Evaluation of certain veterinary drug residues in food

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

World Health Organization
Technical Report Series
799

World Health Organization, Geneva 1990
## CONTENTS

1. Introduction ........................................................................................................... 7

2. General considerations ....................................................................................... 8
   2.1 Principles governing the safety evaluation of residues of veterinary drugs in food ................................................................. 8
   2.2 Decision process for establishing recommended Maximum Residue Limits ........................................................................... 8
   2.3 Bound residues ............................................................................................... 10
   2.4 Assessment of the microbiological risk due to residues of antimicrobial drugs in food ................................................................. 13
      2.4.1 Microbiological risk for the intestinal flora ........................................ 13
      2.4.2 Microbiological risks for the food processing industry ................. 15
   2.5 Allergic potential of residues of veterinary drugs in food ....................... 15
      2.5.1 The nature of allergy .......................................................................... 15
      2.5.2 The role of veterinary drugs in the induction of allergic reactions .... 16
   2.6 Temporary ADIs and MRLs ........................................................................... 17
   2.7 Expression of ADIs ....................................................................................... 18

3. Comments on residues of specific veterinary drugs ........................................... 18
   3.1 Anthelmintic drugs ....................................................................................... 18
      3.1.1 Clonambel ................................................................. 18
      3.1.2 Ivermectin ................................................................. 23
      3.1.3 Levamisole ............................................................................. 31
   3.2 Antimicrobial agents .................................................................................... 37
      3.2.1 Benzylpenicillin ............................................................................ 37
      3.2.2 Oxytetracycline ........................................................................... 41
   3.3 Growth promoters ....................................................................................... 45
      3.3.1 Carbadox ............................................................................... 45
      3.3.2 Olaquindox .............................................................................. 50

4. Recommendations .............................................................................................. 54

References ............................................................................................................... 55

Annex 1. Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives .......... 57

Annex 2. Recommendations on compounds on the agenda .................................. 63

Annex 3. Further toxicological studies and other information required or desired .............................................................................. 65

Annex 4. Divergent opinion on levamisole (Professor A. Somogyi) ............... 68
JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

Rome, 5–14 February 1990

Members

Professor M. Ansay, Chair of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Liège, Brussels, Belgium
Dr J. Boisseau, Director, Laboratory of Veterinary Drugs, National Centre of Veterinary and Nutritional Studies, Fougeres, France (Vice-Chairman)
Dr R. Ellis, Director, Chemistry Division, Food Safety and Inspection Service, Department of Agriculture, Washington, DC, USA (Joint Rapporteur)
Professor A. El Tayeb Ibrahim, Professor of Preventive Medicine and Veterinary Public Health, Faculty of Veterinary Science, University of Khartoum, Sudan
Dr R. Furrow, Deputy Associate Director, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Rockville, MD, USA
Dr Liu Sheng Ming, Head, China Import and Export Commodity Inspection Technology Institute, Chanyang District, Beijing, China
Dr B. MacGibbon, Consultant in Environmental Health to the Department of Health, London, England (Chairman)
Dr J.G. McLean, Dean, Faculty of Applied Science, Swinburne Institute of Technology, Hawthorn, Victoria, Australia (Joint Rapporteur)
Dr D.M. Pugh, Department of Small Animal Clinical Studies, Faculty of Veterinary Medicine, University College, Dublin, Ireland
Dr J.L. Rojas-Martínez, Chief, Toxicology Section, National Centre for Diagnosis and Research in Animal Health, San José, Costa Rica
Professor A. Somogyi, Director, Max von Pettenkofer Institute of the Federal Office of Public Health, Berlin (West)

Secretariat

Dr D. Arnold, Head, Department of Drugs, Animal Nutrition and Residue Research, Institute for Veterinary Medicine, Berlin (West) (WHO Temporary Adviser)
Dr S. Bynnes, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Rockville, MD, USA (FAO Consultant)
Dr G. Burin, Health Evaluation Division, Office of Pesticide Programs, Environmental Protection Agency, Washington, DC, USA (WHO Consultant)
Dr M. Elwell, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA (WHO Temporary Adviser)
Dr J. Finnis, Principal Scientist, International Life Sciences Institute, Risk Science Institute, Washington, DC, USA (WHO Temporary Adviser)
Mr R.A. Hankin, Administrator, Pharmaceuticals and Veterinary Medicines Division, Commission of the European Communities, Brussels, Belgium (WHO Temporary Adviser)
Dr R.J. Heitzman, Science Consultant, Newbury, England (FAO Consultant)
Dr J.L. Herrman, Scientist, International Programme on Chemical Safety, Division of Environmental Health, WHO, Geneva, Switzerland (Joint Secretary)

Dr W.C. Keller, Center for Veterinary Medicine, Food and Drug Administration, Rockville, MD, USA (WHO Temporary Adviser)

Dr R.C. Livingston, Acting Director, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Rockville, MD, USA (FAO Consultant)

Dr D. McGregor, Unit of Carcinogen Identification and Evaluation, International Agency for Research on Cancer, Lyon, France

Professor A. Rico, Professor of Biochemistry, National Veterinary School, Toulouse, France (WHO Temporary Adviser)

Dr F.X.R. van Leeuwen, Laboratory for Toxicology, National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands (WHO Temporary Adviser)

Dr J. Weatherwax, Food Quality and Standards Service, Food Policy and Nutrition Division, FAO, Rome, Italy (Joint Secretary)

Dr K.N. Woodward, Veterinary Medicines Directorate, Central Veterinary Laboratory, New Haw, Weybridge, Surrey, England (WHO Temporary Adviser)

Dr K. Yoshihira, Head, Division of Food Additives, National Institute of Hygienic Sciences, Tokyo, Japan (WHO Temporary Adviser)
Monographs containing summaries of relevant data and toxicological evaluations are available under the title:


Specifications are issued separately by FAO under the title:

*Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/3, in press.

---

**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.
EVALUATION OF CERTAIN
VETERINARY DRUG RESIDUES IN FOOD

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

A meeting of the Joint FAO/WHO Expert Committee on Food Additives was held at FAO Headquarters, Rome, from 5 to 14 February 1990. The meeting was opened by Dr P. Lunven, Director, Food Policy and Nutrition Division, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and of the World Health Organization.

Dr Lunven noted that the Committee was one of the longest-serving expert bodies in the United Nations system, with a high reputation for providing unbiased advice on the safety of substances used or found as residues in food. He recalled the evolution of the Committee’s responsibilities from the evaluation only of direct food additives in 1956 to the present complex evaluations, including veterinary drug residues that may be found in edible animal products. The present meeting was the third to be devoted exclusively to the evaluation of such drug residues.

Dr Lunven noted that the Contracting Parties to the General Agreement on Tariffs and Trade were among those reliant upon the Committee’s evaluations. In the current Uruguay Round of multilateral trade negotiations, those Parties had decided on a long-term programme of harmonization of national regulations dealing with the import and export of foods, based on the recommendations of the Codex Alimentarius Commission, which, for veterinary drug residues, were based on the Committee’s evaluations.

1. INTRODUCTION

In response to the recommendations of the Joint FAO/WHO Expert Consultation held in 1984 (1), two previous meetings of the Joint FAO/WHO Expert Committee on Food Additives have been held to consider veterinary drug residues in food (Annex 1,
references 80 and 85). The present meeting\(^1\) was convened in response to the recommendation made at the thirty-fourth meeting of the Committee that meetings on this subject should be held regularly (Annex 1, reference 85). 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 further elaborate 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 to food-producing animals in accordance with good practice in the use of veterinary drugs;

(b) to evaluate the safety of residues of certain veterinary drugs; and

(c) to discuss matters arising from the report of the fourth session of the Codex Committee on Residues of Veterinary Drugs in Foods (2).

2. GENERAL CONSIDERATIONS

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 the thirty-second and thirty-fourth reports of the Committee (Annex 1, references 80 and 85), and in the report of the Joint FAO/WHO Expert Consultation on Residues of Veterinary Drugs in Foods (1).

2.2 Decision process for establishing recommended Maximum Residue Limits

In recommending a Maximum Residue Limit (MRL) for a specific compound, several factors are taken into account by the

---

\(^1\) 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 33 previous meetings of the Joint FAO/WHO Expert Committee on Food Additives (Annex 1).
Committee. Among them are the results of toxicological and radiolabel residue studies, the bioavailability of bound residues, the identification of target tissue(s), the existence of a residue marker for determining compliance with safe residue limits, residue data from use of the veterinary drug according to good practice in the use of veterinary drugs, withdrawal periods for adequate residue depletion, and practical analytical methods for residue analysis.

