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Evaluation of certain veterinary drug residues in food

Thirty-second Report of the
Joint FAO/WHO Expert Committee on
Food Additives



World Health Organization
Technical Report Series
763



World Health Organization, Geneva 1988

Monographs containing summaries of relevant data and toxicological evaluations are available under the title:

Toxicological evaluation of certain veterinary drug residues in food. Cambridge, Cambridge University Press, 1988 (WHO Food Additives Series, No. 23)

Specifications are issued separately by FAO under the title:

Residues of some veterinary drugs in animals and foods. (To be published as an FAO Food and Nutrition Paper.)

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

The preparatory work for toxicological evaluations of food additives and contaminants by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is actively supported by certain of the member States that contribute to the work of the International Programme on Chemical Safety (IPCS).

The International Programme on Chemical Safety (IPCS) is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. One of the main objectives of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment.

ISBN 92 4 120763 9

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ISSN 0512-3054

PRINTED IN SWITZERLAND

87/7434 - Schüler SA - 7000

90/8415 - Schüler SA - 1000(R)

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JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

Rome, 15-23 June 1987

Members

- Dr H. Blumenthal, Director, Division of Toxicology, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA
- Dr J. Boisseau, Director, National Laboratory of Veterinary Drugs, La Haute Marché, Javené, Fougères, France
- Dr L. Cuerpo, Research Chemist, National Institute of Agriculture and Livestock Technology, Castelar, Argentina
- Dr R. Ellis, Director, Chemistry Division, Food Safety and Inspection Service, United States Department of Agriculture, Washington, DC, USA (*Vice-Chairman*)
- Dr J. Juskevich, Residue Safety Branch, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Rockville, MD, USA
- Dr D.P. Kariuki, Director, Veterinary Research Department, Kenya Agricultural Research Institute, Kikuyu, Kenya
- Dr T. McEwan, Director, Biochemistry Branch, Animal Research Institute, Department of Primary Industries, Brisbane, Queensland, Australia (*Joint Rapporteur*)
- Dr B.H. MacGibbon, Senior Principal Medical Officer, Division of Toxicology and Environmental Protection, Department of Health and Social Security, London, England (*Joint Rapporteur*)
- Dr J.G. McLean, Dean, Faculty of Applied Science, Swinburne Institute of Technology, Hawthorn, Victoria, Australia
- Professor A. Rico, Director, Laboratory of Biochemical and Metabolic Toxicology (National Institute of Agronomic Research), National Veterinary School, Toulouse, France
- Professor A. Somogyi, Director, Department of Drugs, Animal Nutrition and Residue Research, Institute for Veterinary Medicine, Federal Office of Public Health, Berlin (West) (*Chairman*)

Secretariat*

- Dr J.R.P. Cabral, Unit of Mechanisms of Carcinogenesis, International Agency for Research on Cancer, Lyon, France (*WHO Temporary Adviser*)
- Dr L.M. Crawford, Chairman, Codex Committee on Residues of Veterinary Drugs in Foods; and Associate Administrator, Food Safety and Inspection Service, United States Department of Agriculture, Washington, DC, USA (*Member of FAO Secretariat*)
- Mrs B. Dix, Food Standards Officer, Joint FAO/WHO Food Standards Programme Group, Food Quality and Standards Service, Food Policy and Nutrition Division, FAO, Rome, Italy
- Professor C.L. Galli, Head, Toxicology Laboratory, Institute of Pharmacological Sciences, University of Milan, Milan, Italy (*WHO Temporary Adviser*)

- Dr G. Gheorghiev, Nutrition Officer (Consumer Protection), Food Policy and Nutrition Division, FAO, Rome, Italy
- Mr R. Hankin, Commission of the European Communities, Brussels, Belgium
(*WHO Temporary Adviser*)
- Dr G. Heinz, Senior Officer (Meat Technology), Animal Production and Health Division, FAO, Rome, Italy
- Dr J.L. Herrman, Division of Food and Colour Additives, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA (*WHO Consultant*)
- Dr R.C. Livingston, Director, Division of Drug Manufacturing and Residue Chemistry, Food and Drug Administration, Rockville, MD, USA (*FAO Consultant*)
- Dr A.W. Randell, Nutrition Officer (Food Science), Food Policy and Nutrition Division, FAO, Rome, Italy (*Joint Secretary*)
- Dr S.I. Shibko, Associate Director to Toxicological Evaluation, Division of Toxicology, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA (*WHO Temporary Adviser*)
- Dr P. Shubik, Senior Research Fellow, Green College, Oxford, England (*WHO Temporary Adviser*)
- Professor M. Swietlikowski, Animal Production and Health Division, FAO, Rome, Italy
- Professor E. Takabatake, Faculty of Pharmaceutical Sciences, Setsunan University, Hirakata-shi, Osaka, Japan (*WHO Temporary Adviser*)
- Dr G. Vettorazzi, Toxicologist, International Programme on Chemical Safety, Division of Environmental Health, WHO, Geneva, Switzerland (*Joint Secretary*)
- Dr K.N. Woodward, Division of Toxicology and Environmental Protection, Department of Health and Social Security, London, England (*WHO Temporary Adviser*)

* Unable to attend: Dr S. Fitzpatrick, Center for Veterinary Medicine, Food and Drug Administration, Rockville, MD, USA (*WHO Temporary Adviser*).

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EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD

Thirty-second Report of the Joint FAO/WHO Expert Committee on Food Additives

A meeting of a Joint FAO/WHO Expert Committee on Food Additives was held at FAO Headquarters, Rome, from 15 to 23 June 1987. The meeting was opened by Mr J.R. Lupien, Chief, Food Quality and Standards Service, Food Policy and Nutrition Division of FAO, who welcomed the participants on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Mr Lupien stated that the present meeting had been convened especially to consider the subject of residues of veterinary drugs in foods.

Mr Lupien noted that at previous meetings the Committee had evaluated the use of a number of antibiotics and other chemotherapeutic agents that, after administration to animals, could under certain conditions leave residues in foods of animal origin. In addition, in 1982 and 1983, the Committee had evaluated the safety of two xenobiotic agents for promoting growth in meat-producing animals. On the advice of an *ad hoc* Joint FAO/WHO Expert Consultation convened in 1984 to discuss the need for special work in this area, the Codex Alimentarius Commission had established, within its structure, the Codex Committee on Residues of Veterinary Drugs in Foods to make recommendations to governments on acceptable levels for residues of veterinary drugs in foods. At its first session, this Codex Committee had strongly recommended that a Joint FAO/WHO Expert Committee provide it with independent scientific advice.

Mr Lupien noted that the subject of residues of veterinary drugs in food covered a broad range of disciplines, and had significant social and economic implications, since actions taken independently by one country could have a marked impact on the agricultural practices and trade of others. The subject was also of interest to consumers who required reassurance that veterinary drugs were used in ways that ensured safe residue levels in edible animal products.

