not provided.

²Temporary. The following information is required for evaluation in 2002:

1. A radiolabel depletion study in pigs to determine the relationship between free thiamphenicol, thiamphenicol conjugates and total residues in all tissues.
2. A validated analytical method for use in all animal tissues, which incorporates an enzymatic hydrolysis step allowing the determination of the sum of thiamphenicol and thiamphenicol conjugates as free thiamphenicol.

Insecticides

Deltamethrin

Acceptable daily intake: 0 – 10 µg/kg bw (established by the 1982 Joint FAO/WHO Meeting on Pesticide Residues (FAO Plant Production and Protection Paper 46, 1983))

Residue definition: Deltamethrin

Recommended maximum residue limits (MRLs)

Species	Muscle ¹ (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk ¹ (µg/kg)	Egg ¹ (µg/kg)
Cattle	30	50	50	500	30	
Sheep	30	50	50	500		
Chickens	30	50	50	500		30
Salmon	30					

¹No residues were detected. MRLs are for guidance only and are based on two times the limit of quantification of the analytical method.

Phoxim

Acceptable daily intake: 0 – 4 µg/kg bw

Residue definition: Phoxim

Recommended maximum residue limits (MRLs)¹

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk (µg/kg)
Cattle	50	50	50	400	10
Pigs	50	50	50	400	
Sheep	50	50	50	400	
Goats	50	50	50	400	

¹Temporary. The following information is required for evaluation in 2002:

1. Radiolabel studies to relate the marker residue to the total residue in ruminants and pigs following topical application of the formulated product
2. Residue studies in accordance with Good Laboratory Practice using the current recommended treatments in cattle and sheep
3. Validation of available analytical methods for phoxim residues in tissues of cattle, sheep, goats and in milk from cattle

Production aids

Estradiol-17 β , progesterone, and testosterone (see Annex 1)

Acceptable daily intakes

Estradiol-17 β :	0 – 0.05 $\mu\text{g}/\text{kg}$ bw
Progesterone:	0 – 30 $\mu\text{g}/\text{kg}$ bw
Testosterone:	0 – 2 $\mu\text{g}/\text{kg}$ bw

Maximum residue limits: MRLs “not specified”¹ in cattle muscle, liver, kidney, and fat

¹MRL “not specified” means that available data on the identity and concentration of residues of the veterinary drug in animal tissues indicate a wide margin of safety for consumption of residues in food when the drug is used according to good practice in the use of veterinary drugs. For that reason, and for the reasons stated in the individual evaluation, the Committee concluded that the presence of drug residues in the named animal product does not present a health concern and that there is no need to specify a numerical MRL.

Porcine Somatotropin

Acceptable daily intake: ADI “not specified”¹ (applies to Grolene®, Reporcin®, and Somagrepur®)

Maximum residue limit: MRLs “not specified”² in pig muscle, liver, kidney, and fat (applies to Grolene®, Reporcin®, and Somagrepur®)

¹ADI “not specified” means that available data on the toxicity and intake of the veterinary drug indicate a large margin of safety for consumption of residues in food when the drug is used according to good practice in the use of veterinary drugs. For that reason, and for the reasons stated in the individual evaluation, the Committee concluded that use of the veterinary drug does not represent a hazard to human health and that there is no need to specify a numerical ADI.

²See definition of MRL “not specified” under estradiol-17 β , progesterone, and testosterone.

Tranquilizing agent

Azaperone

New information on the method of analysis in tissues of pigs was reviewed. Insufficient characterization on specificity, accuracy, and reproducibility of the method was provided. The Committee recommended that the method be improved and after further development it be forwarded to the Codex Committee on Residues of Veterinary Drugs in Foods for consideration.

NOTE

This document has been distributed prior to publication of the full report of the fifty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to ensure the fast dissemination of information, in particular to the Codex Alimentarius Commission, for which JECFA is the scientific advisory body on matters relating to residues of veterinary drugs in food.

The FAO and WHO Joint Secretaries of JECFA request that further inquiries regarding the compounds evaluated at the fiftieth meeting be made only after the full official report has been published and distributed by WHO in the name of both sponsoring organizations, FAO and WHO. Your cooperation is much appreciated.

The information provided in this annex is the draft report item summarizing the assessment of estradiol-17 β , progesterone, and testosterone at the fifty-second meeting of JECFA, and is included to provide quick dissemination of information. Although the scientific conclusions will not be changed, it is subject to extensive editing.

Annex 1

Estradiol-17 β , progesterone, and testosterone

Estradiol-17 β

Estradiol-17 β is a hormone produced primarily by the developing follicle of the ovary in adult females. Estradiol-17 β in combination with progesterone or testosterone is administered to cattle to increase the rate of weight gain and to improve feed efficiency. Estradiol-17 β was previously reviewed at the thirty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives which considered establishment of an acceptable daily intake (ADI) and maximum residue limits (MRLs) “unnecessary”. Estradiol-17 β was re-evaluated at the present meeting to consider any data that had been generated since the previous review and to provide a quantitative estimate of its safe intake.

Toxicological data

The Committee considered data in the published literature from studies on the bioavailability following oral administration, metabolism, short-term toxicity, reproductive toxicity, genotoxicity, long-term toxicity and carcinogenicity of administered estrogens. Numerous reports on studies of the use of exogenous estrogens in women were considered, with studies in experimental animals on the mechanisms of action of estradiol-17 β . The extensive database from epidemiological studies of women taking oral contraceptive preparations containing estrogens and post-menopausal estrogen replacement therapy was also used to evaluate the safety of estradiol-17 β .

Estradiol-17 β is an 18-carbon steroid and the most potent of the natural estrogens. Estradiol-17 β exerts its biological effects largely by receptor-mediated mechanisms. It binds with high affinity and high specificity to intracellular receptors. Binding of estradiol-17 β acts directly in the growth and development of the reproductive tract, breast, and secondary sex characteristics. In non-pregnant females, estradiol-17 β acts synergistically with progesterone during the luteal phase of the menstrual cycle to initiate events leading to a new cycle. Continued estradiol-17 β production is essential for normal growth and development of the fetus. In addition to its effects on reproductive tissues, estradiol-17 β is an important metabolic hormone, particularly because of its effects on the cardiovascular, skeletal, and gastrointestinal systems.

In general, estradiol-17 β is inactive when given orally because of gastrointestinal and hepatic inactivation. However, fine-particle formulations of estradiol-17 β are effective when given orally and are used therapeutically. The bioavailability of a single 4-mg dose of fine-particle estradiol-17 β administered orally to 14 young women was 5% in comparison with a dose administered intravenously. At least 60% of the oral dose of fine-particle estradiol-17 β appears in the serum as estrone and estrone-sulfate, and is available as part of the endogenous pool.

The acute toxicity of estradiol-17 β after oral administration is very low.

Few conventional short- and long-term studies to examine the systemic toxicity of estrogens in animals given estradiol-17 β orally were available. There was sufficient information to demonstrate that the adverse effects of estradiol-17 β seen in animals are associated with the hormonal activity of estrogens. Because of the specificity and affinity with which estradiol-17 β binds to its receptors, the hormonal effects occur at much lower doses than other toxicological responses, and hence are the most appropriate for use in the safety evaluation of the compound.

In studies of developmental toxicity in rats, estradiol-17 β doses equivalent to 25 mg/kg bw were implanted on day 10 of pregnancy, at which dose all embryos were resorbed. In an interim report of a multi-

generational study of reproductive toxicity in female rats, estradiol-17 β was administered in the feed at doses of 0.003, 0.17, 0.69, or 4.1 mg/kg bw per day. No viable pups were observed at the two highest doses. As the progesterone levels were altered at some times at all doses of estradiol-17 β , a no-observed-effect level (NOEL) was not determined in this study.