The first step in establishing a recommended MRL is the determination of an Acceptable Daily Intake (ADI) based on the available toxicological data. If the use of the veterinary drug according to good practice in the use of veterinary drugs yields concentrations of residues lower than those corresponding to the ADI, the MRL will be reduced accordingly. However, if the residues cannot be measured using a practical analytical method under these conditions of use, the MRL will be raised so that compliance with the MRL may be checked analytically. In no instances, however, will an MRL be recommended at concentrations that significantly exceed the MRL based on toxicological considerations. Fig. 1 describes this decision process.

An important factor to be considered in the establishment of MRLs in various edible tissues and other products of animal origin is the amount of the food item consumed. In order to protect all segments of the population, it is reasonable to use intake data at the upper limit of the range for individual edible tissues and animal products. The Committee based its recommendations on the following daily intake values (see the report of the thirty-fourth meeting of the Committee, Annex 1, reference 83): 300 g of meat (as muscle tissue), 100 g of liver, 50 g of kidney, 50 g of tissue fat, 100 g of egg and 1.5 l of milk.

As a practical example, a review of the establishment of the MRL for zeranol as discussed at the thirty-second meeting of the Committee (Annex 1, reference 80) is highlighted. The Committee established an ADI of 0.0-0.5 µg/kg of body weight. For a 60-kg person consuming 500 g of animal products daily, the maximum permissible level of zeranol residues in meat would be 60 µg/kg of edible bovine tissue. In the cattle studies presented for evaluation, when zeranol was administered according to good practice in the use of veterinary drugs, the maximum mean residue limits, calculated as zeranol equivalents, did not exceed 0.2 µg/kg in muscle or 10 µg/kg in liver at any time after subcutaneous implantation in the ear. However, the MRL was raised to the lowest level consistent with the
Fig. 1. Decision tree for establishing recommended MRLs

Definitions
MRL₁ = Maximum Residue Limit considered to be without hazard to human health, as determined from the Acceptable Daily Intake (ADI)
MRL₀ = Maximum Residue Limit based on residues of the drug when used according to good practice in the use of veterinary drugs
MRLₙ = Maximum Residue Limit based on the availability of a validated practical analytical method for measuring residues of the compound of interest

practical analytical methods available for routine residue analysis to 2 µg/kg for bovine muscle and 10 µg/kg for bovine liver.

This decision-tree approach for recommending MRLs has been used at the present meeting of the Committee.

2.3 Bound residues

The safety evaluation of bound residues of animal drugs is discussed in the report of the thirty-fourth meeting of the Committee (Annex 1, reference 85). If bound residues make up an insignificant portion of the total residue, a suitable extractable residue can usually be selected as a marker compound and used to establish an MRL. However, in some cases, bound residues become a significant portion of the residues of interest, and their impact on the ADI must then be evaluated. Fig. 2 outlines the approach adopted by the
Fig. 2. Suggested approach for assessing the toxicological significance of bound residues

Tissue collected from the test animal species at various times after drug administration (Total residue)

Mild extraction procedure

- Determine percentage of extractable residues for each tissue
  - Identify a marker compound

- Determine percentage of non-extractable residues for each tissue

*in vitro* procedure

- Releasable
  - Determine released products
  - Assess toxicological potential

- Non-releasable
  - Bioavailable
  - Non-bioavailable

*in vivo* procedure

WHO 30689
Committee in assessing the safety of bound residues. The Committee noted that both in vitro and in vivo studies, providing information on releasable residues and bioavailable residues respectively, could be useful for this purpose.

The Committee recognized that analytical procedures for measuring quantities of residues from veterinary drugs in food animals have been developed for several purposes. One type of extraction uses mild techniques for determining compliance with safe residue limits in regulatory control programmes and international trade. A second type of extraction is a more vigorous in vitro procedure for measuring and characterizing the amount of releasable residues from total bound residues. Data from both extraction procedures are useful for assisting in the toxicological evaluation of veterinary drugs and their metabolic products and they may also be relevant for recommending MRLs. The Committee suggests that other in vitro techniques such as incubation of the bound residue with acid or appropriate enzyme preparations that mimic the human digestive system be used to gather information on the qualitative, quantitative, and toxicological nature of the released products. The extraction conditions must be such that the compounds of interest are not destroyed.

In the report of the thirty-fourth meeting of the Committee, the Gallo-Torres procedure was given as an acceptable in vivo procedure to evaluate the extent to which the bound residue is absorbed when the food is ingested (bioavailability). If the bound residue is shown to be unavailable, in many instances it may be subtracted from the total residue of concern.

The results of these in vitro and in vivo studies are evaluated to decide whether further tests are required. If significant amounts of the bound residue are shown to be bioavailable or releasable, then the toxicological significance of those products may need to be assessed. Such additional investigations may involve techniques ranging from structural assessment to in vitro or short-term in vivo tests.

With the exception of the bioavailability study, the Committee has not attempted to enumerate specific studies that will aid in the safety assessment of bound residues, nor does the Committee insist that manufacturers adopt the procedure shown in Fig. 2. However, the Committee does consider that any approach used in the assessment of the bound residues should be clearly justified. The Committee believes that the residue and toxicological data that will
be necessary to assess the safety of a particular bound residue will vary according to the drug and the nature of the bound residue. The amount of data required can depend on factors such as the degree of bioavailability, the amount of non-bioavailable residue, the nature of the bound residue, and the toxicological potency of the parent drug or metabolite on which the ADI is based. In short, the Committee will evaluate each bound residue on a case-by-case basis.

2.4 Assessment of the microbiological risk due to residues of antimicrobial drugs in food

In evaluating the safety of residues of antimicrobial drugs, the specific risks associated with their antimicrobial activity should be considered in addition to their pharmacological properties. The antimicrobial activity could become the determining factor of this safety evaluation if the toxicity of the substance to be considered is such that higher levels of residues could be tolerated in food on a toxicological basis.

In this respect, the Codex Committee on Residues of Veterinary Drugs in Foods has adopted a definition of Maximum Residue Limit for Veterinary Drugs taking into account “other relevant public health risks (that may refer to allergic and microbiological risks) as well as food technological aspects”.

In assessing the microbiological risk, two biological systems need to be considered:

—the intestinal flora of the consumer
—bacteria used in the food processing industry.

The risk being considered by the Committee does not deal with the potential health effects associated with ingestion of food of animal origin that contains resistant bacteria selected under the pressure of antimicrobial therapy, because the Committee’s terms of reference include only the safety assessment of drug residues.

2.4.1 Microbiological risk for the intestinal flora

It is important to consider whether residues of antimicrobial agents ingested in food of animal origin pose a danger to human health by exerting a selective pressure on the intestinal flora, thereby favouring the growth of microorganisms with natural or acquired resistance. The Committee believed that plasmid-mediated resistance was unlikely to develop, on the basis of the available data.
In evaluating the effect of residues of antimicrobial drugs on the human gut flora, the characteristics of the flora should be taken into account. There are approximately $10^{11}$ microbes per gram of faeces, more than 90% of which are anaerobic bacteria. The flora is stable, meaning that the bacterial ecology generates important "barrier effects" (which tend to prevent intrusion by foreign microbes); it is also specific for human beings.

Given these characteristics, the evaluation of the effects of residues of antimicrobial drugs on the human gut flora must be based on available data:

(a) pertaining to the identification of bacteria that constitute human gut flora;
(b) regarding bacteria that are representative of the whole flora;
(c) obtained from in vivo experiments, which take into account the barrier effects.

At present, human epidemiological studies are not able to provide adequate information in this area, given the variations in resistant bacterial flora due to human drug therapy. Nevertheless, published data have demonstrated that experimentation in human volunteers is an appropriate methodology.

If human data are not available, other data based on experiments in animals may be considered. The validation of animal models, such as holoxenic rodents implanted with human gut flora, should be encouraged.

In the absence of in vivo data, in vitro data such as minimum inhibitory concentrations\(^1\) may be used, on a temporary basis, for safety evaluations. Nevertheless, MRLs based on minimum inhibitory concentrations established under standard conditions should incorporate factors such as the impact of gut pH and anaerobiosis, the resorption and degradation of antimicrobial drugs in the gut, and the volume of the ingested bolus of food containing the antimicrobial drug residues.

---

\(^1\) The minimum inhibitory concentration is defined as the minimum concentration of an antimicrobial drug giving complete inhibition of growth of a particular microorganism, as judged by the naked eye after a given period of incubation (WHO Technical Report Series, No. 610, 1977).
2.4.2 Microbiological risks for the food processing industry

The Committee was aware of the important inhibitory effects of residues of antimicrobial drugs on cheese manufacturing. Studies carried out in laboratories and industrial plants, especially those concerned with yoghurt production, have demonstrated that it is possible to determine a concentration of residues of antimicrobial drugs that does not inhibit the microorganisms used in such production. Moreover, such concentrations are readily monitored because the official microbiological methods used for this purpose employ the same microorganisms as those used in cheese manufacturing.