Because of the multidisciplinary nature of the subject Mr Lupien stressed that the Committee should take into account all of the scientific and technical data concurrently, so that Acceptable Residue Levels set by the Committee for animal products and Acceptable Daily Intakes (ADIs) set for human beings would be linked together.

1. INTRODUCTION

A Joint FAO/WHO Expert Consultation was held in Rome in November 1984 (1), in response to a recommendation of the fifteenth session of the Codex Alimentarius Commission, to consider various issues relating to the presence in food of chemicals used in animal husbandry and veterinary medicine. Those present at the Consultation recommended *inter alia* that immediate consideration should be given by the Codex Alimentarius Commission to the establishment of a Codex Committee on Residues of Veterinary Drugs in Foods. They also recommended that the Directors-General of FAO and WHO be requested to give earliest consideration to the convening of an appropriate scientific body, from time to time and as necessary, to advise Member governments and the Codex Committee on questions pertaining to residues of veterinary drugs in foods of animal origin, in terms of both potential public health hazards and barriers to international trade.

The present meeting of the Joint FAO/WHO Expert Committee on Food Additives¹ was convened in response to this latter recommendation and those made at the twenty-sixth and twenty-seventh meetings of the Committee (Annex 1, references 59, 62). At its present meeting, the Committee's purpose was to provide guidance to FAO and WHO Member States and to the Codex Alimentarius Commission on public health issues pertaining to residues of veterinary drugs in foods of animal origin. The specific tasks before the Committee were:

(a) to establish principles for evaluating the safety of residues of veterinary drugs in foods and for determining acceptable and safe levels for such residues when the drugs in question are administered

¹ As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in 1955 (FAO Nutrition Meeting Report Series, No. 11, 1956; WHO Technical Report Series, No. 107, 1956), there have been thirty-one previous meetings of the Joint FAO/WHO Expert Committee on Food Additives (Annex 1).

to animals in accordance with good veterinary and animal husbandry practices;

(b) to determine criteria for appropriate methods of analysis for detecting or quantifying residues of veterinary drugs in foods;

(c) to evaluate or re-evaluate the safety of residues of certain veterinary drugs; and

(d) to discuss and provide advice on matters arising from the report of the first session of the Codex Committee on Residues of Veterinary Drugs in Foods (2).

The Joint FAO/WHO Expert Committee on Food Additives has previously considered the use and assessed the safety of certain veterinary drugs and their residues. At its twelfth meeting, the Committee evaluated the safety of, and developed specifications for, several antibiotics that may, as a result of their addition to animal feed or use by veterinarians to treat infection, be present in animal tissues consumed as human food. Recommendations regarding the use of these agents and for further studies were made in the report of the Committee (Annex 1, reference 17).

At various meetings, the Committee has devoted considerable attention to the safety assessment of hormones in animal production. General principles for the assessment of both hormones of natural origin (and their derivatives) and synthetic compounds with hormonal activity were elaborated at the twenty-fifth meeting of the Committee (Annex 1, reference 56). The specific xenobiotic anabolic agents trenbolone acetate and zeranol were evaluated at the twenty-sixth and twenty-seventh meetings of the Committee (Annex 1, references 59 and 62). After considering the results of detailed studies on the nature and levels of residues of these agents and their metabolites and the results of toxicological studies, the Committee provisionally accepted the use of trenbolone acetate and zeranol as anabolic agents for the production of meat for human consumption in accordance with good animal husbandry practice (Annex 1, reference 62). Further studies on these agents were considered at the present meeting of the Committee (see section 3.2.2).

Guidelines established at previous meetings when the Committee evaluated the safety of anabolic agents used in animal production have been consolidated in Annex II of *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference 76).

2. GENERAL CONSIDERATIONS

As this was the first meeting of the Committee devoted exclusively to the evaluation of residues of veterinary drugs in foods, the Committee discussed procedures that would ensure that its reputation for impartial scientific evaluation of data was maintained. In this regard, the members of the Committee noted that (a) during their term of office, they were responsible only to the two organizations concerned, FAO and WHO; (b) they were invited as participants in their individual capacities and not as representatives of their governments or institutions; and (c) their discussions were confidential (3).

2.1 Principles governing the safety evaluation of residues of veterinary drugs in food

In making recommendations on the safety of residues of veterinary drugs in food, the Committee took into consideration the principles contained in *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference 76), in previous reports of the Committee in which residues of veterinary drugs in food had been considered (Annex 1, references 56, 59, and 62), and in the report of the Joint FAO/WHO Expert Consultation on Residues of Veterinary Drugs in Foods (1). The Committee considered it appropriate and helpful to outline these general principles (see sections 2.1.2 and 2.1.3), but believed that it was desirable to encourage innovation and further developments in such areas as toxicology and residue analysis, and to this end did not wish to be unduly rigid in its requirements for information and its interpretation of the principles.

2.1.1 Definitions

The Committee accepted the following definitions, which had been adopted by the Codex Committee on Residues of Veterinary Drugs in Foods (2):

A "veterinary drug" is "any substance applied or administered to any food-producing animal, such as meat or milk-producing animals, poultry, fish or bees, whether used for therapeutic, prophylactic or diagnostic purposes or for modification of physiological functions or behaviour".

“Residues of veterinary drugs” include “the parent compounds and/or their metabolites in any edible portion of the animal product, and [include] residues of associated impurities of the veterinary drug concerned”.

2.1.2 *Information needed on drugs to be evaluated*

General characteristics

The Committee must be assured that any veterinary drug it evaluates is well characterized, with regard to both the active substance and its major impurities. Details of chemical and physical characteristics of the drug, including impurities, are needed. In addition, the manufacturing process should be described and the consistency and quality of the final product demonstrated; the process must be capable of yielding a reproducible product.

Substances to be evaluated by the Committee should be registered as veterinary drugs in at least one country.

Use patterns

Data should be submitted on the patterns of use of each veterinary drug, including information about what are recognized as good veterinary or animal husbandry practices under different local conditions. Information is required on the purposes for which the product is used, doses used, methods of administration, target species, and recommended withdrawal times.¹ In certain cases it may be necessary for the Committee to be assured that the recommended withdrawal times can be observed in usual practice.

Pharmacological characteristics

Information on the pharmacological activities of the drug and its mechanisms of action is desirable.

Analytical criteria

Methods of analysis should be specified. These are needed to: detect, quantify, and positively identify residues of veterinary drugs; support toxicological, drug metabolism, and pharmacokinetic studies; support residue studies of compounds to be evaluated by the Committee; and satisfy the needs of public health agencies. The

¹ The withdrawal time after administration of a drug is the time during which animals or animal products should not be harvested (by fishing, milking, slaughtering, egg collection, etc.) for human consumption.

performance of analytical methods should be assessed, as appropriate, according to some or all of the following criteria:

- (a) accuracy,
- (b) precision,
- (c) specificity,
- (d) sensitivity,
- (e) reproducibility,
- (f) reliability,
- (g) cost-effectiveness.