The Committee reviewed short-term studies that examined the genotoxic potential of estradiol-17 β . Estradiol-17 β did not cause gene mutations *in vitro*. In some other assays, sporadic but unconfirmed positive results were obtained. There was more consistent evidence for the induction of micronuclei *in vitro*, aneuploidy *in vitro*, cell transformation *in vitro*, oxidative damage to DNA *in vivo*, and DNA single-strand breakage *in vivo* by estradiol-17 β . The Committee concluded that estradiol-17 β has genotoxic potential.

A normal biological function of estrogens is to increase the number of proliferating cells in the endometrium and breast. This effect is exerted by binding with high affinity to estrogen receptors. These receptors, of which there are several forms, are found in many tissues. In cultured human breast cancer cells containing estrogen receptors, estradiol-17 β stimulates growth at concentrations from 10⁻¹² mol/L up to a maximum response at about 10⁻¹⁰ mol/L. Estradiol-17 β does not stimulate growth of cultured human breast cancer cells that do not contain estrogen receptors. At higher concentrations, estrogens also stimulate cell proliferation in rat liver *in vivo* and in cultured rat hepatocytes. Any factor that increases mitotic activity reduces the time available for repair before the next cell division of DNA damage induced by any means. The mutagenic events that are required for neoplasia are not necessarily induced by the same agent that causes the cell proliferation. Consequently, if receptor-mediated stimulation of cell growth is an important mechanism of neoplasia induced by estradiol-17 β , it would be expected that late-stage carcinogenic activity would be the dominant process. Experimental carcinogenicity studies in rodents, in which estradiol-17 β was administered in conjunction with known carcinogens, support this mechanism, as do the observations on human cancer incidence with post-menopausal hormone replacement therapy. In long-term carcinogenicity studies in animals reviewed at the thirty-second meeting, oral and parenteral administration of estradiol-17 β increased the incidences of tumours only in hormone-dependent tissues, including the kidneys of the male Syrian hamster. The Committee concluded that the effects of estradiol-17 β on carcinogenesis are most probably a result of interaction with its hormonal receptors.

The commonest uses of estrogens in humans are for oral contraception and post-menopausal replacement therapy. For oral contraception, the xenobiotic estrogen ethinylestradiol is usually used. Fine-particle estradiol-17 β and conjugated equine estrogen preparations are commonly used for post-menopausal estrogen replacement therapy. Healthy post-menopausal women were given 0.3, 0.62, 1.2 or 2.5 mg conjugated equine estrogens for two weeks followed by no medication for three weeks. When this regimen was repeated four times, 0.3 mg/day was the NOEL for changes in the serum concentrations of corticosteroid-binding globulin (CBG). These data show that there is a threshold level for estrogen administered orally below which there is no increase in serum concentrations of CBG. In a study involving 23 healthy post-menopausal women receiving various estrogen preparations orally, 0.3 mg/day of conjugated equine estrogens had no effect on serum concentrations of follicle-stimulating hormone, angiotensinogen, sex-hormone-binding globulin, or CBG. Conjugated equine estrogens and fine-particle estradiol-17 β were equipotent for all four hormone-dependent end-points. Thus, 0.3 mg/person, equivalent to 5 μ g/kg bw per day, was the NOEL for the hormonal effects of estradiol-17 β described above. Further support for this NOEL is that 0.3 mg/day of estradiol-17 β administered orally to women was not associated with relief of the symptoms of menopause.

A study of approximately 7700 infants whose mothers reported taking oral contraceptives during pregnancy showed no evidence that estrogens present a teratogenic hazard.

Because of differences in pharmacokinetic and pharmacodynamic properties of natural and xenobiotic estrogen preparations, the Committee concluded that data on the use of estrogens for post-menopausal replacement therapy are more appropriate than data on their use for oral contraception for evaluating the safety of estradiol-17 β . Epidemiological studies on women who took estrogens, either alone or in combination with progestogens and androgens, showed that the risks for cancers at most sites were unaffected; the risks for cancer of the endometrium and of the breast were increased. Among women who had ever used post-menopausal estrogen-only therapy, the relative risk for endometrial cancer was 2.3 (95% confidence limit, 2.1-2.5) in a meta-analysis of 30 epidemiological studies. The relative risk of women taking post-menopausal estrogen-only therapy for more than 10 years was 9.5 (95% confidence limit, 7.4-12.3). The addition of progestogens to post-menopausal estrogen-only therapy reduced the excess risk substantially, although the

excess risk may not be completely eliminated. In a review of 51 epidemiological studies of women on hormonal replacement therapy, the relative risk for cancer was increased by a factor of 1.023 (95% confidence interval, 1.011-1.036) for each year of use. These relative risk estimates are based on studies of women who used post-menopausal estrogen replacement therapy preparations containing either conjugated equine estrogens (average dose, 0.625 mg/day) or estradiol-17 β (1-2 mg/day). Overall, the available data suggest that the increased cancer incidence among women receiving post-menopausal estrogen replacement therapy is due to the hormonal effects of estrogens.

The Committee established an ADI of 0-50 ng/kg bw per day on the basis of the NOEL of 0.3 mg/day (equivalent to 5 μ g/kg bw per day) in studies of changes in several hormone-dependent parameters in post-menopausal women. A safety factor of 10 was used to account for normal inter-individual variation, and an additional factor of 10 was added to protect populations of various sensitivities.

Progesterone

Progesterone is a hormone produced primarily by the corpus luteum of the ovary in adult females. It is administered in combination with estradiol to cattle to increase the rate of weight gain and to improve feed efficiency. Progesterone was reviewed previously at the thirty-second meeting of the Committee, which considered establishment of an ADI and MRLs to be "unnecessary". Progesterone was re-evaluated at the present meeting in order to consider any data that have been generated since the previous review and to provide a quantitative estimate of its safe intake.

Toxicological data

The Committee considered data in the published literature from studies on the oral bioavailability, metabolism, short-term toxicity, reproductive toxicity, genotoxicity, long-term toxicity, and carcinogenicity of progesterone. Numerous reports of studies on progesterone in humans were considered. In addition, the extensive database derived from use by women taking progestogens as a component of oral contraception, as injectable progestogen-only contraception, and post-menopausal hormone replacement therapy was used to support the safety evaluation.

Progesterone is a 21-carbon steroid that is the only important natural progestogen. The normal role of progesterone is to prepare the uterus for implantation and to maintain pregnancy. The production of progesterone by the corpus luteum is controlled by the pituitary luteinizing hormone. Continued production of progesterone is necessary to maintain pregnancy. In non-pregnant females, an elevated concentration of progesterone inhibits the cyclic release of luteinizing hormone and higher levels inhibit production of follicle-stimulating hormone. Progesterone opposes some of the effects of estrogens. It exerts its biological effects by receptor-mediated mechanisms. Prior stimulation with estrogens is essential for progesterone to elicit its biological response. Progesterone binds with high affinity and high specificity to an intracellular receptor protein. Binding of progesterone activates the receptor, resulting in activation of specific genes.

Progesterone has low bioavailability (< 10%) when given by the oral route due to gastrointestinal and/or hepatic inactivation. The acute oral toxicity of progesterone is low.