2.5 Allergenic potential of residues of veterinary drugs in food

In response to a recommendation in the report of the thirty-fourth meeting of the Committee (Annex 1, reference 85), the Committee considered the possible hazards to human health arising from residues in food derived from veterinary drugs with allergenic properties. It recognized that several drugs employed in human and animal medicines had produced allergic reactions when used in humans (3). However, despite the widespread use of veterinary medicines, there were very few publications concerning the possible involvement of residues of veterinary drugs with allergic reactions in consumers.

2.5.1 The nature of allergy

The Committee noted the classification system of allergic reactions proposed by Coombs & Gell (types I-IV) and the underlying mechanisms of action (4). It recognized that, in general, initial sensitization of a susceptible individual occurred following the administration of a relatively large dose of a drug with allergenic potential and that subsequent challenge, often with a much smaller dose, could elicit an allergic response. These true allergic reactions, which are immunologically mediated, should be differentiated from cases of drug intolerance, due to pharmacological or toxicological properties of the drug, and drug idiosyncrasy, caused by factors such as enzyme deficiency in the individual leading to an unusual adverse response.
2.5.2 The role of veterinary drugs in the induction of allergic reactions

The Committee concluded that the very small amount of any drug with allergenic potential likely to be encountered as a residue in food of animal origin was unlikely to lead to sensitization. Sensitization was only likely to occur after exposure to relatively large quantities of an allergenic drug (5), for example after use in human medicine or following occupational exposure. However, in theory, the small amount of an allergenic drug present as a residue could elicit an allergic response in a previously sensitized person, although any preformed allergens (e.g., penicillin–protein conjugates) would probably be poorly absorbed following ingestion (5).

Although a significant number of farm milk samples have been found to contain small amounts of penicillin, the Committee noted that there have been very few published reports of apparent allergic reactions associated with exposure of milk consumers to residues (6–16). The few available data suggested an association between penicillin residues in dairy products, mainly milk, and the occurrence of chronic urticaria. There was a single report of an anaphylactic reaction which was possibly due to penicillin in pork (17). Unfortunately, the presence of penicillin in the suspect food has rarely been confirmed using analytical procedures, although in a number of the reported cases, urticaria was ameliorated by treating food with penicillinase, which appeared to confirm the presence of a penicillin-like molecule (7). Two case reports implicated tetracycline and streptomycin in the production of adverse reactions following the ingestion of food believed to be contaminated with residues of these drugs (18, 19).

A report of an anaphylactic reaction following the consumption of a soft drink contaminated with penicillin (20) showed that care was needed in determining the origin of a contaminating allergenic drug, since it was not possible to implicate veterinary drug use as the cause in this case. Similarly, an anaphylactic reaction to penicillin present in a frozen meal containing steak may have been due to microbial contamination (21).

The Committee suggested the following criteria for establishing a causal relationship between residues of veterinary drugs and an allergic reaction in consumers:

(a) that residues of the veterinary drug were present in the food causing the allergic reaction;
(b) that the patient was not allergic to the same food without the veterinary drug residues;
(c) that an immunological mechanism could be demonstrated for the response; and
(d) that the origin of the drug residue in food was likely to have been from use in animals.

Although there is no evidence from which threshold doses for such effects can be determined, the Committee concluded that hypersensitivity reactions due to the ingestion of food of animal origin containing allergenic drug residues were unlikely to be of major health significance. This view was supported by the small numbers of reports in the published literature. Nevertheless, the Committee recognized that reactions could occur in highly sensitized individuals and therefore recommended that residues of drugs with known or suspected allergenic properties be kept as low as practicable, particularly penicillin and other β-lactam antibiotics such as the cephalosporins.

2.6 Temporary ADIs and MRLs

The Committee has encountered many instances in which only limited data are available for particular veterinary drugs. When the Committee is confident that the consumption of residues of such drugs is safe (i.e., without toxicological hazard) over the relatively short period of time required to generate and evaluate further data, but is not confident that their consumption is safe over a lifetime, it establishes temporary ADIs. Similarly, temporary MRLs are sometimes established to provide time to generate and evaluate further data on the nature and quantification of residues.

When the Committee establishes temporary ADIs or temporary MRLs it specifies what information is required to resolve outstanding issues, and sets a date by which these data should be submitted to the Committee. The Secretariat will then place the drug on the agenda of an appropriate future meeting of the Committee. At that time the Committee has the option of establishing a full ADI (MRL), extending the temporary ADI (MRL), or not extending the temporary ADI (MRL).
2.7 Expression of ADIs

When establishing the numerical expression of the ADI, the Committee has decided to express it to only one significant figure. If an ADI is calculated from a no-observed-effect level that has more than one significant figure, the number will therefore be rounded to one significant figure, consistent with acceptable rounding procedures.

3. COMMENTS ON RESIDUES OF SPECIFIC VETERINARY DRUGS

The Committee re-evaluated the safety and residues of two antimicrobial agents. It evaluated for the first time the safety and residues of three anthelminthic drugs and two growth promoters. The recommendations made with regard to the compounds on the agenda are given in Annex 2, while details of further toxicological studies and other information required or desired are given in Annex 3.

3.1 Anthelminthic drugs

3.1.1 Closantel

This is the first occasion on which closantel has been reviewed by the Committee. Closantel is used primarily in cattle and sheep for the treatment and control of adult and immature liver flukes, nematodes and larval stages of some arthropods.

Toxicological data

The toxicological data considered by the Committee included the results of studies on metabolism, of short-term studies and of studies on carcinogenicity, genotoxicity and effects on reproduction and development.

In studies in rats and sheep, closantel was shown to be strongly bound to the plasma proteins. The compound was poorly metabolized, mainly to 3- and 5-monoiodoclosantel. About 90% of the administered dose was excreted in the faeces, and about 90% was unchanged.

In a study in rats in which closantel was administered in the diet at levels up to the equivalent of 40 mg per kg of body weight per day
for 13 weeks, spermatic granulomas in the epididymis and fatty changes in the liver were observed at the highest dose level. The no-observed-effect level was 2.5 mg per kg of body weight per day.

Dogs were dosed for three months by oral administration of the compound in capsules at levels up to 40 mg per kg of body weight per day. There were fatty changes in the liver at the highest dose level. The no-observed-effect level for this study was 2.5 mg per kg of body weight per day.

A carcinogenicity study was conducted in mice, which received closantel in the diet at levels up to 80 mg per kg of body weight per day for 18 months. There were no dose-related effects on the total numbers of tumours nor on any individual type of tumour, nor were any other effects observed that were related to treatment.

In a carcinogenicity study in rats in which closantel was administered in the diet at levels up to 40 mg per kg of body weight per day for 24 months, there was no effect on the overall incidence of tumours. However, by comparison with concurrent controls, there was a statistically significant increase in the incidence of haemopoietic tumours in male rats at 10 mg per kg of body weight per day. This incidence was nevertheless within the historical control range, while the incidences found in the control and high-dose groups were significantly lower than in the historical controls. Spermatic granulomas were observed. The no-observed-effect level for this study was 2.5 mg per kg of body weight per day.

Adverse effects on reproduction were probably associated with toxicity to the male reproductive organs, and were seen only at doses of 2.5 mg per kg of body weight per day and above. In a fertility study in rats in which closantel was administered in the diet, there was a reduced pregnancy rate in untreated females paired with males receiving the highest dose of 40 mg per kg of body weight per day. A three-generation reproduction study was conducted in male and female rats, in which the compound was administered once monthly by gavage. There was a decrease in the pregnancy rate and number of implants per animal at the highest dose of 40 mg per kg of body weight. Spermatic granulomas were seen at 10 and 40 mg per kg of body weight, but not at 2.5 mg per kg of body weight.

In teratogenicity studies in rabbits and rats no teratogenic or toxic effects were evident at doses up to 40 mg per kg of body weight per day. The rabbits received closantel by gavage on days 6 to 18 of pregnancy, while the rats were given the compound in the diet on days 6 to 15 of pregnancy.
Closantel gave negative results in a range of in vitro and in vivo mutagenicity studies. No tests for clastogenicity were performed, but the Committee noted that carcinogenicity studies had been carried out in two species.

Limited clinical reports were available from 33 patients who were treated with a single oral or parenteral dose of closantel at 2.5–10 mg per kg of body weight. There were no adverse effects.

An ADI of 0–0.03 mg per kg of body weight was established for closantel based on the no-observed-effect level of 2.5 mg per kg of body weight per day in rats and a safety factor of 100.

Residue data
The Committee considered data on the metabolism of closantel in sheep and cattle, and on the depletion of residues from the edible tissue of sheep and cattle following treatment with closantel at dose levels normally associated with good practice in the use of veterinary drugs. The Committee used these data to recommend MRLs in sheep and temporary MRLs in cattle.