For analytical methods used in toxicological, drug metabolism, and pharmacokinetic studies, criteria (a) to (e) are most important. For methods used in residue depletion studies done under field conditions (for example to permit the setting of an Acceptable Residue Level for a drug used in accordance with good animal husbandry practice), criteria (a) to (f) are important. Analytical methods to be used by public health agencies for residue testing of foods of animal origin must be assessed on all seven criteria for each residue of interest.

The criteria listed should be considered in assessments of qualitative as well as quantitative (including confirmatory) analytical methods. In addition, ongoing quality assurance and quality control programmes are essential to ensure the acceptable performance of analytical methods according to the necessary criteria.

Metabolism and pharmacokinetics

Before the safety of residues of a particular veterinary drug can be assessed, appropriate metabolic studies in the food-producing animal are generally required to identify and quantify the residues. These studies should simulate the conditions of use of the drug in animal husbandry as closely as is practical. The pharmacokinetics of the residues should be examined between the time of administration of the drug and the time the animals enter the human food supply.

Metabolic studies may also be required in the animal species used for toxicological investigations, in order to ensure that these laboratory animals are exposed to the same array of compounds as are human consumers of the animal products.

Toxicological data

For any drug to be assessed by the Committee, it is essential to have toxicological data on the most important residues found in the food-producing species, i. e., the parent compound or its metabolites or both. The safety assessment of a residue depends on the quantity present, its bioavailability, and its potential toxicity.

Criteria for the testing requirements for the safety assessment of food additives and contaminants have been established (Annex 1, reference 76). Many of these criteria are applicable to veterinary drug residues. The extent of toxicological data needed depends not only on the likely dietary exposure of human beings to the compound of interest, but also on, for example, whether the compound is normally present in human tissues (see section 3.2.1).

When drugs used for veterinary purposes are also used therapeutically in human beings, information from human case reports or epidemiological studies may be available. Such data not only influence the amount of toxicological information required from animal studies but may provide important evidence of possible adverse reactions in human beings that are not detectable in animal models (see, for example, section 3.1 on chloramphenicol).

The Committee emphasized the importance of improving understanding of the mechanisms involved in toxic effects, rather than following a check-list approach to toxicological testing requirements. For example, studies not normally required for evaluation, such as immunotoxicity or neurotoxicity studies, may sometimes be useful.

Residue depletion studies under field conditions

The Committee agreed that field trials of veterinary drugs meeting the following criteria will usually provide adequate information on residue depletion and allow the setting of acceptable withdrawal periods for the drugs under good veterinary or animal husbandry practices:

(a) The mode of administration, dosage, and formulation of the drug should be the same as those proposed for its intended use(s) in food-producing animals.

(b) Animal groups should be large enough to allow meaningful statistical assessment of the data.

(c) After animals have been treated with the drug, tissues and biological fluids should be collected at appropriate times for residue analysis, so that a recommended withdrawal period can be set.

2.1.3 *Assessment of data*

End-points of assessment

Where possible and appropriate an ADI based on determination of a no-observed-effect level from toxicological or human data, and application of a suitable safety factor, is used as the end-point of the toxicological evaluation. However, inadequacies in the toxicological data may sometimes make it difficult to establish a no-observed-effect level. In other instances it may be inappropriate to set an ADI, for example when a compound being assessed as a residue of a veterinary drug is also present normally in the human body (see, for example, discussion on estradiol-17 β in section 3.2.1).

Assessment of toxicity

Residues of veterinary drugs may be present as a combination of three major forms: free, conjugated, and covalently bound to cellular constituents.

The Committee recognized that covalently bound residues make up a substantial portion of the total residues for certain compounds. It proposed that principles for assessing the biological impact, including the bioavailability, of these types of residues be a topic for discussion at a future meeting.

An ADI for a drug is usually based on the toxicity of the parent drug rather than on its metabolite(s). However, it may sometimes be necessary to calculate ADIs for individual metabolites.

Acceptable residue levels

When an ADI is established, consideration of the estimated intakes of the relevant foods by human beings allows an assessment to be made of a safe and acceptable residue level for the relevant animal tissue(s).

If the levels of residues estimated from supervised trials, when the drug is administered according to good animal husbandry practice, are *below* those considered toxicologically acceptable, then the levels determined by good practice will dictate the Acceptable Residue Level recommended by the Committee, provided that practical analytical methods are available for routine residue analysis.

If the levels of residues found in practice *exceed* those determined to be acceptable from the toxicological evaluation and consumption data, then drug use in the food-producing animals may need to be modified to reduce residue concentrations in edible tissues to acceptable levels. Possible modifications include extending the withdrawal periods and changing the drug dosage form or method of delivery.

When it has been determined that an ADI is unnecessary because the compound of interest is produced endogenously in human beings and animals, then the establishment of an Acceptable Residue Level is also unnecessary. At the other extreme, when an ADI has not been allocated because, on toxicological grounds, the safety of the compound cannot be assured, then no Acceptable Residue Level should be established either.

Microbiological aspects

The principles outlined here apply to the evaluation of residues of all veterinary drugs. For the establishment of tolerance limits for residues of certain chemotherapeutic agents, however, the antimicrobial properties of the residues must also be taken into account. Antimicrobial properties will indeed become the determining factor in safety evaluation when the toxicity of the substances to be considered (for example tetracyclines, β -lactam antibiotics) is so low that their residues in food could, from the toxicological viewpoint, be tolerated even at the height of therapeutically effective tissue concentrations, i.e., without any withdrawal period.

At the microbiological level, concern for food safety is centred on the question of whether or not *residues* of antimicrobial agents ingested via food of animal origin pose a danger to human health by exerting a selective pressure on the intestinal flora and thus favouring the growth of microorganisms with natural or acquired resistance.

Considering the significance of this issue, the Committee recommended the preparation of a detailed working paper to be considered at a future meeting.

2.2 Principles governing the evaluation of substances on the agenda

Residues of veterinary drugs may be present in food as a consequence of the use of drugs as therapeutic and prophylactic

agents in veterinary practice or as growth promoters. The Committee recognized that certain hormonally active substances employed as growth promoters are used in animals for other purposes as well. The Committee concluded that residues left after the use of a drug for growth promotion should be considered separately from residues left after the use of that drug for other purposes, because in the latter case (a) the administration of the drug might be by a different route, and (b) a different withdrawal period in conformity with good veterinary practice might first have to be established and observed. The Committee therefore did not consider residues of hormonally active drugs used for purposes other than growth promotion.

3. COMMENTS ON RESIDUES OF SPECIFIC VETERINARY DRUGS

The Committee re-evaluated the safety of one antimicrobial substance and two xenobiotic hormonally active growth promoters; it evaluated for the first time the safety of three endogenous hormones used as growth promoters.

3.1 Antimicrobial agents

Chloramphenicol

Chloramphenicol was previously considered at the twelfth meeting of the Committee (Annex 1, reference 17). At the present meeting, the Committee considered residue data, extensive toxicological data, and reports of the results of human exposure to the drug for therapeutic purposes. Human exposure to chloramphenicol can give rise to aplastic anaemia, a rare but often fatal condition. The Committee concluded that no dose-response relationship could be established for this effect. The mechanism for the pathogenesis of aplastic anaemia is unknown, and no suitable animal model exists. Thus, a no-effect level could not be established and an ADI could not be allocated for chloramphenicol because it was not possible to give an assurance that residues in foods of animal origin would be safe for sensitive subjects. Therefore, the Committee did not recommend an Acceptable Residue Level.