No conventional studies of toxicity in animals treated with progesterone orally were available, and few other studies were found. Because progesterone binds specifically and with high affinity to its specific receptor, the hormonal effects are the most sensitive toxicological end-points in these studies. Studies of toxicity after administration by other routes suggest that the effects seen in animals are associated with hormonal activity. Although equivocal results have been reported for the induction of single-strand DNA breaks and DNA adducts have been seen in some *in vivo* and *in vitro* studies, it was not mutagenic. On balance the Committee concluded that progesterone has no genotoxic potential.

Mouse pups given five daily subcutaneous injections of 100 μ g progesterone per pup (equivalent to 200 mg/kg bw per day) beginning 36 h after birth and observed for up to one year had increased incidences of mammary gland tumours. Female rabbits given an average dose of 8mg per kg bw intramuscularly every second week for two years developed endometrial cysts, which were sometimes associated with atypical hyperplasia, but no significant changes were observed in other tissues.

Developmental toxicity was not seen in studies in rats and rhesus monkeys. Rats dosed at 5–25 mg/kg bw on days 14–19 of gestation delivered pups that showed no evidence of masculinization. Rhesus monkeys given progesterone at 5 mg/kg bw intramuscularly on five days per week beginning at one month of pregnancy and continuing to birth delivered healthy offspring with no evidence of abnormalities. The Committee noted that exogenous progesterone has been used to maintain pregnancy with no evidence of toxicity, and it does not interfere with the normal conclusion of pregnancy. No multigenerational study of reproductive toxicity was available on progesterone.

The commonest uses of progestogens for humans are in contraception and in post-menopausal hormone replacement therapy, in which synthetic progestogens are usually used either alone or in combination with estrogens. In a study designed to explore anti-proliferatory and secretory end-points in the endometrium in women, 300 or 600 mg/day (300 mg twice a day) of fine-particle progesterone was administered orally for two weeks after pretreatment with estrogens for 30 days. The group receiving 300 mg/day showed incomplete conversion of the uterus to full secretory activity, while the group receiving 600 mg/day showed full secretory conversion of the uterus. In studies using 200 or 300 mg progesterone orally for one or five years, there was no evidence of endometrial hyperplasia or carcinoma. In humans, oral administration of a single dose of 200 mg of fine particle progesterone (equivalent to 3.3 mg/kg bw) provided concentrations in blood similar to those found during the luteal phase of the ovulatory cycle. This was considered to be the lowest-observed-effect level (LOEL) in humans.

There is extensive literature on the use of synthetic progestogens for oral contraception in combination with estrogens. While synthetic progestogens differ from natural products in their pharmacokinetic and pharmacodynamic properties, tissue specificity, and potency, a prominent feature of the synthetic agents is a protective effect against the untoward effects of estrogens. No increase in cancer risk at any site was shown in women using oral progesterone or other progestogens alone for contraception. When used in combination with estrogens as post-menopausal replacement, progesterone reduced the excess risk for endometrial cancer found with estrogen alone but did not alter the increased risk for breast cancer.

The Committee established an ADI of 0-30 µg/kg bw for progesterone on the basis of the LOEL of 200 mg per day (equivalent to 3.3 mg/kg bw) based on changes in the uterus. A safety factor of 100 was used to allow for extrapolation from a LOEL to a NOEL and to account for normal inter-individual variation.

Testosterone

Testosterone is a hormone produced primarily by the testes. Testosterone propionate in combination with estradiol is administered to cattle to increase the rate of weight gain and to improve feed efficiency. Testosterone was previously reviewed at the thirty-second meeting of the Committee, which considered establishment of an ADI and MRLs for testosterone to be “unnecessary”. Testosterone was re-evaluated at the present meeting to consider any data that have been generated since the previous review and to provide a quantitative estimate of its safe intake.

Toxicological data

The Committee considered data in the published literature from studies on the oral bioavailability, metabolism, short-term toxicity, reproductive toxicity, genotoxicity, long-term toxicity, and carcinogenicity of testosterone. Reports of studies in humans were also considered.

Testosterone is a 19-carbon steroid that has potent androgenic properties including maintenance of testicular function and growth and differentiation of secondary sex characteristics. It exerts its biological effects through receptor-mediated mechanisms. Testosterone binds with high affinity and high specificity to an intracellular receptor protein, the androgen receptor. Binding of testosterone activates the receptor, resulting in activation of specific genes. In certain target tissues, testosterone is metabolized to 5 α -dihydrotestosterone, which has higher binding affinity for the androgen receptor.

Androgens have marked anabolic effects that include increased protein synthesis in muscle and bone. This results in an increased rate of body growth. In females, androgens have actions in the breast, uterus, and vagina similar to those of progestogens. Luteinizing hormone and follicle-stimulating hormone from the pituitary gland control the production of testosterone by the testes. Testosterone in turn modulates the

concentration of follicle-stimulating hormone and luteinizing hormone, thus controlling the circulating levels of testosterone through a feedback mechanism.

Testosterone has low bioavailability when given by the oral route, owing to gastrointestinal and hepatic inactivation. Plasma measurements made after oral administration of 25 mg testosterone to young women indicated that approximately 4% of the dose was bioavailable.

The acute toxicity of testosterone after oral administration is very low.

Few studies have been conducted of the toxicity of testosterone in animals treated by the oral route. Short- and long-term studies of toxicity in animals demonstrate that the adverse effects of testosterone are due to its hormonal activity. Therefore, the most sensitive toxicological targets are hormone-sensitive tissues, such as the prostate. Six adult male baboons received weekly intramuscular injections equivalent to 8 mg/kg bw of testosterone enanthate for up to 28 weeks. At the end of the study, histological evidence of non-neoplastic alterations of the prostate was found. Female rabbits given an average dose of testosterone equivalent to 6 mg/kg bw intramuscularly every second week for two years developed endometrial cysts and secretions from the mammary gland. No significant changes were found in non-reproductive tissues.

In studies of developmental toxicity, testosterone was embryotoxic; in rats, a dose equivalent to 25 mg/kg bw of testosterone administered as subcutaneous implants on day 10 of pregnancy resulted in complete embryonic resorption. Multigenerational studies of reproductive toxicity have not been conducted on testosterone as, while normal circulating levels of testosterone are required for normal reproductive function in males, elevated levels of testosterone interfere with normal reproductive function in both males and females.

The genetic toxicity of testosterone has been evaluated in mammalian cells. No chromosomal aberrations, mutations, or DNA adducts were found with testosterone alone, and the Committee concluded that testosterone has no genotoxic potential. No additional studies of carcinogenicity have been performed with testosterone in animals since the previous evaluation. The Committee re-affirmed that the increased rate of prostate cancer detected in rats was consistent with the hormonally mediated effects of testosterone and its metabolites.

In men, physiological circulating levels of testosterone range from 3-10 ng/ml. In women circulating testosterone values are less than 1 ng/ml and the majority of circulating testosterone is derived from conversion of dihydroepiandrosterone and androstenedione of adrenal and ovarian sources. Androgens are used therapeutically in men with deficient testicular function to restore normal testosterone levels. The effects of excess androgens, particularly in young boys, include deepening voice, acne, and growth of facial hair, while in women hair loss and menstrual irregularities may be seen. In a clinical trial involving five eunuchs, a dose of 100 mg/day of an orally administered fine-particle formulation of testosterone had no effect on sexual function indexes, while a dose of 400 mg/day of orally administered testosterone was fully effective in restoring sexual function. Thus, oral administration of a 100 mg/day dose (equivalent to 1.7 mg/kg bw per day) was the NOEL in this study. Studies on post-menopausal women receiving the testosterone analogue, methyltestosterone, alone or in combination with estrogens, indicate that 10 mg/day can induce virilizing signs (e.g. acne, hirsutism) in a sizeable proportion of exposed women. The effects were dose- and time-dependent. The Committee noted that methyltestosterone is more potent than testosterone when given by the oral route.