Sheep. Limited data on total residue depletion were available from ten sheep, to which a single intramuscular dose of 5 mg per kg of body weight \(^{14}\)C-closantel or a single oral dose of 10 mg per kg of body weight was given. One animal from each group was killed at 14, 21, 35, 42 and 56 days after treatment. The edible tissues were analysed by scintillation counting or a high-pressure liquid chromatographic method for parent closantel. Similar results for residues of closantel in muscle, kidney and fat were obtained after oral or intramuscular administration. Virtually no metabolism occurred in those tissues. In liver, closantel accounted for about 60% of the total residue following intramuscular administration or 70% following oral administration. The concentration of residues in the tissues decreased slowly, with a half-life of about three weeks, and after 56 days withdrawal time was 1–2 mg/kg in kidney (residue concentrations were lower in other tissues).

The Committee noted that the depletion of residues of closantel from plasma paralleled that from edible tissues of sheep, i.e. the tissue:plasma ratio remained reasonably constant with time. The ratios observed were approximately 0.03 for muscle, 0.09 for liver, 0.16 for kidney and 0.01 for fat. The extrapolation of concentrations of closantel residues from plasma to edible tissues therefore seems
feasible. Therefore, testing for residues in plasma may be of potential use in determining when treated animals may be marketed as food.

In the sheep livers from the above study, and in a metabolism study, two metabolites were found to account for the remaining residues: 3- and 5-monoiodoclosantel. Reductive deiodination, therefore, is a major metabolic pathway in liver.

For a 60-kg person, the permitted daily intake of closantel residues would be 1.8 mg contributed by 500 g of animal-derived food based on the ADI of 0-0.03 mg per kg of body weight. For all dose levels studied in sheep, the ADI would not be exceeded at 14 days withdrawal. However, the doses used were 10 mg per kg of body weight given orally or 5 mg of body weight intramuscularly, whereas the maximum permitted doses are 15 mg per kg of body weight given orally or 7.5 mg per kg of body weight intramuscularly. If the estimated amount of closantel residues ingested is increased by 50% to take account of these maximum permitted doses, then the ADI of 1.8 mg would be met at a withdrawal time of approximately 14 days. At 28 days withdrawal, the intake of residues of closantel—even with this adjustment of 50%—is well below the ADI. On the basis of the data from the studies that were reviewed, the Committee recommended an MRL of 1.5 mg/kg for all edible tissues of sheep. This MRL will ensure that the daily intake of residues of closantel will not exceed the ADI (see Table 1).

*Cattle.* Data on the depletion of total residues were not available for cattle. However, in view of the metabolism data in sheep and rats, the Committee believed it reasonable to estimate that closantel accounted for not less than 50% of the total residues in muscle, kidney and fat, and not less than 30% of the total residue in liver. Using these estimates to adjust observed closantel concentrations to a total residue basis, the Committee concluded that the ADI would not be exceeded at 42 days withdrawal time. This conclusion was derived from studies in which cattle were given a single intramuscular dose of 2.5 mg per kg of body weight, yielding an estimate that consumers would ingest about 0.74 mg of total residues of closantel daily from 500 g of edible cattle tissues. At 42 days, the concentrations of parent closantel were 0.29 mg/kg in muscle, 1.39 mg/kg in kidney and 0.56 mg/kg in liver. Accordingly, the Committee recommended temporary MRLs of 0.5 mg/kg in muscle, 2 mg/kg in kidney, and 1 mg/kg in liver (see Table 2). An MRL was not established for fat because of unacceptable variability in the data
### Table 1. Recommended MRLs for closantel in sheep

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Daily food intake</th>
<th>Recommended MRL as parent drug (mg/kg)</th>
<th>Daily total residue intake based on MRL (mg)</th>
<th>Concentration of closantel at 28 days withdrawal time (mg/kg)</th>
<th>Daily total residue intake based on observed residue levels (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>oral dose*</td>
<td>intramuscular dose*</td>
<td></td>
<td>oral dose</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.3 kg</td>
<td>1.5</td>
<td>0.45</td>
<td>&lt;0.4</td>
<td>1.1</td>
</tr>
<tr>
<td>Liver</td>
<td>0.1 kg</td>
<td>1.5</td>
<td>0.25</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.5)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.05 kg</td>
<td>1.5</td>
<td>0.075</td>
<td>0.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Fat</td>
<td>0.05 kg</td>
<td>1.5</td>
<td>0.075</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Total</td>
<td>0.85</td>
<td></td>
<td></td>
<td></td>
<td>0.31</td>
</tr>
</tbody>
</table>

*Equal to the concentration of closantel in the tissues at 28 days after a single oral dose of 10 mg per kg of body weight.

*Equal to the concentration of closantel in the tissues at 28 days after a single intramuscular dose of 5 mg per kg of body weight.

*Number in parentheses refers to estimate of total residues. Following intramuscular administration, closantel accounted for approximately 80% of the total residues in liver.

Number in parentheses refers to estimate of total residues. Following oral administration, closantel accounted for approximately 70% of the total residues in liver.

### Table 2. Recommended temporary MRLs for closantel in cattle

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Daily food intake</th>
<th>Recommended temporary MRL as parent drug (mg/kg)</th>
<th>Daily total residue intake based on MRL (mg)</th>
<th>Concentration of closantel at 42 days withdrawal time (mg/kg)*</th>
<th>Daily total residue intake based on observed residue values (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>oral dose*</td>
<td>intramuscular dose*</td>
<td></td>
<td>oral dose</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.3 kg</td>
<td>0.5</td>
<td>0.30*</td>
<td></td>
<td>0.29 (0.58)*</td>
</tr>
<tr>
<td>Liver</td>
<td>0.1 kg</td>
<td>1.0</td>
<td>0.33*</td>
<td></td>
<td>0.56 (1.87)*</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.05 kg</td>
<td>2.0</td>
<td>0.25*</td>
<td></td>
<td>1.39 (3.76)*</td>
</tr>
<tr>
<td>Fat</td>
<td>0.05 kg</td>
<td>NE*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.83</td>
<td></td>
<td></td>
<td></td>
<td>0.50</td>
</tr>
</tbody>
</table>

*Equal to the concentration of closantel in the tissues at 42 days after a single intramuscular dose of 2.5 mg per kg of body weight.

*Based on an estimate that closantel accounted for 50% of the total residues in muscle, kidney and fat.

*Based on an estimate that closantel accounted for 30% of the total residues in liver.

*Not established. An MRL was not established for fat because of variability in the data, on the basis of available data, fat is not expected to contribute more than about 0.25 mg to the daily intake of residues of closantel at day 42 of withdrawal.

that were provided. The Committee did not increase these temporary MRLs to correspond more closely to the ADI because (1) the residue studies were carried out using a dose of 2.5 mg/kg whereas the maximum permitted dose is 7.5 mg/kg, and (2) the lack of total residue depletion and metabolism studies in cattle is a major deficiency. As recommended, the temporary MRLs should ensure an adequate margin of safety. The Committee may consider...
establishing full MRLs for cattle tissues when the results of total
residue and metabolism studies are submitted.

Although the Committee was not aware that an analytical
method had been validated thoroughly, a high-pressure liquid
chromatographic procedure is available that appears capable of
measuring the recommended MRLs in the above tissues.

The Committee requires results of the following studies by 1991:

1. A metabolism study in cattle that characterizes the total residue,
in particular to show the relationship between the concentration
of closantel and total residues at various times.
2. A study of total residue depletion in cattle using \([{}^{14}\text{C}]\)closantel, at
the maximum use level.
3. Adequate studies of residue depletion in cattle using unlabelled
closantel at the maximum use levels to assess the concentrations
of residues that occur under field conditions.

3.1.2 Ivermectin

Ivermectin had not been previously evaluated by the Committee.
The product is widely used as a broad-spectrum antiparasitic drug
against nematodes and arthropods in food-producing animals. In
human medicine, it is mainly used for the treatment of oncho-
cerciasis.

Ivermectin is a mixture of two homologous compounds with no
less than 80% 22,23-dihydroavermectin B\(_{1a}\) (H2B1a) and no more
than 20% 22,23-dihydroavermectin B\(_{1b}\) (H2B1b) (Fig. 3). The
homologues differ chemically by one methylene group at position 26.
Ivermectin is derived from abamectin, a natural fermentation
product of *Streptomyces avermitilis* NRRL B165. The only difference
in structure is that abamectin has a double bond between carbon
atoms 22 and 23, whereas ivermectin has a single bond in this
position.

The mode of action of ivermectin in parasites has remained
evasive. The avermectins appear to act at multiple sites, including
both \(\gamma\)-aminobutyric acid-dependent and independent chloride ion
channels. The mechanisms of the toxic action of ivermectin in
mammalian species have not been elucidated.

Toxicological data

The Committee reviewed toxicological data from studies on
pharmacokinetics, biotransformation, acute and short-term toxicity,
Effects on reproduction and development, genotoxicity, and observations in humans. Aspects of the comparative toxicities of ivermectin and abamectin were also considered.