The Committee noted that the limit of detection of readily available and reliable methods of analysis at the time of the evaluation was around 10 µg/kg.

The Committee recommended that efforts should be made to replace or prohibit the use of chloramphenicol in food-producing animals, particularly in laying birds and lactating animals where high levels of residues in eggs and milk were major problems.

Monographs were prepared on the toxicological and residue data.

3.2 Growth promoters

The Committee evaluated the safety of residues, in foods of animal origin, of five hormonally active substances used as growth promoters. It was aware of the therapeutic use of some of these substances in animals but did not consider residues arising from such uses (see section 2.2).

The Committee noted that several of the hormonally active substances on the agenda were used in combination one with another, and recommended that, where substances having similar physiological activities were combined, evidence that their hormonal effects were additive, rather than synergistic, should be provided. The Committee agreed that data on the residues of each of the substances that are used in combination should be available for evaluation, whether or not their physiological activities were similar.

3.2.1 *Endogenous growth promoters*¹

Estradiol-17 β

At its twenty-fifth meeting (Annex 1, reference 56), the Committee considered the use of hormonally active substances in animal production, and concluded that there was unlikely to be any cause for concern when estradiol-17 β was properly used in animal production. The basis for this conclusion was that the ingestion of meat from animals treated with estradiol-17 β made only a small contribution to the overall normal dietary intake of estrogenic substances, and that the amount of estradiol-17 β ingested in meat was small in comparison with the amount produced endogenously in human subjects. Estradiol-17 β was evaluated by the International Agency for Research on Cancer in 1974, 1979, and 1987 (4-6).

At the present meeting, the Committee considered the use of estradiol-17 β in cattle as a growth promoter, taking into account

¹ Bibliographical references are included in this section since toxicological monographs (which would normally list such references) have not been prepared for estradiol-17 β , progesterone, or testosterone.

information on use patterns, residues in animals, and analytical methodology, toxicological data from laboratory animal experiments, and observations in human subjects.

Estradiol-17 β , alone or in combination with other hormonally active substances, is administered to bovine animals by subcutaneous implant, usually in the ear, to improve the rate of weight gain and feed efficiency.¹ The release rate from one type of commercial implant is approximately 60 μ g per animal daily (7).

The results of studies of the biological activity (8-14), carcinogenicity (15-28), embryotoxicity (29), and mutagenicity (30-32) of estradiol-17 β were available to the Committee. Oral and parenteral administration of estradiol-17 β can increase the incidence of tumours in experimental animals (15-28, 33-36). These tumours largely occur in tissues with high levels of specific hormone receptors that are normally responsive to stimulation by the particular hormone concerned. The Committee concluded that the carcinogenic response was related to the hormonal activity of estradiol-17 β at levels considerably higher than those required for a physiological response. The results of the mutagenicity studies were negative.

The Committee noted that estradiol-17 β occurs naturally in all mammals including human beings. The hormone is therefore normally present in the edible products of mammals that have not been treated with implants. These background levels vary widely with the age and sex of each animal species. The highest naturally occurring levels are found in pregnant animals. Studies with radiolabelled materials show that the use of estradiol-17 β as a growth promoter may produce twofold to fivefold increases in the levels in individual animals, depending on the tissue analysed. The maximum increases occur in the liver and fat of steers and calves given implants, while muscle levels are not affected. Despite being increased, the levels of estradiol-17 β in these animals fall well within the normal range found in untreated bovine animals of different types and ages. Insufficient data were available on normal levels in some classes of animals likely to be treated with estradiol-17 β , in particular in veal calves, to determine whether or not the increased levels produced by implants of estradiol-17 β would fall within the normal ranges for untreated animals in each class.

¹ Feed efficiency is the efficiency of conversion of feed into edible tissues.

The Committee compared the intake of estradiol-17 β that would result from ingestion of meat from treated animals with the normal daily production of estradiol-17 β in human beings. For example, the average amounts of estradiol-17 β produced daily in men (48 μ g, 37) and in pregnant women (37.8 mg, 38) are, respectively, about 15 000 and several million times the amount contained in a 500 g portion of meat from an animal treated with the hormone according to good animal husbandry practice. Even in prepubertal boys, the amount of estradiol-17 β produced daily (6.5 μ g, 39) is a thousand times the amount derived from ingestion of 500 g of treated meat. The Committee therefore concluded that the amount of exogenous estradiol-17 β ingested in meat from treated animals would be incapable of exerting a hormonal effect, and therefore any toxic effect, in human subjects.

The Committee considered an ADI unnecessary for a hormone that is produced endogenously in human beings and shows great variation in levels according to age and sex. The Committee concluded that residues arising from the use of estradiol-17 β as a growth promoter in accordance with good animal husbandry practice are unlikely to pose a hazard to human health.

The Committee recognized that most methods of analysis for estradiol-17 β are radioimmunoassays, which usually have a large coefficient of variation at the concentrations being measured. While these methods may be satisfactory for measuring estradiol-17 β levels in experimental situations, improvements would be needed if routine analytical methods for the control of residues were required.

On the basis of its safety assessment of residues of estradiol-17 β , and in view of the difficulty of determining the levels of residues attributable to the use of this hormone as a growth promoter in cattle, the Committee concluded that it was unnecessary to establish an Acceptable Residue Level.

A monograph was prepared summarizing the residue data. No toxicological monograph was prepared.

Progesterone

At its twenty-fifth meeting (Annex 1, reference 56), the Committee concluded that, for the safety assessment of residues, similar considerations applied to progesterone and estradiol-17 β ; it had concluded that there was unlikely to be any cause for concern when the latter hormone was properly used in animal production

(see p. 17). Progesterone was evaluated by the International Agency for Research on Cancer in 1974, 1979, and 1987 (40-42).

At the present meeting, the Committee considered the use of progesterone in cattle as a growth promoter, taking into account information on use patterns, residues in animals, and analytical methodology, toxicological data from laboratory animal experiments, and observations in human subjects.

Progesterone, in combination with estradiol-17 β , is administered to steers and calves by subcutaneous implantation in the ear to improve the rate of weight gain and feed efficiency.

The data available to the Committee included the results of studies of biological activity (9, 10, 12, 41, 43, 44), carcinogenicity (21, 23, 36, 41, 42, 45-47), and mutagenicity (48). All of the carcinogenicity studies were carried out with parenteral administration of progesterone, and were designed to test the effect of progesterone in combination with known carcinogens or with other hormones. Among the small number of animals treated with progesterone alone in these studies, the incidences of tumours of the mammary gland, ovary, uterus, and vagina were higher than in control animals (42, 45, 49). These effects on tumour production occurred only with doses of progesterone causing obvious hormonal effects. The Committee concluded that the effect of progesterone on tumour production was directly related to its hormonal activity and therefore tumours would not result from ingestion of progesterone at levels that did not produce any hormonal effects.