No epidemiological studies on long-term treatment of humans were available. Therapeutic doses of testosterone given for treatment of aplastic anaemia or hypogonadism have resulted in the induction of liver cysts and hepatomas.

The Committee established an ADI of 0–2 µg/kg bw for testosterone on the basis of the NOEL of 100 mg/day (equivalent to 1.7 mg/kg bw per day) in the study of eunuchs and a safety factor of 1000. The large safety factor was used to protect populations with various sensitivities and because of the small number of subjects in the study used to identify the NOEL.

Residue data on estradiol-17 β , progesterone, and testosterone

Estradiol-17 β alone or in combination with progesterone, testosterone, or trenbolone acetate is given to cattle to improve their rate of weight gain and their efficiency of conversion of feed into edible tissues. The approved route of administration is subcutaneous implantation into the ear. When estradiol-17 β benzoate or testosterone propionate is used instead of their free forms, the esters are rapidly hydrolysed in the animal after release from the implant. The rates of release of the substances vary from one implant type to the other. In a typical study 60 μ g per day were released into the animal.

Description of the available data base and the products evaluated

The present report relies on data obtained using the implants characterized in Table 1. The majority of the available studies had been conducted to support approved uses. However, the review also included several investigations in which experimental fixed combinations of trenbolone acetate and estradiol-17 β or sequential implantation of such products was used.

Table 1

Product Name	Comparison of the composition [mg/implant] of certain implants used for growth promotion ^a						
	Estradiol-17 β (E ₂)	Estradiol Benzoate (E ₂ -b)	Testosterone	Testosterone propionate	Progesterone	Trenbolone acetate	Target animals
Compudose	24 45						cattle
Synovex® S		20			200		steers
Synovex® H		20		200			heifers
Synovex® C		10		100			calves
Steeroid®	20				200		steers
Heiferoid®	20			200			heifers
Implix® BM	20				200		steers
Implix® BF	20		200				heifers
Torelor®	40					200	steers
Revalor® lactose	20					140	calves
Revalor® G	8					40	steers
Revalor® S	24					120	steers
Revalor® H	14					140	heifers
Finaplix-S®						140	steers

^aUse of Torelor® and concomitant or sequential use of Implix®/Revalor® is not approved.

Since most of the products used were combinations and since the concentrations found in tissues reflected both product-specific kinetic and dynamic properties (e.g. negative feedback of endogenous production of certain hormones) the Committee decided to evaluate residues of the administered substances product by product rather than substance by substance.

Analytical Methods Performance

The Committee critically examined the available information on both the scope and the performance of the analytical methods used for the determination of residues. The radioimmunoassay methods which were developed about 20 years ago have been most thoroughly validated and were supported by an excellent database. For example, in a large number of the available residue studies the methods were able to quantify

estrogenic hormone concentrations below 10^{-9} g/kg of meat. Certain more recently developed methods, particularly those that had been used in investigational studies with experimental combinations of hormones, did not achieve the same sensitivities. However, all methods that were described were generally found valid for the estimation of the hormone residues for which they had been developed by the authors. It was, however, found that some of the methods had not been designed to determine both the free hormones, their relevant metabolites, and the relevant conjugates in all relevant edible tissues. This was, however an indispensable requirement for a sufficiently accurate analysis of both the endogenously produced and the exogenously administered estrogenic hormonal compounds. Estrone and estradiol-17 β are interconvertible and have comparable metabolism. Their estrogenic potencies are similar. The oral bioavailabilities of the glucuronides and sulfates of these substances are in the same order.

The differences in scope and performance of the analytical methods with respect to these criteria added a further argument for a product by product review of all three substances together.

Statistical evaluations and intake calculations

The results of the residue studies were statistically evaluated. The distributions of the residues were described by a number of characteristics including the mean, standard deviation, geometric mean and median. The assumption of normal distribution of the hormone concentrations could not be defended. Taking into consideration that tissue concentrations were sometimes below the limits of detection or the limits of quantification of the methods, the median was the most stable and convenient parameter to provide an estimate of a central tendency of the data without excluding any individual result or making specific assumptions on substitute values for results like "below the limit of quantification". Appropriate median values (see below) were therefore also used as the basis for the calculation of theoretical maximum daily intakes.

The objective of the intake calculations was to obtain conservative estimates of the theoretically possible excess dietary intakes of preferential eaters of meat which could be attributed to the approved uses of the products reviewed. The calculations were, therefore, performed in the following stepwise manner:

- For every given time point of every study the median hormone concentrations found in tissues of control animals and of treated animals were multiplied by the respective high daily consumption figures for "meat" conventionally used by JECFA (300 g muscle, 100 g liver, 50 g kidney, and 50 g fat/person per day). The median value of a residue or contaminant in food is the appropriate value to be used if lifetime dietary intake is to be assessed. The procedure used here is not directly comparable to the one used when the Committee recommends numerical MRLs, which serve as the basis for estimating the concentrations of residues in food commodities.
- The results obtained in this way for muscle, liver, kidney and fat were summed up to calculate a figure for the total intake for 500 g of "meat".
- If data for several time points after implantation were available, the time points with the highest values were used. This was done to account for the fact that withdrawal periods have not been established for the use of any approved product. If the highest values did not coincide in time for all hormones, the time point with the highest results for estrogen intake was selected. This ensures a conservative approach because the ADI for estradiol-17 β is lower than for the other two compounds. The effects of this selection on the estimates obtained for the other hormones were negligible. For the purpose of this report these figures are referred to as *Theoretical Maximum Daily Intakes*.
- In order to estimate the above defined excess intakes, the Theoretical Maximum Daily Intakes calculated for the concurrent untreated control population was subtracted from the figure obtained using the corresponding data of implanted animals.

Table 2 summarizes the final results of all relevant intake calculations. It also provides information on excess intakes of estrogens, the most relevant group of residues.

For total estrogens the highest excess intakes calculated in this way were in the order of 30-50 ng/person per day (see Table 2, Synovex® in heifers in conjunction with comment 1 and Finaplex® in heifers in conjunction with comment 5). This range of excess intakes is less than 2% of the ADI for estradiol-17 β

(3000 ng for a 60 kg person). In studies carried out with experimental combinations, the resulting excess intakes were more than twice as high (about 4% of the ADI) if compared with the approved uses.

For progesterone the highest excess intake of the parent compound (which represents the only relevant hormonally active residue) was approximately 500 ng/person per day for its approved uses (see Table 2, Synovex® in calves). This excess intake corresponds to approximately 0.003% of the ADI for progesterone (1800 µg for a 60 kg person).

For testosterone the highest excess intake of the free hormone was in the order of 60 ng/person per day for all approved uses (see Table 2, Synovex® in heifers). This intake corresponds to approximately 0.05% of the ADI for testosterone (120 µg for a 60kg person). The intake of other possibly relevant metabolites, which are not precisely known, could theoretically be in the same order of magnitude.

Conclusions and recommendations

The Committee noted that the hormone concentrations found in individual populations of treated animals – despite the fact that they typically were statistically significantly higher than the corresponding values of the concurrent controls – were within the physiological range of these substances in cattle and that the calculated excess intakes contributed only a small additional hormonal burden to the background dietary intakes resulting from the consumption of other normal foods of both animal and plant origin.