Pharmacokinetic data were available from studies in mice, rats, dogs, rhesus monkeys, and human volunteers. In mice, peak plasma levels were reached approximately four hours after a single oral dose of 51 mg per kg of body weight. The average plasma to brain ratio of the concentrations of the drug was approximately 11:1. When ivermectin was administered at 0.1 to 0.5 mg per kg of body weight per day for 35 days, steady-state concentrations were observed from day 21. The concentration in the plasma and brain was proportional to the dose.

In a study in rats in which H2B1a was administered orally at 0.06 to 0.75 mg per kg of body weight, the dose and residue levels in plasma and tissues were also shown to be well correlated. In a study in which [\(^1\)H]ivermectin was given orally at 0.3 mg per kg of body weight the residue concentrations were highest in fat, followed by liver, kidney, and muscle. The main route of excretion was via the faeces.
In female rats aged eight weeks at initiation of dosing and receiving daily oral doses of 2.5 mg per kg of body weight for 61 days and then throughout mating, gestation, and until day 9 postpartum, steady-state plasma concentrations were reached on day 10 of treatment. On day 1 postpartum, however, the plasma concentration was three to four times the steady-state concentration, probably due to an increased mobilization of body fat. When treatment was restricted to days 1 to 9 postpartum, the concentration of ivermectin in the plasma increased gradually throughout the lactation period, and concentrations in milk were at least three or four times the corresponding concentrations in plasma. Under these conditions, the concentrations in the plasma of the offspring increased dramatically between days 1 and 6 postpartum, and on day 10 they were up to three times the concentrations found in maternal plasma. On days 1 and 4 postpartum, residue levels in brain tissue from offspring were similar to plasma concentrations. The results of this study suggested that the transfer of the drug via the milk was probably responsible for the increase in neonatal mortality observed in multigeneration studies.

In a 36-day study in the beagle dog in which ivermectin was administered orally at 0.5 and 2.0 mg per kg of body weight per day, the concentrations of H2B1a in the plasma increased dramatically between days 2 and 8 of treatment and reached steady state after approximately three weeks. A fourfold increase in the dose resulted in an average eightfold increase in plasma levels. In a comparative study with abamectin and ivermectin in immature rhesus monkeys, higher plasma concentrations were reached with ivermectin at all the dose levels investigated (2, 8 and 24 mg per kg of body weight). For both substances the concentrations in plasma were related to the dose, but the relationship was not linear.

In a study with human volunteers in which various formulations of ivermectin were administered orally, peak plasma concentrations were reached within approximately four hours. Administration of [3H]ivermectin showed that approximately 49% of the dose was eliminated in the faeces within five days. In a clinical study in lactating women treated with a single dose of ivermectin, a maximum concentration of 0.1 μg/l of the drug was found in milk on the day after treatment. This level decreased to less than 0.1 μg/l approximately one week after treatment. Although plasma levels were not reported for this particular study and the data determined from other studies were not directly comparable, it appears that
concentrations in human milk are similar to or slightly less than those in plasma.

Most of the studies on biotransformation were conducted using \(^3\)Hivermectin. When rat liver microsomes were incubated in vitro with the individual components and an NADPH-regenerating system, more than 70% of the radioactivity was associated with the corresponding parent compound. The major polar metabolite was identified as the 24-desmethyl-24-hydroxymethyl alcohol. The corresponding monosaccharide was also detected. These findings correlated well with the results of in vivo liver metabolism studies. In addition, a group of nonpolar metabolites was detected in fat, which yielded polar products on hydrolysis that were similar to the ivermectin metabolites present in liver.

Acute toxicity studies were conducted in mice, rats, rabbits, dogs, rhesus monkeys and a variety of target species (pigs, sheep, cattle and horses). The typical signs of acute toxicity of ivermectin were attributed to its effects on the central nervous system. These were most severe in CF\(_1\) mice, which exhibited ataxia, bradypnoea and tremors. Death occurred from approximately one hour to six days after dosing. Ivermectin was more toxic in neonatal rats than in young adult rats. This was believed to be due to postnatal completion of the blood–brain barrier in this species.

In beagle dogs, mydriasis was the most sensitive indicator of toxicity. More severe signs included ataxia and tremors. Deaths were preceded by a comatose-like state. Approximately 30% of collies tested were highly sensitive to ivermectin (as estimated from reports from non-recommended use of the drug). In immature rhesus monkeys no tremors or convulsions occurred. The most sensitive indicator was vomiting, which occurred in one of four monkeys given ivermectin at 2.0 mg per kg of body weight. The steep dose–response curve in rodents for the toxicity of ivermectin was not reproduced in monkeys.

Short-term studies were considered in rats, dogs, and monkeys. In a 14-week study in rats in which ivermectin was administered orally to pregnant dams, splenic enlargement and bone-marrow hyperplasia were noted in the offspring at 0.8 and 1.6 mg per kg of body weight per day. The no-observed-effect level was 0.4 mg per kg of body weight per day. These changes did not occur in other species which received ivermectin.

In a 14-week study in beagle dogs in which the compound was given orally, mydriasis and slight loss of body weight were observed
at 1.0 and 2.0 mg per kg of body weight per day (each group consisted of four females and four males). Four dogs in the groups receiving ivermectin at 2.0 mg per kg of body weight per day developed tremors, ataxia, anorexia, and dehydration, and were killed prior to scheduled necropsy. No other treatment-related effects were found. The no-observed-effect level was 0.5 mg per kg of body weight per day.

In a two-week study in which ivermectin was administered orally to neonatal monkeys at 0.04 and 0.1 mg per kg of body weight per day, and to immature monkeys at 0.3, 0.6 and 1.2 mg per kg of body weight per day, no drug-related effects were observed.

Three multigeneration studies were initiated in rats, but the first two were halted prior to scheduled termination because neonatal toxicity was apparent at all dose-levels tested. In the final (three-generation) study, the highest dose level was 0.4 mg per kg of body weight per day. The results indicated that ivermectin was toxic to neonatal rats at doses of 0.4 mg per kg of body weight per day or above (administered to adult females) as evidenced by increased neonatal mortality up to approximately ten days postpartum, and by the decreased weights of surviving offspring. The results of a cross-fostering study indicated that the neonatal toxicity was not related to in utero exposure but to postnatal exposure via maternal milk.

The developmental toxicity of ivermectin has been investigated in mice, rats, rabbits, and dogs. The results demonstrated that teratogenic effects (cleft palates in mice, rats, and rabbits; clubbed fore-paws without skeletal alterations in rabbits) were produced only at dose levels similar to those causing severe toxic effects in pregnant animals. The no-observed-effect level for teratogenicity in the most sensitive species and strain, the CF1 mouse, was 0.2 mg per kg of body weight per day, while for maternal toxicity it was 0.1 mg per kg of body weight per day.

Ivermectin was negative in three in vitro assays for genotoxicity. The Committee noted that no test of clastogenicity had been performed.

There were no carcinogenicity studies available on ivermectin. The Committee noted the very close structural similarities of ivermectin and abamectin. Extensive toxicological tests had been conducted on both compounds by one particular manufacturer, using the same strains of test animals over the same period of time. The Committee therefore reviewed several aspects of the comparative toxicology of the two products. The compounds were
indistinguishable at the level of receptor binding. Clinical signs of the
toxicity of both compounds included mydriasis in dogs, vomiting in
monkeys, and tremors, convulsions, and coma at higher doses in
most species. CF1 mice were most sensitive to the compounds.
In general, ivermectin was slightly less toxic than abamectin in
laboratory animals (2–4-fold higher threshold). In 14-week studies in
rats in which ivermectin and abamectin were administered orally at
0.4 mg per kg of body weight per day, no adverse effects were
observed. In a 14-week study with ivermectin in dogs, mydriasis was
seen at 1.0 mg per kg of body weight per day and above, and
tremors, ataxia and anorexia at 2.0 mg per kg of body weight per
day. In a 12-week study with abamectin in dogs, mydriasis occurred
at 1.0 mg per kg of body weight per day and above and extreme
weight loss at 2.0 mg per kg of body weight per day and above. In
multigeneration studies, toxicity in pups was the most sensitive
indicator, and occurred at 0.4 mg per kg of body weight per day. The
no-observed-effect levels for the formation of cleft palates in CF1
mice were the same. Both compounds were negative in a number of
in vivo tests for genotoxicity. Abamectin was also negative in in vivo
tests, including elastogenicity. Carcinogenicity studies with
abamectin were negative at maximum tolerated doses in mice and
rats. The Committee therefore concluded that it was unnecessary to
request data from long-term toxicity and carcinogenicity studies on
ivermectin.

Ivermectin is widely used in humans for the treatment of
onchocerciasis at single doses of 0.2 mg per kg of body weight.
Tolerance to the compound has been assessed in healthy volunteers
and in patients; adverse effects are usually mild and transient. In
particular, in patients no effects on the central nervous system were
observed.