Residues in edible tissues had been studied after administration of radiolabelled progesterone to cattle, and metabolic products analysed by high-performance liquid chromatography. In liver, the major metabolites were 3 α -hydroxy-5 β -pregnan-20-one and 5 β -pregnane-3 α ,20 β -diol, while in kidney the principal metabolites were 20 β -hydroxypregn-4-en-3-one, 3 α - and 3 β -hydroxy-5 α -pregnan-20-one, and 5 α -pregnane-3 β ,20 β -diol. In fat the major component was unchanged progesterone. Progesterone levels were also measured in tissues from treated steers using a radioimmunoassay technique sensitive at the low ng/kg level, and were found to be about 0.4 μ g/kg in muscle, liver, and kidney, and 3.5 μ g/kg in fat; these levels can be compared with normal levels of approximately 0.2 μ g/kg in muscle, liver, and kidney, and approximately 2.5 μ g/kg in fat from untreated animals.

Progesterone, like estradiol-17 β and testosterone, occurs naturally in mammals, and is normally present in the dairy products

and the tissues of untreated animals (50). In the edible tissues of animals treated with progesterone in combination with estradiol-17 β , residue levels are up to twice as high as in the tissues of untreated animals. However, the levels of progesterone found in meat from animals treated with implants according to good animal husbandry practice are extremely low when compared with the amounts of endogenous progesterone produced daily in human beings. Even in prepubertal boys, the 300 ng additional progesterone derived from a 500 g portion of meat from treated animals is considerably less than the amount of endogenous progesterone produced daily (about 150 μ g) (51). In addition, for those animal classes studied, the progesterone residue levels in treated animals fall well within the normal range of levels found in untreated bovine animals of different types and ages.

The Committee concluded that the amount of exogenous progesterone ingested in meat from treated animals would not be capable of exerting a hormonal effect, and therefore any toxic effect, in human beings.

The Committee deemed it unnecessary to set an ADI for a hormone that is produced endogenously in human beings and shows marked physiological variation in levels according to age and sex. The Committee concluded that residues arising from the use of progesterone as a growth promoter in accordance with good animal husbandry practice are unlikely to pose a hazard to human health.

On the basis of its safety assessment of residues of progesterone, and in view of the difficulty of determining the levels of residues attributable to the use of this hormone as a growth promoter in cattle, the Committee concluded that it was unnecessary to establish an Acceptable Residue Level.

A monograph was prepared on the residue data. A toxicological monograph was not prepared.

Testosterone

At its twenty-fifth meeting, the Committee concluded that, for the safety assessment of residues, similar considerations applied to testosterone and estradiol-17 β ; it had concluded that there was unlikely to be any cause for concern when the latter hormone was properly used in animal production (see p. 17). Testosterone was evaluated by the International Agency for Research on Cancer in 1974, 1979, and 1987 (52-54).

At the present meeting, the Committee considered the use of testosterone in cattle as a growth promoter, taking into account information on use patterns, residues in animals, and analytical methodology, toxicological data from laboratory animal experiments, and observations in human subjects.

Testosterone, in combination with estradiol-17 β , is administered to heifers by subcutaneous implantation in the ear to improve the rate of weight gain and feed efficiency.

Residues in the edible tissues of non-pregnant heifers had been studied by radioimmunoassay. Thirty days after implantation of testosterone and estradiol-17 β , mean levels of testosterone in fat had increased from 26 to 340 ng/kg, in muscle from 20 to 100 ng/kg, in liver from 13 to 34 ng/kg and in kidney from 190 to 450 ng/kg. These levels then progressively decreased to reach levels expected for the endogenous hormone only at 120 days.

The Committee considered the results of studies on carcinogenicity (55-63), mutagenicity (32, 64-66), and embryotoxicity (67-70). The carcinogenicity studies involved administration of testosterone by subcutaneous injection (55-61, 63) or implantation (59, 61, 62) in rodents and rabbits. No results from feeding studies were available. In rodents treated with high doses of testosterone, the incidence of uterine tumours was "surprisingly high" (61) and the incidence of prostatic tumours was higher than in control animals (62). The Committee considered that these tumours resulted from the hormonal activity of testosterone.

Testosterone is normally produced in all mammalian species. When heifers are treated in accordance with good animal husbandry practice, the levels of residues in edible tissues may be increased about twofold, but these levels are extremely low when compared with the amounts of testosterone normally produced by human beings (71-73). Even in prepubertal girls, the amount of endogenous testosterone produced daily (32 μ g) is almost a thousand times the amount of testosterone that would be ingested in a 500 g portion of meat derived from a treated animal (40 ng). The Committee concluded that the amount of exogenous testosterone ingested in edible tissues from treated animals would not be capable of exerting a hormonal effect, and therefore any toxic effect, in human beings.

The Committee considered an ADI unnecessary for a hormone that is produced endogenously in human beings and shows great physiological variation in levels according to sex and age. The Committee concluded that residues resulting from the use of

testosterone as a growth promoter in accordance with good animal husbandry practice are unlikely to pose a hazard to human health.

On the basis of its safety assessment of residues of testosterone, and in view of the difficulty of determining the levels of residues attributable to the use of this hormone as a growth promoter in cattle, the Committee concluded that it was unnecessary to establish an Acceptable Residue Level.

A monograph was prepared summarizing the residue data. A toxicological monograph was not prepared.

3.2.2 *Xenobiotic growth promoters*

Trenbolone acetate

Trenbolone acetate (TBA) was previously evaluated at the twenty-sixth and twenty-seventh meetings of the Committee (Annex 1; references 59 and 62). The use of TBA as an anabolic agent was considered provisionally acceptable, and the results of ongoing studies to determine a no-hormonal-effect level in the nonhuman primate were requested.

At the present meeting, the Committee considered information on patterns of use, residues in animals, and analytical methodology, and toxicological data.

TBA is a synthetic steroid with anabolic properties. At the 17-position in the molecule two epimers, α and β , are possible. The β -epimer of TBA is the commercial product. It is administered to cattle either alone or in combination with estradiol-17 β or zeranol as a subcutaneous implant in the ear to improve body weight, feed conversion, and nitrogen retention. It is usually implanted 60–90 days before the intended date of slaughter.

Radioimmunoassays can detect free and conjugated α - and β -trenbolone (α - and β -TBOH) at levels of 75 ng/kg in tissues. Enzyme immunoassays have the same low detection limits. Chemical methods utilizing high-performance liquid chromatography and gas chromatography–mass spectrometry are sensitive in the range of 1–10 μ g/kg. Although these chemical methods do not have the low detection limits of radioimmunoassays, they are more specific.