Taking into consideration that the available data on the identity and concentrations of residues of the approved veterinary drugs in animal tissues indicate a wide margin of safety for consumption of residues in food when the products are used according to good practice in the use of veterinary drugs the Committee concluded that there would be no need to specify numerical MRLs for the three hormones and recommended MRLs “not specified”¹ in cattle tissues. The Committee recommended, however, that the total intake of estrogenic residues resulting from the use of any approved hormonal product be kept below the above calculated excess intakes.

¹ MRL “not specified“ means that available data on the identity and concentration of residues of the veterinary drug in animal tissues indicate a wide margin of safety for consumption of residues in food when the drug is used according to good practice in the use of veterinary drugs. For that reason, and for the reasons stated in the individual evaluation, the Committee concluded that the presence of drug residues in the named animal product does not present a health concern and that there is no need to specify a numerical MRL.

Table 2

Product	Animals	Comments	Description of the treatment of the animals	Theoretical Maximum Daily Intake [ng/person per day]					
				E ₁	E ₂ -17 α	E ₂ -17 β	Excess E ₁ +E ₂ - β	P	T
Synovex-S (E ₂ -b+P)	Steers	1	Control animals Animals slaughtered 15 days after implantation	1.0 2.0		0.5 6.3	6.8	190 254	
Synovex H (E ₂ -b+T-p)	Heifers	1	Control animals Animals slaughtered 15 days after implantation	1.4 3.9		1.5 15	16		17 70
Synovex-C (E ₂ -b+T) Synovex-H (E ₂ -b+T-p)	Calves a) female	1	Control animals, slaughtered on day 61 Control animals, slaughtered on day 119 Control animals, slaughtered on day 240 Control animals, slaughtered on day 301 Control animals, slaughtered on day 329 Control animals, slaughtered on day 360 implanted day 0; slaughtered on day 119	1.1 1.2 0.8 1.4 0.7 2.0 3.0		3.5 2.2 1.7 4.4 2.1 3.0 3.7	3.3	22 53	13 22 22 51
Synovex-C (E ₂ -b+T-p) Synovex S (E ₂ -b+P)	Calves b) castrated males		Control animals, slaughtered on day 61 Control animals, slaughtered on day 119 Control animals, slaughtered on day 240 Control animals, slaughtered on day 301 Control animals, slaughtered on day 329 Control animals, slaughtered on day 360 implanted on days 0, 118, 240; slaughtered on day 301	0.8 0.4 1.1 0.5 1.2 0.8 3.7		0.7 0.5 1.2 0.9 0.7 1.0 11	13	501 552 669 421 536 1170 540	
Synovex H (E ₂ -b+T-p)	Pregnant heifers	2	120 days pregnant , <u>unsynchronized</u> controls 120 days pregnant , synchronized controls 120 days pregnant , 61 days implanted 180 days pregnant , synchronized controls 180 days pregnant , 61 days implanted 240 days pregnant , synchronized controls 240 days pregnant , 61 days implanted	93 113 34 280 107 326 377		16 16 15 48 24 139 49	-80 -197 -39		203 172 233 282 237 377 326
Steer-oid (E ₂ + P) Heifer-oid (E ₂ +T-p)	Steers Heifers	3	Control animals Animals slaughtered 15 days after implantation Control animals Animals slaughtered 15 days after implantation			21 25 16 18	4 2	299 375	43 48
Compudose (E ₂)	Steers	4	Control animals Animals implanted 70-180 days	4.4 7.4		4.5 5.7	4.2		

Product	Animals	Comments	Description of the treatment of the animals	Theoretical Maximum Daily Intakes [ng/person per day]					
				E ₁	E ₂ -17 α	E ₂ -17 β	Excess E ₁ +E ₂ - β	P	T
Compudose (E ₂)	heifers	4	Control animals Animals implanted 84 days	3.1 3.6		3.7 4.3			
Compudose (E ₂)	Bull calves	4	Control animals Animals implanted	4.0 9.0		4.0 14	15		
	Bulls	4	Control animals Animals implanted	3.8 5.0		3.3 5.9	3.8		
	ZEBU Steers	4	Control animals Animals implanted	6.1 4.4		3.6 3.4	-1.9		
FINAPLIX (Tb-ac)	Heifers	5	implanted animals, slaughtered on day 15	4.6		23	27.6		
			implanted animals, slaughtered on day 30	5.2		29	34.2		
			implanted animals, slaughtered on day 60	3.9		13	16.9		
			implanted animals, slaughtered on day 75	3.7		14	17.7		
TORELOR (Tb-ac+E ₂)	Steers	5	Control animals Animals implanted on day 0; slaughtered on day 30	3.1 11		19 66	54.9		
			Animals implanted on days 0, 60; slaughtered on day 90	7.3		89	74.2		
Revalor (Tb-ac +E ₂)	Heifers	6	Control animals Animals implanted 30 days			1.0 6.6	5.6		
	Steers		Control animals Animals implanted 15 days			2.0 3.4	1.4		
Revalor (Tb-ac +E ₂) Implix BM (E ₂ +P)	Calves	7	Male control animals, slaughtered on day 30		57	6.6		628	
Male control animals, slaughtered on day 80				69	7.7	493			
Revalor implanted, slaughtered on day 80				132	63	55.3			
Revalor/Implix implanted, slaughtered on day 100				233	97	82.7	667		
Revalor (Tb-ac +E ₂) Implix BF (E ₂ +T)	Calves	7	Female control animals, slaughtered on day 30		60	6.5			19
Female control animals, slaughtered on day 80				59	8.6	493			
Revalor implanted, slaughtered on day 80				111	61	52.4			
Revalor/Implix implanted, slaughtered on day 15				179	99	83.9		208	

E₁, estrone; E₂, estradiol; P, progesterone; T, testosterone; T-p, testosterone propionate; Tb-ac, trenbolone acetate

Comments to Table 2

1 The calculations of intakes are based on determinations of the concentrations of free hormones in muscle, liver, kidney and fat. The fractions of the conjugated hormones were not determined. To obtain an estimate of the degree of under-estimation of the "true" Theoretical Maximum Daily Intake (TMDI), information from a study with implants containing ¹⁴C-labelled hormones/hormone esters in the same proportions as in the commercial products can be used. Based on total radioactivity found in tissues of animals slaughtered 15 days after implantation and on the fractions of total residues identified as conjugates the individual contributions in percent of the TMDI of the free vs. conjugated fractions present in the four standard edible tissues have been calculated as shown in the table below. From these data it appears justifiable to multiply the estimates of TMDIs for consumption of tissues from steers/heifers implanted with Synovex® S/Synovex® H by a factor of two. This correction is probably not relevant in the case of progesterone, where the tentatively identified conjugated metabolites have no significant gestagenic properties.