The Committee concluded that the most relevant effect for the
safety evaluation of residues of ivermectin was its effect on the
mammalian nervous system. An ADI of 0–0.0002 mg per kg of body
weight was established based on a no-observed-effect level of 0.1 mg
per kg of body weight per day for maternal toxicity in the CF1
mouse. A safety factor of 500 was selected on the basis of the absence
of neurological effects in patients. This also provided a 1000-fold
margin of safety for the developmental toxicity of ivermectin.
Residue data

The Committee considered data from radiometric studies on the depletion of residues of ivermectin from the tissues of cattle, sheep, pigs, rats, and horses. Data were also provided for residues in milk, but these data were not evaluated because ivermectin is not approved for use in lactating animals.

In the species tested the drug was almost exclusively excreted in the faeces, with less than 1% eliminated in the urine.

The concentration of residues in liver and fat was much higher than in muscle and kidney in food-producing animals. Fat and liver were recommended as the most suitable tissues for residue control purposes and the depletion of total residues in these tissues is shown in Table 3.

Table 3. Total residues of ivermectin in liver and fat

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dose of ivermectin (mg per kg of body weight)</th>
<th>Route of administration of drug</th>
<th>Withdrawal time (days)</th>
<th>Estimated total residues (μg per kg of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>liver</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>fat</td>
</tr>
<tr>
<td>Cow</td>
<td>0.3</td>
<td>subcutaneous</td>
<td>7</td>
<td>622</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28</td>
<td>34</td>
</tr>
<tr>
<td>Cow</td>
<td>0.3</td>
<td>intratuminal</td>
<td>7</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>42</td>
<td>0</td>
</tr>
<tr>
<td>Sheep</td>
<td>0.3</td>
<td>intratuminal</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>Pig</td>
<td>0.4</td>
<td>subcutaneous</td>
<td>7</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28</td>
<td>3</td>
</tr>
</tbody>
</table>

The parent drug, a mixture of H2B1a and H2B1b, accounted for a major proportion of the total residues and H2B1a was considered a satisfactory marker compound for measuring total residues. The percentage of total residues present as marker compound H2B1a was similar in sheep and cattle, and was higher than in pigs. In fat tissue, the percentage of total residues present as H2B1a decreased as the withdrawal time increased, but H2B1a was considered to be the most satisfactory marker compound available for measuring total residues in fat. A relatively non-polar fraction (more polar
nevertheless than the parent drug) accounted for 26-55% of the residues in fat. The residues in these fractions were tentatively identified as acyl-esters of the major metabolites and their deposition in fat may account for the high concentrations of and the persistence of residues in fat.

Virtually all of the radiolabelled residue in the edible tissues may be extracted by means of organic solvents. Thus, there are very few, if any, bound residues and no further consideration of the bound residues is necessary.

The metabolism of ivermectin was evaluated in liver tissue. The metabolic profile differed between some species. In cattle, sheep, and rats the 24-hydroxyl-methyl derivatives formed significant amounts of the residues. These derivatives were not detected in pigs, but significant amounts of the 3’-O-desmethyl derivatives were present.

A satisfactory analytical method is available for measuring residues of H2B1a in the range 5–60 μg/kg. The method involves: (1) tissue extraction with addition of an internal standard; (2) fluorogenic derivatization; (3) reverse-phase high-performance liquid chromatography; and (4) calculation of the concentration of H2B1a from standard curves. The limit of detection of this method is 3 μg/kg.

**Maximum Residue Limits.** The following points were considered by the Committee in recommending MRLs for ivermectin:

(a) The ADI for total residues is 0.0002 mg per kg of body weight.
(b) The marker compound is H2B1a.
(c) H2B1a accounts for approximately 50% of total residues in liver, muscle, and kidney in cattle and sheep, and about 40% of total residues in swine liver, muscle, and kidney.
(d) As withdrawal times increase, the proportion of H2B1a in the total residues in fat decreases. At 28 days after ivermectin administration, H2B1a accounts for approximately 20% of total residues.
(e) The drug is widely used, with practical withdrawal times of at least 28 days.
(f) Liver and fat are the tissues of choice for determining compliance with safe residue limits.
(g) A suitable analytical method is available for measuring residues in liver and fat.
An MRL for H2B1a of 0.015 mg/kg for liver and 0.02 mg/kg for fat is recommended for all species.

The residue marker for ivermectin, H2B1a, represents approximately 50% of the total residues in muscle, liver and kidney, and 20% of the total residues in fat. On this basis, the recommended maximum daily intake of ivermectin is 3 µg for liver and 5 µg for fat (taking into account food intake data on page 9). The remaining 4 µg per day (from the 12 µg/day allowed by the ADI for a 60-kg adult) yields a concentration of H2B1a of 6 µg/kg in both muscle and kidney. These concentrations will not be exceeded in food-producing animals if the recommended MRLs are observed in liver and fat.

No further studies on residues of ivermectin were requested.

3.1.3 Levamisole

Levamisole had not been previously reviewed by the Committee. The compound is used as an anthelminthic in cattle, sheep, goats, swine, and poultry. It is effective against lungworms and gastrointestinal nematodes. It is also used as adjuvant therapy in the treatment of human cancer.

Toxicological data

The Committee considered results from studies on metabolism, short-term studies, and studies on carcinogenicity, effects on reproduction and development, and mutagenicity, as well as from clinical reports in humans.

The absorption, distribution, excretion, and biotransformation of levamisole were studied primarily in rats. The results showed that the drug was rapidly absorbed and metabolized, with only 45% of the radioactivity in the plasma being accounted for by the parent compound after one hour. More than 90% of the radioactivity was excreted in the urine and faeces within 8 days. Over 50 metabolites were identified from these studies and four major metabolic pathways have been proposed. Following oral administration of levamisole to humans, the half-life was estimated to be 4 hours, while the half-life for total radioactivity was 16 hours. Most of the radioactivity was excreted in the urine, with 4% as unchanged levamisole and the remainder as metabolites.

In a 13-week study in rats, in which levamisole was administered in the diet, reduced weight gains were noted at doses of 40 and 160 mg per kg of body weight per day in both sexes. This effect was
also observed at 10 mg per kg of body weight per day in female rats. In a 90-day study in dogs, no drug-related effects were noted at doses of up to 6 mg per kg of body weight per day.

Groups of three dogs of each sex were dosed for one year (six days per week) by oral administration of the compound in capsules. During the eighth week, all six dogs that received levamisole at 20 mg per kg of body weight per day and one dosed at 5 mg per kg of body weight per day developed severe haemolytic anaemia. The haematocrit level and total erythrocyte counts were decreased, while the numbers of erythroblasts and immature granulocytes were increased. Treatment was discontinued in these affected dogs, and their haematological parameters returned to normal about two weeks later. However, anaemia developed again when treatment was resumed. A levamisole-dependent erythrocyte-agglutinating factor was demonstrated in vitro in the serum of these dogs. No other treatment-related effects were found. The no-observed-effect level was 1.25 mg per kg of body weight per day in this study.

The Committee noted the deficiencies in the study, including the low number of animals per group, completion of the study with only two treated groups and uncertainty regarding the mechanism for levamisole-induced haemolysis, which appeared to have an immunological basis.

In a chronic toxicity study in rats in which levamisole was administered in the diet for 12 or 18 months, a significant reduction in weight gain was observed at 80 mg per kg of body weight per day in both sexes. This effect occurred to a lesser degree at 20 mg per kg of body weight per day. In general, absolute organ weights were decreased, while relative organ weights were increased at 80 mg per kg of body weight per day. Other changes observed in this treatment group included slight increases in alkaline phosphatase (in females) and bilirubin (at 12 months), and degeneration of the germinal epithelium of the testis. The no-observed-effect level for this study was 20 mg per kg of body weight per day.

In an 18-month carcinogenicity study in mice in which levamisole was administered in drinking-water, no treatment-related effects were evident with respect to the number of tumour-bearing mice or the incidence of various types of tumour. Dose levels of up to 80 mg per kg of body weight per day were used in this study, but the Committee noted the lack of toxicity data provided to support selection of these doses. There was no evidence for a carcinogenic effect in female mice. However, the survival rate of male mice beyond
12–15 months was poor. In addition, the Committee noted that a high percentage of the male mice had not been examined microscopically, which further reduced the sensitivity of the study. The Committee concluded that this study did not fully assess the carcinogenic potential of levamisole in male mice.

In a 24-month carcinogenicity study in rats in which levamisole was administered in feed at doses up to the equivalent of 40 mg per kg of body weight per day, no treatment-related effects with respect to the number of tumour-bearing rats or incidence of various tumour types were reported. There was no evidence of a carcinogenic effect in female rats and survival was not affected by treatment. However, taking into account the low survival rate beyond 18 months, the Committee concluded that this study did not fully assess the carcinogenic potential of levamisole in male rats.