After administration to cattle, TBA is rapidly hydrolysed to TBOH, the major metabolite being α -TBOH, occurring in the excreta, bile, and liver. In muscle most of the TBOH is present as β -TBOH. Experiments with implantation of 200 mg of radiolabelled TBA in calves and heifers showed that maximum levels of residues

occurred about 30 days after implantation. The highest mean concentration of residues as TBOH equivalents was 50 µg/kg in liver, while muscle contained 3 µg/kg.

The results of the studies requested at the twenty-seventh meeting had been submitted. In addition new toxicological data were available on reproduction and mutagenicity. The Committee also reviewed previously available data including metabolic, teratogenicity, and carcinogenicity data.

Acute toxicity studies in several species showed TBA to be of low toxicity when given orally.

Experiments had been performed in rats to assess the effect of TBA on pregnancy, on the reproductive function of multiple generations, and on the development of the offspring to weaning. Treatment at 0.3 and 0.5 mg/kg in the diet, equivalent to about 20–30 µg/kg of body weight per day, was associated with mean weekly body weights for female rats that were only slightly higher than those of controls, and marginal differences in litter parameters, while treatment with TBA at 3.0 and 18.0 mg/kg in the diet was associated with a hormonal effect. It was considered that TBA exerted no effect on reproductive performance in the rat at 0.5 mg/kg in the diet, equivalent to 30 µg/kg of body weight per day. No teratogenic effect was seen in two feeding studies in rats at very high doses of TBA. In a comprehensive range of *in vivo* and *in vitro* mutagenicity studies, all tests were negative for TBA, β-TBOH, and TBA's major metabolite, α-TBOH, with the exception of the mutation assay in mouse lymphoma cells, which gave equivocal results with β-TBOH and α-TBOH. The Committee also noted a report of an equivocal result in a transformation study with β-TBOH in Syrian hamster embryo fibroblasts and took into account the recognized difficulty in interpreting results from this type of study.

The Committee reaffirmed the opinion expressed at its twenty-seventh meeting regarding the results of long-term feeding studies with TBA with rats and mice (Annex 1, reference 62). It considered that the liver hyperplasia and tumours in mice fed high doses of TBA (0.9–9 mg/kg of body weight per day) and the slight increase in the incidence of islet-cell tumours of the pancreas of rats fed TBA at 1.85 mg/kg of body weight per day (the highest dose in the study) arose as a consequence of the hormonal activity of TBOH.

The Committee therefore concluded that its safety assessment could be based on establishing the no-hormonal-effect level. It reviewed a study with castrated male rhesus macaque monkeys

administered β -TBOH orally, and considered that this model could be relevant to the human population. The castrated male rhesus monkey is highly sensitive to compounds with antigonadotropic activity; the Committee therefore adopted a conservative approach by using this study as the basis for establishing an ADI for human beings. Despite the small numbers in each group of monkeys studied, and the advanced age of the animals used, the Committee set 2 $\mu\text{g}/\text{kg}$ of body weight per day as a no-hormonal-effect level, based on assessment of histological changes in the seminal vesicles. In the intact female rhesus monkey, TBA had a clear no-hormonal-effect level of 10 $\mu\text{g}/\text{kg}$ of body weight per day. The Committee also considered that the pig was a sensitive model for assessing hormonal effects, and noted that here too TBA had a no-hormonal-effect level of 2 $\mu\text{g}/\text{kg}$ of body weight per day, based on assessment of pathological changes in the testes. Another study in the pig demonstrated that the hormonal activity of β -TBOH was about ten times that of α -TBOH. No data on individual animals were available for any of the pig studies.

In the absence of satisfactory toxicological data the Committee was unable to establish a separate no-effect level for the α -TBOH metabolite. It also noted that this metabolite was not produced in significant amounts in the rat, which made it inadvisable to extrapolate from data generated from β -epimer experiments in that species.

The Committee established a temporary ADI of 0–0.01 $\mu\text{g}/\text{kg}$ of body weight for TBA based on a no-hormonal-effect level of 2 $\mu\text{g}/\text{kg}$ of body weight per day.

The Committee requested that the following information be submitted by 1990:

(a) the final reports, with supporting data, for the tissue residue studies in which TBA was administered to heifers and TBA in combination with estradiol-17 β was administered to steers;

(b) data on individual animals from the three hormonal studies in pigs that were reviewed by the Committee;

(c) results from a 90-day study, in an appropriate species, with orally administered α -TBOH.

The Committee recommended a temporary Acceptable Residue Level of 1.4 $\mu\text{g}/\text{kg}$ for β -TBOH in bovine meat on the basis of a daily intake by a 70 kg person of 500 g of meat.

The major residue in liver and kidney is α -TBOH. As this epimer has one-tenth of the hormonal activity of β -TBOH, the Committee

established a temporary Acceptable Residue Level of 14 µg/kg in liver and kidney.

The Committee recognized that further assessment of data related to bound residues of TBA was necessary before a final evaluation of residue data was possible.

Monographs were prepared on the toxicological and residue data.

Zeranol

Zeranol was evaluated at the twenty-sixth and twenty-seventh meetings of the Committee (Annex 1, references 59 and 62), when it provisionally accepted the use of this compound as an anabolic agent for the production of meat, and requested submission of results from studies on the no-hormonal-effect level in non-human primates, and adequate carcinogenicity studies in two rodent species.

At the present meeting, the Committee considered information on use patterns, residues in animals, and analytical methodology, and toxicological data.

Zeranol is a non-steroidal anabolic agent administered to cattle by subcutaneous implant in the ear to improve the rate of weight gain and feed efficiency. The Committee was aware that in some countries zeranol is also used in sheep. However, the Committee evaluated only the use in cattle.

Studies with radiolabelled zeranol orally administered to rats and monkeys and implanted in cattle have shown that zeranol is metabolized to zearalanone and taleranol. In cattle the rate of depletion of the implant peaks at 5–15 days and slows with time. At 65 days, approximately 60% of the initial dose remains at the implant site.

In the cattle study, when zeranol was administered according to good animal husbandry practice, the maximum mean residue levels, calculated as zeranol equivalents, did not exceed 0.2 µg/kg in muscle, 10 µg/kg in liver, 2 µg/kg in kidney, and 0.3 µg/kg in fat at any time after implantation.

Sensitive analytical methods are available for zeranol and some of its metabolites. Details of several radioimmunoassay methods using either polyclonal or monoclonal antibodies have been published. Other recently developed methods make use of chemiluminescence and enzymic reactions. Chemical methods include capillary gas chromatography of the trimethylsilylether derivative of zeranol and confirmation of the derivative by ion-trap detection, with linearity over the range of 1–10 µg/kg, precision of $\pm 15\%$, and recovery of

90%. Another chemical method incorporates improved extraction procedures for zeranone and some of its metabolites, and permits their analysis at the 1 µg/kg level, quantification, and confirmation by gas chromatography-mass spectrometry using on-column derivatization and internal standards.

The toxicological data available to the Committee included results from the requested studies, as well as mutagenicity, reproduction, and teratogenicity data.