Substance implanted	Testosterone propionate		Estradiol benzoate				Progesterone	
	Heifers		Heifers		Steers		Steers	
fraction of total labelled residue	free	conjugated	free	conjugated	free	conjugated	Free	conjugated
	Contribution [%] to the TMDI							
Muscle	6.7	5.3	5.3	3.7	5.9	3.1	3.0	1
Liver	29.9	38.1	36.5	13.5	21.5	28.5	17.2	15.8
Kidney	3.8	4.2	9.9	18.1	9.9	18.1	26.5	22.5
Fat	11.0	1.0	12.2	0.8	11.6	1.4	13.2	0.8
Total	51.4	48.6	63.9	36.1	48.9	51.1	59.9	40.1

- 2 The calculations of intakes are based on determinations of the concentrations of free hormones in muscle, liver, kidney and fat. The fractions of the conjugated hormones were not determined. The data could not be corrected owing to a lack of relevant information; however, in view of the well established significant reduction in the TMDI as a consequence of implantation of pregnant heifers such a correction is apparently unnecessary.
- 3 The calculations of intakes are based on determinations of the concentrations of free hormones in muscle and fat. The given figures most likely greatly underestimate the "true" TMDIs. No information was available to correct these estimates.
- 4 The method used includes the extraction and deconjugation of conjugates. The estimated intake figures, therefore, represent total parent compound and can be used as they are given in the table.
- 5 The free and conjugated fractions of estradiol-17 β were determined in all tissues; however, estrone (free fraction only) was determined only in liver and fat. The "true" TMDIs for estrogens, therefore, could well be 50% higher than the values given in the table. Data on which a more precise estimate of a correction factor could be based were not available. The study on Torelor® represents research and does not reflect an approved use.
- 6 From the method description it appears that conjugates are not included in the determination of the residues. In view of the effects of trenbolone-estradiol combinations on estrogen concentrations seen in other studies, it cannot be excluded that the data given in the table significantly underestimate the "true" TMDI for estrogens.
- 7 No description of the analytical method was given in the report. The values for estradiol-17 α are based on concentrations found in the liver only. The study represents research and does not reflect an approved use.

Annex 2

General consideration items

1. Evaluation of antimicrobial agents

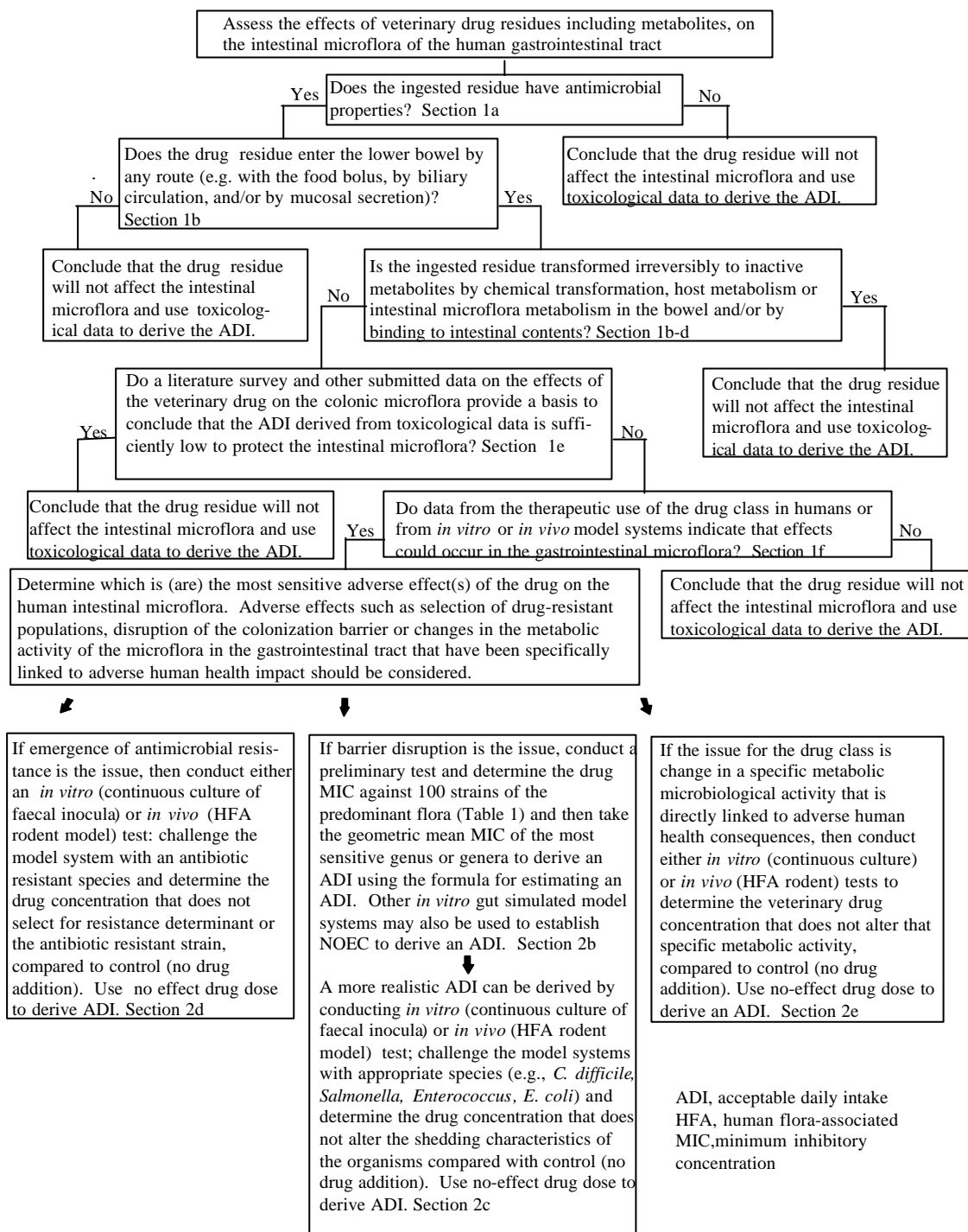
The microbiological risk associated with residues of antimicrobial agents in food resulting from their use in animals was addressed at the thirty-second, thirty-sixth, forty-second, forty-fifth and forty-seventh meetings of the Committee. At the present meeting, with continued interest in evaluating the public health impact of use of antimicrobial agents in food-producing animals, the Committee prepared a more systematic approach to assessing the effects and safety of antimicrobial drug residues on the human intestinal microflora.

Recommendations for the evaluation of veterinary drug residues for their potential to affect human intestinal microflora

Given the current state of science, in the absence of validated predictive models but with the need to determine an ADI, the Committee proposed a comprehensive, tiered decision-tree system that will permit use of all relevant data from model test systems *in vitro* and *in vivo* in addition to minimum inhibitory concentrations (MICs). Use of the procedure will provide a method for the systematic evaluation of the safety of residues of antimicrobial veterinary drugs in food (see Figure 1).

1. Additional microbiological data are not required if there is evidence of at least one of the following:
 - (a) The veterinary drug and its residues in milk and edible tissues do not have antimicrobial properties.
 - (b) The ingested residue does not enter the colon. Data on the excretion of oral doses and their pharmacokinetics and metabolism may be useful.
 - (c) Ingested residues are transformed to inactive metabolites before entering the lower bowel. Pharmacokinetic and metabolic studies may be used for this evaluation.
 - (d) Ingested veterinary drug residues are transformed quantitatively to microbiologically inactive metabolites or bound quantitatively to the colon contents, resulting in drug inactivation soon after entry into the lower bowel.
 - (e) A literature survey of the effects of the veterinary drug *in vitro* or *in vivo* on colonic microflora provides a basis for concluding that the ADI derived from toxicological data is sufficiently low to protect the intestinal microflora. Data relevant for making this assessment would include, for example, changes in predominant colonic populations, changes in the colonization barrier, changes in resistant bacterial populations in continuous culture, the drug's effects on the predominant gastrointestinal microbial flora in animals or humans in a colonization barrier model, or changes in the incidence of antimicrobial resistance in the gut microflora as end-points.
 - (f) Human clinical data show that therapeutic use of the drug results in an incidence of toxicological effects that is substantially higher than that of any gastrointestinal side-effects due to disruption of the microflora. Thus, an ADI would be driven by toxicological data, not microbiological data.

Fig. 1. Decision tree on determining the adverse microbiological effects of residues of antimicrobial drugs in food-producing animals.