In three reproduction studies in rats in which levamisole was administered in the diet, there was decreased maternal weight gain, increased incidence of stillbirth, reduced birth weight, and reduced weight gain by the pups during suckling, and decreased survival at 3 weeks at 80 mg per kg of body weight per day. The no-observed-effect level for effects on reproduction was 20 mg per kg of body weight per day.

Levamisole was further evaluated for effects on development in studies in which it was administered to rats and rabbits during all or part of gestation. In rats, the incidence of resorptions was increased slightly after oral administration at 80 mg per kg of body weight per day, which was the highest dose tested. In the rabbit, the highest dose of 40 mg per kg of body weight per day caused a marked reduction in maternal weight gain and an increase in the incidence of fetal death and abnormalities. The no-observed-effect levels for these studies were 20 mg per kg of body weight per day for the rat and 10 mg per kg of body weight per day for the rabbit.

The genotoxic potential of levamisole was investigated in a number of test systems. Positive results were reported in the chromosomal aberration test and the sister chromatid exchange test using human lymphocytes. Negative results were reported in a series of Ames tests, a further chromosomal aberration test, a mouse micronucleus test, and a dominant lethal assay.

The Committee considered data from numerous clinical reports on the use of levamisole in human therapy. Adverse effects were rare, the most important being agranulocytosis and neutropenia. Although these haematological disorders were generally reversible,
certain factors, such as the concurrent use of other drugs, made the interpretation of occasional fatalities extremely difficult. Fatalities were usually associated with concurrent infection before agranulocytosis had been recognized. The Committee noted that the increase in the number of IgM antibodies observed in some patients suggested an immunological basis for the granulocytopenia; this effect was not associated with the single dose of 2.5 mg per kg of body weight used for the treatment of human parasites. Thrombocytopenia and haemolytic anaemia have also been reported in patients being treated with levamisole.

The Committee noted that there were a large number of published reports concerning the induction of granulocytopenia or agranulocytosis by levamisole, primarily in patients being treated for rheumatoid arthritis or cancer. However, the data were inadequate for the purposes of establishing a no-observed-effect level for the induction of these haematological effects. The results of a survey showed a 0.1% incidence of agranulocytosis in patients receiving levamisole at 50–200 mg on three days every other week. However, agranulocytosis and thrombocytopenia have been reported in a cancer patient treated only with levamisole at 2.5 mg per kg of body weight on two consecutive days each week for 13 weeks. Although the bone marrow was initially depressed, the patient's immunological indicators returned to normal within two weeks of the cessation of treatment. The immunological basis for this condition was further supported by the presence of levamisole-dependent leukoagglutinins correlating with the acute phase of granulocytopenia. A report of a patient with a history of prior treatment with levamisole, who developed mild granulocytopenia following the administration of a single challenge dose of approximately 0.2 mg per kg of body weight, suggested that this dose may be near the no-observed-effect level in humans.

The Committee considered that levamisole-induced haemolysis in dogs and agranulocytosis in humans may have a similar immunological basis. However, there was insufficient evidence to confirm or exclude this possibility. It was noted that thrombocytopenia and haemolytic anaemia have been observed in both dogs and human patients treated with levamisole. However, neither the data from case reports on human therapeutic use nor those from the study in dogs were considered adequate for the purpose of establishing a full ADI. In the former instance, most of the patients were suffering from cancer or diseases associated with an
underlying autoimmune disorder, and had been exposed to a variety of therapeutic regimens before receiving levamisole; furthermore, there was no information about the threshold dose at which agranulocytosis occurred. In the study in dogs, which used an inadequate number of animals, haemolysis rather than agranulocytosis was the end-point, and there was uncertainty whether the pathogenesis of this effect was identical to that responsible for the agranulocytosis in humans.

A temporary ADI of 0–0.003 mg per kg of body weight was established for levamisole, based on a no-observed-effect level of 1.25 mg per kg of body weight per day for the induction of haemolysis in dogs, and a safety factor of 500, which the Committee selected for this compound after taking into consideration the uncertainty regarding the relevance of the dog model and the fact that no threshold could be established from the human data.

The Committee noted that the lowest level of levamisole reported to be associated with even mild granulocytopenia in a compromised (i.e., ill) human patient was at least 60 times the temporary ADI. The Committee assumed that residues other than levamisole have the same potential toxicity as the parent drug.

One member of the Committee, Professor A. Somogyi, disagreed with the conclusions of the majority. His personal opinion is given in Annex 4.

Residue data

The Committee considered data on the metabolism of levamisole and depletion of residues of levamisole from the edible tissue of chickens, cattle, sheep and swine.

As noted earlier, metabolism studies in rats have resulted in the identification of at least 50 metabolites in urine. Four major metabolic pathways have been proposed to account for these metabolites.

The Committee noted that only limited data on the metabolism of levamisole in pigs and goats were available. However, the results were similar to those observed in rats (see page 31): the drug was rapidly excreted, a large proportion of the dose was eliminated in the urine, and extensive metabolism appeared to have occurred. The Committee considered that the metabolism of levamisole in food-producing animals may be qualitatively similar to that in rats, although this would need to be demonstrated in adequately conducted studies. However, it was not possible to determine a
relationship between a suitable marker compound and the total residues in tissues, on the basis of data available from studies in pigs and goats.

No data on total residue depletion were available from studies in food-producing animals dosed with radiolabelled levamisole. Studies in rats demonstrated an initial rapid decline of total residues of levamisole from the tissues of treated rats (half-life of about 1 day), and a slower second phase (half-life of approximately 4–5 days). The total residue concentrations in rats reached 100 μg/kg on day 4 in muscle, on day 14 in liver, and on day 10 in kidney.

The depletion of residues of levamisole from the edible tissues of cattle, sheep, swine and chickens has only been investigated using the unlabelled drug. The drug was administered in various formulations, including bolus, soluble powder, pour-on, and injection. The concentration of residues of the parent drug in edible tissues fell below the 100 μg/kg level at 2–5 days withdrawal time. In cattle, the concentration of residues of parent levamisole in milk was less than 10 μg/kg at 60 hours following oral, parenteral, or dermal administration of the drug. The tissue samples in these studies were analysed for levamisole using gas chromatography, polarography, and high-pressure liquid chromatography.

For a 60-kg person, the permitted daily intake of total residues of levamisole would be 180 μg contributed by 500 g of animal-derived meat and 1.5 l of milk. On the basis of the available data in rats, the manufacturer estimated that levamisole would represent approximately 10% of the total residues in food-producing animals, which would give an estimated maximum daily intake of 18 μg of parent drug. Since this amount would be contributed through 2 kg of dietary meat and milk, a suitable analytical method for measuring residues of levamisole would require a detection limit of 9 μg/kg. The limit of detection of the method used by the manufacturer was 10 μg/kg. The Committee, therefore, recommended a temporary MRL of 0.01 mg/kg for tissues of all species and milk.

The Committee requires the following by 1994:

1. A comprehensive assessment of the incidence of granulocytopenia and agranulocytosis, thrombocytopenia and haemolytic anaemia in humans receiving levamisole, together with dose–response information.

2. The results of studies that demonstrate that the mechanisms for the production of haemolytic anaemia in dogs and neutropenia or
agranulocytosis in humans are related phenomena, such as experiments in which the specificity of the antibody response and of the target cells is studied.

3. A comparison of the metabolites of levamisole produced in humans, laboratory animals, and food-producing animals; if differences in major metabolites are demonstrated, evidence should be obtained on the potential of the metabolites that are produced in food-producing animals to induce haematological effects.

4. The results of a metabolism study in food-producing animals that characterizes the total residues, which should include data on the relationship between a suitable marker compound and the total residues after different withdrawal periods.

5. The results of radiometric studies on the depletion of total residues in food-producing animals.

6. The results of depletion studies using the maximum recommended dose levels of unlabelled levamisole, with sufficient animals to permit an assessment of the residues that occur under field conditions.

3.2 Antimicrobial agents

3.2.1 Benzylpenicillin

Benzylpenicillin had been previously evaluated at the twelfth meeting of the Committee (Annex 1, reference 17).

Benzylpenicillin is one of the most widely used antibiotics in both animals and humans. It is primarily used for the control of mastitis in dairy cows and for treating infections of the urinary tract, gastrointestinal system, and respiratory tract. Benzylpenicillin is also administered as a feed additive to pigs to control streptococcal meningitis and is included as an additive in the drinking-water of poultry.

Toxicological data

No toxicological studies were available for review. Among the adverse reactions which had been reported in people consuming food containing benzylpenicillin residues, hypersensitivity reactions were most common. The overall prevalence of allergy to penicillin, taking into account various reports of allergic reactions in different populations and using a variety of test procedures, was estimated to
be 3–10%. There was no evidence of sensitization caused by benzylpenicillin residues in food. The Committee evaluated the available data on allergic reactions caused by penicillin residues. Only four cases were considered to be adequately documented to demonstrate that hypersensitivity reactions could be caused by ingestion of less than 40 μg of the drug.