Zeranone was shown to be a weak estrogen in long-term studies in the mouse, rat, dog, and monkey. Most of the changes noted occurred in mammary glands and organs of the reproductive tract. Zeranone did not cause changes in other reproductive parameters in rats, and was not teratogenic in mice or rats. Zeranone and its metabolites zearalanone and taleranone were not mutagenic in a number of tests in bacterial and mammalian systems. Zeranone (at an unspecified concentration) gave a positive result in the Rec-assay (*Bacillus subtilis*) and taleranone gave a positive result in the test with Chinese hamster ovary cells in the absence of activation, but a negative result with activation.

In the carcinogenicity study performed in rats, dietary levels of zeranone up to 25 mg/kg (equivalent to 1.25 mg/kg body weight per day) had estrogenic but not carcinogenic effects. In the study performed in mice, zeranone had significant estrogenic effects in male mice in the highest dose group, receiving zeranone at 15 mg/kg in the diet (equivalent to 2.25 mg/kg of body weight per day); these mice also showed a higher incidence of anterior lobe tumours of the pituitary gland than did mice in the negative control group. Such tumours rarely occur spontaneously in mice but are known to result from administration of estrogenic hormones. The positive control group receiving a diet containing 2.5 mg/kg estradiol-17β showed a higher incidence of anterior lobe tumours of the pituitary than did either animals receiving zeranone or animals in the negative control group. Thus the Committee concluded that the tumorigenic effect of zeranone was associated with its estrogenic properties, and that the determination of a no-hormonal-effect level for tumours would permit an estimate of safe levels of exposure to be made.

A no-hormonal-effect level could not be established in male cynomolgus monkeys since no estrogenic effects were observed even at the highest dose administered (5 mg/kg of body weight per day). In intact female cynomolgus and rhesus macaque monkeys, 5 mg/kg of body weight per day was established as a no-hormonal-effect

level. In ovariectomized female cynomolgus monkeys the no-hormonal-effect level was 0.05 mg/kg of body weight per day. The Committee concluded that this model could be relevant to the human population and, since the ovariectomized female cynomolgus monkey is highly sensitive to estrogenic substances, adopted a conservative approach by using this study as a basis for setting an ADI for human beings.

An ADI of 0–0.5 µg/kg of body weight was established for zeranol.

For a 70 kg person consuming 500 g of meat daily, the maximum permissible level of zeranol residues in meat would be 70 µg/kg of edible tissue. However, the Committee wished to point out that levels of residues of zeranol in meat from animals treated according to good husbandry practice would be below this figure. Therefore an Acceptable Residue Level was established for zeranol when used according to good animal husbandry practice: 10 µg/kg for bovine liver and 2 µg/kg for bovine muscle. (The latter level is higher than that observed in cattle treated according to good animal husbandry practice, but is the lowest level consistent with the practical analytical methods available for routine residue analysis.)

Monographs were prepared on the toxicological and residue data.

4. MATTERS ARISING FROM THE FIRST SESSION OF THE CODEX COMMITTEE ON RESIDUES OF VETERINARY DRUGS IN FOODS

The Committee considered specific matters brought to its attention by the first session of the Codex Committee on Residues of Veterinary Drugs in Foods (2). Many of these have been addressed in section 2 of the present report. The Committee noted the following additional points.

Acceptable Residue Levels

The Committee considered various approaches to the establishment of Acceptable Residue Levels (see section 2.1.3), but noted that a precise definition of this term had not been adopted. The Committee used the following working definition at the present meeting:

The "Acceptable Residue Level" of a veterinary drug in food is the highest acceptable concentration of residues in food. It is determined from the ADI established by the Joint FAO/WHO Expert Committee on Food Additives and from estimated daily intakes of the relevant foods, and is adjusted as necessary to be consistent with good veterinary and animal husbandry practice and practical analytical methods.

This definition, together with alternative approaches to establishing Acceptable Residue Levels, should be reviewed at a future meeting of the Committee.

Code of Practice for the Use of Veterinary Drugs

The Committee noted that this code was under preparation by the Codex Committee, and asked to be kept informed of progress in its development.

Priority list of veterinary drug residues

In noting the establishment of a procedure for setting priorities for the consideration of residues of veterinary drugs in food proposed by Codex member countries, the Committee drew attention to the many veterinary drugs used in tropical countries for which not many data are available on the safety of residues. It recognized that the evaluation of the residues of such drugs could pose problems if adequate data were not submitted and encouraged all interested parties to provide the best possible information when requested to do so on these drugs.

5. FUTURE WORK

1. A detailed working paper should be prepared, for consideration at a future meeting of the Committee, on the possible hazard to human health arising from the ingestion of residues of antimicrobial agents administered to food-producing animals.
2. Consideration should be given at a future meeting of the Committee to determining the biological impact of veterinary drug residues covalently bound to cellular constituents in animal tissues.

6. RECOMMENDATIONS TO FAO AND WHO

1. In view of the large number of veterinary drugs requiring evaluation, meetings of the Joint FAO/WHO Expert Committee on Food Additives should be held regularly to evaluate them.

2. Guidelines should be prepared on the types of data on veterinary drugs to be submitted to the Committee to enable it to assess the safety of residues of such drugs in foods.

3. An important consideration in setting priorities for placing substances on the agenda should be that they are registered as veterinary drugs in at least one country.

4. Information on intakes by human beings of drug residues in food should be provided to the Committee for those veterinary drugs that are referred to it for evaluation.

5. The biological potency of certain veterinary drugs means that their use might have implications for the activities of authorities other than those directly concerned with food and agriculture, for example authorities responsible for occupational health. Such implications should be drawn to the attention of these authorities as the need arises.

6. For determining acceptable levels of drug residues in animal tissues, the continued development of simple, rapid methods of analysis should be encouraged.

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Annex 1

REPORTS AND OTHER DOCUMENTS RESULTING FROM PREVIOUS MEETINGS OF THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

1. *General principles governing the use of food additives* (First report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. *Procedures for the testing of international food additives to establish their safety for use* (Second report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. *Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants)* (Third report of the Expert Committee). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, vol. I. *Antimicrobial preservatives and antioxidants*. Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. *Specifications for identity and purity of food additives (food colours)* (Fourth report of the Expert Committee). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, vol. II. *Food colours*. Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. *Evaluation of the carcinogenic hazards of food additives* (Fifth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
6. *Evaluation of the toxicity of a number of antimicrobials and antioxidants* (Sixth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).
7. *Specifications for the identity and purity of food additives and their toxicological evaluation; emulsifiers, stabilizers, bleaching and maturing agents* (Seventh report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).
8. *Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants* (Eighth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).
9. *Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants*. FAO Nutrition Meetings Report Series, No. 38A, 1965; WHO/Food Add/24.65 (out of print).
10. *Specifications for identity and purity and toxicological evaluation of food colours*. FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.
11. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour-treatment agents, acids, and bases* (Ninth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).