2. The types of microbiological studies recommended for establishing an ADI, if none of the above can be demonstrated, are:

- (a) First, if data are not available to address items 1-6 above and new information is needed, the drug class should be considered to determine whether the main concern is emergence of resistance or disruption of flora (see Figure 1).
- (b) If colonization barrier effects are a concern, in the absence of other relevant data, the MIC of the veterinary drug against a total of 100 bacterial strains, comprising 10 isolates of suitable organisms to represent relevant genera (such as *Bifidobacterium* spp., *Bacteroides* spp., *Clostridium* spp., *Eubacterium* spp., *Fusobacterium* spp., *Enterococcus* spp., *Lactobacillus* spp., *Peptostreptococcus* spp., *Peptococcus* spp., and *Ruminococcus* spp.) of the microflora in the human gastrointestinal tract (Table 1), can be determined as a conservative estimate of the ADI. The relevance of the MIC of a veterinary drug against *E. coli* can be debated. It is the twenty-second most abundant species commonly detected and represents 1.2% of the total isolates from the human gastrointestinal tract (Moore & Moore, 1995). Therefore, *E. coli* should not be considered one of the most relevant species in screening typical intestinal microflora for the effects of antimicrobial compounds; however, an increase in the number of *Enterobacteria* can be used to indicate colonization resistance in the gastrointestinal tract (Corpet, 1993), since they are easy to culture and an increase in number may suggest the potential for infection by pathogens.

Standardized MIC tests using US National Committee for Clinical Laboratory Standard methods or equivalent should be used at inoculum levels of 10^7 and 10^9 colony-forming units per ml. When multiple strains of the same species are studied, the convention is to quote MIC₅₀ and MIC₉₀ values, the MIC necessary to inhibit 50 and 90%, respectively, of the tested strains of a given species. Since 10 species (or multiple strains of several species within a particular genus) may be tested for purposes of the formula used for estimating an ADI, the MIC₅₀ and geometric mean in this context refers to the summaries calculated for each bacterial genus. In practice, if 100 relevant strains are tested as noted above and the overall geometric mean of the most sensitive bacterial genera is used, then it is not necessary to use a safety factor. To ensure the greatest margin of safety, the choice of microorganism should be restricted to the most sensitive relevant genera that are normally considered sensitive to the test compound.

If other suitable, scientifically defensible data are available, the formula used for estimating an ADI should not be the primary or sole criterion for determining an ADI. For example, using results from *in vitro* models like the semicontinuous culture system to monitor population changes of target microorganisms within the normal microflora, changes in volatile fatty acids, bacterial enzymes linked to specific adverse effects in humans, emergence of resistance, or barrier disruption, a no-effect concentration (NOEC) can be established to derive an ADI (see Figure 1).

- (c) If colonization barrier disruption is the concern and no data are available, then information should be provided to show at least one of the following:
 - i. Addition of the veterinary drug in a range of concentrations covering the colonic concentrations expected for an ADI does not alter the barrier afforded by continuous culture of faecal inocula against colonization by an added microorganism (e.g. *C. difficile*, zoonotic pathogens, or *Pseudomonas* challenge). Since there is no disruption of the colonization barrier, this concentration would be used as the NOEC.
 - ii. Oral administration of the veterinary drug to a monogastric animal (e.g. rat, mouse, or human flora-associated rodent) to deliver colonic concentrations expected for an ADI in humans shows “no effect” on the colonization barrier to challenge oral doses of *C. difficile* or zoonotic or opportunistic pathogens.

Table 1**Relevant bacterial species isolated from the human gastrointestinal tract^a**

<i>Bacteroides vulgatus</i>	<i>Peptococcus</i> spp.
<i>Bacteroides uniformis</i>	
<i>Bacteroides stercoris</i>	<i>Peptostreptococcus anaerobius</i>
<i>Bacteroides fragilis</i>	<i>Peptostreptococcus productus</i>
<i>Bacteroides ovatus</i>	<i>Peptostreptococcus parvulus</i>
<i>Bacteroides caccae</i>	<i>Peptostreptococcus micros</i>
<i>Bacteroides distasonis</i>	<i>Peptostreptococcus prevotii</i>
<i>Bacteroides thetaiotaomicron</i>	
<i>Bacteroides capillosus</i>	<i>Prevotella</i> spp.
<i>Bacteroides merda</i>	
	<i>Ruminococcus bromii</i>
<i>Fusobacterium prausnitzii</i>	<i>Ruminococcus obeum</i>
<i>Fusobacterium russii</i>	<i>Ruminococcus gnavus</i>
	<i>Ruminococcus callidus</i>
<i>Bifidobacterium adolescentis</i>	<i>Ruminococcus torques</i>
<i>Bifidobacterium longum</i>	<i>Ruminococcus albus</i>
<i>Bifidobacterium catenulatum</i>	
<i>Bifidobacterium infantis</i>	<i>Enterococcus faecium</i>
<i>Bifidobacterium angulatum</i>	<i>Enterococcus faecalis</i>
	<i>Enterococcus siraeum</i>
<i>Eubacterium aerofaciens</i>	
<i>Eubacterium rectale</i>	<i>Lactobacillus acidophilus</i>
<i>Eubacterium bifforme</i>	<i>Lactobacillus fermentum</i>
<i>Eubacterium eligens</i>	
<i>Eubacterium lentum</i>	<i>Escherichia coli</i> ^b
<i>Eubacterium ventriosum</i>	
	<i>Propionibacterium acnes</i>
<i>Clostridium perfringens</i>	
<i>Clostridium butyricum</i>	
<i>Clostridium ramosum</i>	
<i>Clostridium indolis</i>	

^aAdapted from Moore & Holdeman (1974); Holdeman et al. (1977); Finegold et al. (1983); Drasar & Duerden (1991); Carman et al. (1993); Committee for Veterinary Medicinal Products (1995); Moore & Moore (1995)

^bSee Section 2b

- (d) If emergence of antimicrobial resistance from consumption of residues is the concern, then *in vitro* (continuous culture of faecal inocula) or *in vivo* (mouse, rat, human flora-associated rodent, or pig) data to show that expected residue concentrations in the colon do not change the antibiotic resistance of resident populations of *E. coli* or other bacteria appropriate for the drug class should be provided.
- (e) If changes in enzymatic activity that are specifically linked to an adverse consequence in humans are observed, then that microbiological end-point may be appropriate for some drugs.

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2. Safety of residues at the injection site

The Committee was asked by the Codex Committee on Residues of Veterinary Drugs in Foods to re-consider the safety of residues of drugs that may be present at the injection site and to consider the establishment of an acute reference dose (acute RfD) in such cases. The acute RfD has been defined by a joint FAO/WHO consultation¹ as "... the estimate of the amount of a substance in food or drinking-water, expressed on a body weight basis, that can be ingested over a short period of time, usually during one meal or one day, without appreciable health risk to the consumer on the basis of all the known facts at the time of the evaluation. It is usually expressed in milligrams of the chemical per kilogram of body weight".

The primary objective in performing a safety assessment is to ensure that the average daily consumption of residues over a lifetime does not exceed the ADI and that consumption on a single day does not exceed the acute RfD. The Committee noted that while the safety of residues in edible tissues is usually assessed on the basis of long-term intake, drug residues at the injection site may pose an acute hazard and may therefore require the establishment of an acute RfD. The Committee expressed the view that under some circumstances identification and removal of injection sites after slaughter may not be a practical means of ensuring that humans are not exposed to injection site residues that exceed the acute RfD. The Committee concluded that when a single intake of residue from an injection site is likely to exceed the acute RfD and a withdrawal period is specified, it should be sufficient for residues at the injection site to deplete below the acute RfD.