12. *Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour-treatment agents, acids, and bases.* FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29, 1967.
13. *Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances* (Tenth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.
14. *Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non-nutritive sweetening agents* (Eleventh report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.
15. *Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.
16. *Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.
17. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics* (Twelfth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.
18. *Specifications for the identity and purity of some antibiotics.* FAO Nutrition Meetings Report Series, No. 45A, 1969; WHO/Food Add/69.34.
19. *Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances* (Thirteenth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.
20. *Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances.* FAO Nutrition Meetings Report Series, No. 46A, 1970; WHO/Food Add/70.36.
21. *Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives.* FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37.
22. *Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents* (Fourteenth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971.
23. *Toxicological evaluation of some extraction solvents and certain other substances.* FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39.
24. *Specifications for the identity and purity of some extraction solvents and certain other substances.* FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40.
25. *A review of the technological efficacy of some antimicrobial agents.* FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41.
26. *Evaluation of food additives: some enzymes, modified starches, and certain other substances: toxicological evaluations and specifications and a review of the technological efficacy of some antioxidants* (Fifteenth report of the Expert

- Committee). FAO Nutrition Meetings Report Series, No. 50, 1972; WHO Technical Report Series, No. 488, 1972.
27. *Toxicological evaluation of some enzymes, modified starches, and certain other substances*. FAO Nutrition Meetings Report Series, No. 50A, 1972; WHO Food Additives Series, No. 1, 1972.
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 29. *A review of the technological efficacy of some antioxidants and synergists*. FAO Nutrition Meetings Report Series, No. 50C, 1972; WHO Food Additives Series, No. 3, 1972.
 30. *Evaluation of certain food additives and the contaminants mercury, lead, and cadmium* (Sixteenth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum.
 31. *Evaluation of mercury, lead, cadmium, and the food additives amaranth, diethylpyrocarbonate, and octyl gallate*. FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.
 32. *Toxicological evaluation of certain food additives with a review of general principles and of specifications* (Seventeenth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).
 33. *Toxicological evaluation of certain food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents*. FAO Nutrition Meetings Report Series, No. 53A, 1974; WHO Food Additives Series, No. 5, 1974.
 34. *Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers*. FAO Food and Nutrition Paper, No. 4, 1978.
 35. *Evaluation of certain food additives* (Eighteenth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum.
 36. *Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives*. FAO Nutrition Meetings Report Series, No. 54A, 1975; WHO Food Additives Series, No. 6, 1975.
 37. *Specifications for the identity and purity of some food colours, flavour enhancers, thickening agents, and certain food additives*. FAO Nutrition Meetings Report Series, No. 54B, 1975; WHO Food Additives Series, No. 7, 1975.
 38. *Evaluation of certain food additives: some food colours, thickening agents, smoke condensates, and certain other substances* (Nineteenth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975.
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44. *Evaluation of certain food additives* (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.
45. *Summary of toxicological data of certain food additives*. WHO Food Additives Series, No. 12, 1977.
46. *Specifications for identity and purity of some food additives, including antioxidants, food colours, thickeners, and others*. FAO Nutrition Meetings Report Series, No. 57, 1977.
47. *Evaluation of certain food additives and contaminants* (Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 631, 1978.
48. *Summary of toxicological data of certain food additives and contaminants*. WHO Food Additives Series, No. 13, 1978.
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61. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 25, 1982.
62. *Evaluation of certain food additives and contaminants* (Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda.
63. *Toxicological evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 18, 1983.
64. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 28, 1983.
65. *Guide to specifications—General notices, general methods, identification tests, test solutions, and other reference materials*. FAO Food and Nutrition Paper, No. 5, Rev. 1, 1983.
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67. *Toxicological evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 19, 1984.
68. *Specifications for the identity and purity of food colours*. FAO Food and Nutrition Paper, No. 31/1, 1984.
69. *Specifications for the identity and purity of food additives*. FAO Food and Nutrition Paper, No. 31/2, 1984.
70. *Evaluation of certain food additives and contaminants* (Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 733, 1986.
71. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 34, 1986.
72. *Toxicological evaluation of certain food additives and contaminants*. Cambridge, Cambridge University Press, 1987 (WHO Food Additives Series, No. 20).
73. *Evaluation of certain food additives and contaminants* (Thirtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 751, 1987.
74. *Toxicological evaluation of certain food additives and contaminants*. Cambridge, Cambridge University Press, 1987 (WHO Food Additives Series, No. 21).
75. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 37, 1987.
76. *Principles for the safety assessment of food additives and contaminants in food*. Geneva, World Health Organization, 1987 (WHO Environmental Health Criteria, No. 70).
77. *Evaluation of certain food additives and contaminants* (Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 759, 1987.
78. *Toxicological evaluation of certain food additives*. Cambridge, Cambridge University Press, 1988 (WHO Food Additives Series, No. 22).
79. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 38, 1988.

Annex 2

RECOMMENDATIONS ON COMPOUNDS ON THE AGENDA

Substance	Acceptable Daily Intake for human beings	Acceptable Residue Level
Antimicrobial agent Chloramphenicol	Not allocated ¹	Not allocated ¹
Growth promoters		
<i>Endogenous</i>		
Estradiol-17 β	Unnecessary ²	Unnecessary ²
Progesterone	Unnecessary ²	Unnecessary ²
Testosterone	Unnecessary ²	Unnecessary ²
<i>Xenobiotic</i>		
Trenbolone acetate	0-0.01 $\mu\text{g}/\text{kg}$ of body weight ³	1.4 $\mu\text{g}/\text{kg}$ (bovine tissue) for β -trenbolone; ^{3, 4} 14 $\mu\text{g}/\text{kg}$ (bovine liver and kidney) for α -trenbolone ^{3, 5}
Zeranol	0-0.5 $\mu\text{g}/\text{kg}$ of body weight	10 $\mu\text{g}/\text{kg}$ (bovine liver); ⁶ 2 $\mu\text{g}/\text{kg}$ (bovine muscle) ⁷

¹ No ADI or Acceptable Residue Level could be established because it was not possible to give an assurance that residues would be safe for sensitive subjects, who could develop aplastic anaemia.

² Establishing an ADI and an Acceptable Residue Level for a hormone that is produced endogenously at variable levels in human beings was considered unnecessary by the Committee. Residues resulting from the use of this substance as a growth promoter in accordance with good animal husbandry practice are unlikely to pose a hazard to human health.

³ Temporary acceptance. The following information was requested, to be submitted by 1990: (a) the final reports, with supporting data, for the tissue residue studies in which trenbolone acetate was administered to heifers and trenbolone acetate in combination with estradiol-17 β was administered to steers; (b) data on individual animals from the three hormonal studies in pigs that were reviewed by the Committee; and (c) results from a 90-day study in an appropriate species with orally administered α -trenbolone.

⁴ Based on consumption of 500 g meat per day by a 70 kg person.

⁵ Based on the finding that α -trenbolone had one-tenth of the hormonal activity of β -trenbolone in a study in pigs.

⁶ Based on a level consistent with good animal husbandry practice, as determined by studies with radiolabelled zeranol, since this level is lower than that calculated from the ADI as the maximum permissible on toxicological grounds.

⁷ Based on the lowest level consistent with the practical analytical methods available for routine residue analysis.