3. General topics on MRLs

At its forty-eighth meeting, the Committee considered several initiatives to make its process for setting MRLs more transparent. These issues were initially discussed at the fiftieth meeting of JECFA and at that meeting the Committee refined the issues into a series of specific policy and technical questions on the establishment of recommended MRLs for veterinary drug residues in food. Assignments were made for preparation of background and working papers for consideration at the present meeting, and the Committee took the indicated action on the following items:

Guidelines on the establishment of MRLs for minor animal species

Nearly all MRLs for veterinary drug residues established to date by the Committee have been for edible tissues as well as for milk and eggs of major animal species (i.e. cattle, sheep, pigs, and chickens). Very few MRLs have specifically been established for minor animal species (e.g. deer and

¹ Food consumption and exposure assessment of chemicals. Report of a FAO/WHO Consultation. Geneva, 10-14 February 1997. World Health Organization, 1997 (WHO/FSF/FOS/97.5).

rabbits). This paper outlines proposed guidelines to be used by JECFA when MRLs for veterinary drug residues are to be considered for such minor species. The FAO Secretariat will forward this paper for consideration at the next session of the Codex Committee with a request for comments on this approach.

Residue considerations for establishing MRLs for fish

Establishing MRLs for fish presents several problems, including the definition of edible tissues and the complex pharmacokinetic properties and metabolism of veterinary drugs in fish. This paper provides a preliminary outline of the various issues involved. A more detailed paper will be prepared for consideration at the fifty-fourth meeting of the Committee.

Guidelines on the establishment of MRLs in honey and other bee products

Honey and other bee products are food items that are not a significant part of the usual diet. However, veterinary drugs are used to treat or control diseases in bees, and residues in the products can result. This paper proposes a practical approach to the establishment of MRLs for such residues. The FAO Secretariat will refer this paper to the Codex Committee for review with a request for comments on the approach.

Expression of maximum residue limits

This paper discusses the principles by which numbers are expressed and the issues involved when a numerical MRL is recommended by the Committee. A final paper will be prepared for consideration at the fifty-fourth meeting of the Committee.

Guidance on tissues for which MRLs are to be established for animal food products in international trade

Since its thirty-eighth meeting the Committee has, whenever possible, identified two target tissues for establishment of MRLs. One is either muscle or fat (for international trade) and the other is either liver or kidney (commonly used for residue control by national authorities). Some national authorities, however, have expressed the need for MRLs in all tissues when examining imported animal food products. This paper provides proposed guidance on this issue and the FAO Secretariat will provide it to the Codex Committee for review and comment.

Guidance on the establishment of MRLs for minor species when no MRL exists for the drug residue in a major animal species

A veterinary drug may be proposed for a use in a minor animal species that has never been considered for use in a major species. The drug may be useful, for example, in a species with unique therapeutic needs (e.g. bees) or in a minor species that constitutes a significant portion of meat consumed in a given country (e.g. goats or salmon). In such cases, both the toxicological and residue data may not be available in the quality or quantity normally expected when the Committee considers drugs for major animal species. This paper proposes an approach to this problem as well as a strategy that is both practical and protective of public health. A final version of this paper will be considered at the fifty-fourth meeting of the Committee.

4. Statistical approaches for determining MRLs

In order to improve the transparency and scientific basis for recommending MRLs, the Committee has been developing two approaches that are complementary, yet individually applicable and are dependent on the quality and quantity of the data on residues that are available. When the residue database is limited (e.g. three animals per group) on a veterinary drug with a long history of use, the Committee has agreed in principle to use mean values and consider incorporating three standard deviations for determining upper limits for residues at an individual time point. This approach was used with two compounds that were evaluated at the present meeting.

When a large amount of highly reliable data that permits a comprehensive statistical analysis is available, a more highly refined statistical approach may be applied. This approach was used at the fiftieth meeting of the Committee in the determination of MRLs for eprinomectin. The Committee will continue to evaluate various approaches for determining the most suitable MRLs for a particular substance. It should be noted, however, that the Committee will continue to determine MRLs on a case-by-case basis, dependent upon specific considerations of a substance. The FAO Secretariat will make summaries of these two procedures available and will provide them to the Codex Committee for review and comment.

5. Evaluation policies for residues of veterinary drugs in food

The Committee considered a paper which compiled and summarized all of the policies related to establishing ADIs and recommending MRLs contained in reports of the Committee on residues of veterinary drugs that have been held to date. This document gathers in one place for the first time all of the policies related to the evaluation of veterinary drug residues since the thirty-second meeting that was held in 1987 (the first meeting that dealt exclusively with veterinary drugs). The Committee requested that the document be published by FAO in a form that can be updated on a periodic basis as needed. The Committee also recommended that the document be made available as soon as possible on the FAO and WHO web pages in either PDF or HTML format. The FAO Secretariat will forward the document to the Codex Committee for review and comment.

6. Requirements for validation of analytical methods

The Committee reviewed a document on the requirements for validation of methods for generating pharmacokinetic and depletion data that are used for evaluating residues of veterinary drugs in food. The Committee concluded that the document reflects current policy for assessing the adequacy of methods used in studies that are reviewed and for assessing the suitability of methods proposed for regulatory use in support of MRLs. Recognizing that the Codex Committee on Residues of Veterinary Drugs in Foods has an important role to play in establishing requirements for suitable validation of methods to be used in regulatory programs in support of Codex MRLs, the document will be published and made available to the Codex Committee, sponsors, and to other interested parties.

Protocols for recognized analytical methods, appropriate analytical standard materials and, in some cases, reagents or other test materials that are not commercially available from sources other than the sponsor are of critical importance to official regulatory laboratories. The Committee recommended that the Codex Committee consider elaborating procedures to ensure the availability of such information and materials.

7. Harmonization with the Joint FAO/WHO Meeting on Pesticide Residues on substances used both as veterinary drugs and pesticides

Some substances are used both as a veterinary drug and a pesticide. Application to plant commodities that are used as animal feed or dermal application to animals for control of pests may result in residues in edible animal tissues. Residues may also arise in animal tissues from the veterinary administration of the same substance to food animals. Because of the differences that have evolved in the respective evaluation processes used by the Joint FAO/WHO Expert Committee on Food Additives and the Joint FAO/WHO Meeting on Pesticide Residues, divergent MRLs, and to a lesser extent ADIs, have sometimes resulted for the same chemical.

The Codex Committee on Pesticide Residues at its Thirtieth Session in 1998 recommended that the Joint FAO/WHO Meeting on Pesticide Residues and the Joint FAO/WHO Expert Committee on Food Additives work to harmonize residue definitions, sampling procedures, and other factors that are taken into consideration when recommending MRLs. The Eleventh Session of the Codex Committee on Residues of Veterinary Drugs in Foods held in 1998 also recognized the problem and recommended that

the joint secretaries of the Joint Meeting and the Expert Committee convene an informal meeting of experts from both groups to address the issues. This informal meeting was held prior to the present meeting of the Committee.

The informal meeting based its deliberations on issue papers prepared by the invited experts. These papers addressed approaches for recommending MRLs of lipid-soluble compounds in animal tissues and milk, residue definitions of products collected for residue analysis, sampling procedures, estimation of dietary intake, and risk assessment procedures used for determining MRLs. A set of 24 recommendations to be considered by the Joint FAO/WHO Expert Committee on Food Additives and the Joint FAO/WHO Meeting on Pesticide Residues and the relevant Codex Committees was developed. The report of the meeting will be forwarded to the Codex Committees for their consideration.