Residues of benzylpenicillin can also inhibit starter cultures used in the production of yoghurt, cheese and other milk products.

The Committee concluded that allergy was the determining factor in the safety evaluation of residues of benzylpenicillin. In the absence of adequate data to establish a no-effect-level, the Committee recommended that the daily intake from food be kept as low as practicable, and in any case, below 30 μg of the parent drug. The risk associated with the occurrence of mild hypersensitivity reactions at this level was considered to be insignificant.

**Residue data**

Benzylpenicillin is distributed throughout the body and enters the extracellular fluid of tissues. The drug is rapidly cleared from the blood via the kidneys and into the urine. Following intravenous, intramuscular or subcutaneous administration of the drug in a variety of species, more than 60% of the dose was excreted in the urine within a few hours; when administered by these routes to lactating dairy cattle, less than 0.03% of the dose was excreted in milk. However, most of the dose was excreted in the milk following intramammary administration of the drug.

The persistence of residues in milk depends on the formulation and route of administration, but in the various studies examined by the Committee the residues did not persist beyond 5 days after the end of treatment.

The analytical bioassay methods used for measuring and controlling residues in milk are well established and have detection limits of 1–10 μg/l. The Committee agreed that it was both practical and possible to use methods with a detection limit of 4 μg/l in milk.

The Committee noted that only limited data on residues in meat were available, from studies in cattle. No tissue depletion studies were available. The results of two separate residue studies, on single cows injected intramuscularly with a single dose of benzylpenicillin at 6 and 6.7 mg per kg of body weight, respectively, and killed 2 hours later, are shown in Table 4. The resulting concentrations of
Table 4. Concentration of benzylpenicillin residues in cattle, two hours after intramuscular administration of the parent drug

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Concentration of benzylpenicillin residues (mg/kg)</th>
<th>Based on total residues*</th>
<th>Based on a bioassay method*</th>
<th>Based on a high-performance liquid chromatographic method*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>2.7</td>
<td>0.79</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Bile</td>
<td>117</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>14</td>
<td>0.58</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>30*</td>
<td>0.55</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>0.37-0.76 (5)*</td>
<td>0.03-0.05 (4)*</td>
<td>0.06-0.18 (4)*</td>
<td></td>
</tr>
</tbody>
</table>

*Based on data from a cow given a single intramuscular dose of [**C**]benzylpenicillin at 6.7 mg/kg using a radiometric assay.
*Based on data from a cow given a single intramuscular dose of benzylpenicillin at 6 mg/kg. Assays carried out on same samples.
*Not measured.
*Kidney cortex.
*Kidney medulla.
*Number in parentheses refers to the number of samples tested.

residues were several orders of magnitude higher than the current residue limits for meat.

The results demonstrated that:

(a) The concentration of benzylpenicillin is about 100 times higher in liver and kidney than in muscle, based on the results obtained by the high-performance liquid chromatographic method.

(b) There was poor correlation between the results obtained with different assay methods. The concentrations of residues were highest in the radiometric study (in which the total residues of benzylpenicillin were measured), which could mean that large amounts of the residues were microbiologically inactive metabolites.

(c) The residue values measured by the high-performance liquid chromatographic procedure were higher than those measured using the bioassay because the extraction procedures employed for tissues analysed by the former method were more vigorous. It is therefore important to consider whether the quantity of benzylpenicillin residues in tissues may be underestimated in some bioassays, because not all of the benzylpenicillin residues are available to the bacteria in the test system.

The Committee considered information on residues of benzylpenicillin in calves. The calves were injected intramuscularly with a single dose of benzylpenicillin at 10 mg per kg of body weight or two doses of 3 mg per kg of body weight 24 hours apart, and killed
24 hours later. The residues of benzylpenicillin exceeded 40 µg/kg in muscle, liver and kidney when assayed using the bioassay method.

The Committee noted that bioassay methods were widely used to screen meat for residues of benzylpenicillin. In the European Economic Community, muscle tissue is screened using the Four Plate Test (limit of detection 30–60 µg/kg). The Food Safety and Inspection Service of the United States Department of Agriculture uses two bioassay methods (limit of detection 12.5 µg/kg)—the Swab Test, which uses exudate from kidney, and a method for kidney, liver, and muscle that involves a simple extraction procedure.

These bioassays are not specific for benzylpenicillin and the antibiotic must therefore be identified using a specific chemical method, preferably with the same analytical sensitivity. Several such methods are available, with detection limits of 50–100 µg/kg in meat and 10–50 µg/l of milk. However, these methods are not as sensitive as bioassays and they cannot be used to confirm benzylpenicillin residues at levels of 4–10 µg/l in milk. The Committee emphasized the need to improve the sensitivity of chemical methods for benzylpenicillin.

In reaching a decision on a recommended MRL, the Committee considered the following points:

(a) The drug is used in both meat- and milk-producing animals.
(b) The maximum permitted daily intake of benzylpenicillin is 30 µg.
(c) Bioassay methods for screening benzylpenicillin are widely used and are likely to be the methods of choice for most Member States in the foreseeable future.
(d) The analytical methods that are available for measuring residues in milk are much more sensitive than those for meat.
(e) Chemical assay methods for confirming bioassay results are becoming increasingly available, but at present they are less sensitive than bioassay methods.

An MRL for total residues of benzylpenicillin of 0.05 mg/kg for liver, kidney, and muscle (all species) and an MRL of 0.004 mg/kg for milk are recommended.

Thus, the estimated maximum daily intake of benzylpenicillin residues is 15 µg for muscle, 5 µg for liver, 3 µg for kidney, and 6 µg for milk (taking into account food intake data on page 9), yielding a total maximum daily intake of 29 µg. The concentration of residues in fat is zero.

The Committee recommends:
1. The provision of further information and the results of new studies on the depletion of residues of benzylpenicillin from the edible tissues of food-producing animals.
2. Investigation of the accuracy and precision of the assays used to measure residues of benzylpenicillin.
3. The development of more sensitive chemical assays for benzylpenicillin.

3.2.2 Oxytetracycline

Oxytetracycline had been previously evaluated at the twelfth meeting of the Committee (Annex 1, reference 17), at which a temporary ADI of 0–0.15 mg per kg of body weight was established. The MRLs were set at the limit of detection of the microbiological method of analysis: 100 µg/kg for milk, 250 µg/kg for meat, and 300 µg/kg for eggs.

Oxytetracycline is a broad-spectrum antibiotic used for the treatment and control of a variety of bacterial infections in humans and animals. It is also used as a growth-promoting agent in animals.

Toxicological data

The Committee considered pharmacokinetic data and results from short-term studies in rats, mice, and dogs, a multigeneration study in rats, teratogenicity studies in mice, rats, rabbits, and dogs, long-term/carcinogenicity studies in mice and rats, mutagenicity tests and studies on microbiological effects in laboratory animals and humans.

Pharmacokinetic studies demonstrated that about 60% of ingested oxytetracycline was absorbed from the gastrointestinal tract in humans, compared to 4–9% in mice and swine. Following absorption by various routes of administration, oxytetracycline was widely distributed in the body, particularly in the liver, kidney, bones, and teeth. Systemically available oxytetracycline was primarily excreted in the urine, as parent drug.

In the short-term toxicity studies, oxytetracycline was incorporated into the diets of mice and rats at levels up to the equivalent of 7500 and 2500 mg per kg of body weight per day respectively. Decreased body weights were observed in mice at 3750 and 7500 mg per kg of body weight per day and a non-dose-related incidence of minimal periacinar fatty metamorphosis of the liver was observed in male rats at all dose levels.
In a study in dogs that received oxytetracycline at 0, 5 or 10 g/kg in the diet, degenerative changes in the germinal epithelium of the testes were noted at 10 g/kg. However, these findings were not confirmed in a second study. The no-observed-effect level was equivalent to 250 mg per kg of body weight per day. No effects on reproductive performance were observed in a two-generation study in rats in which the compound was incorporated at a level of 360 mg/kg in the diet, equivalent to 18 mg per kg of body weight per day. In studies in rats, which received the compound orally at 48, 240, and 480 mg per kg of body weight per day on days 1 to 21 of gestation, no teratogenic effects were observed. However, an increase in mortality rate and number of fetal resorptions and a decrease in fetal ossification were noted at all dose levels tested. In a study in mice in which the compound was administered orally, the highest dose of 2100 mg per kg of body weight per day caused maternal toxicity. There was no evidence of a teratogenic effect.

In teratogenicity studies in rats and rabbits in which oxytetracycline was administered intramuscularly at 41 mg per kg of body weight, there was no evidence of a teratogenic effect. However, an increased number of fetal resorptions was noted in rabbits. Intramuscular administration of the compound in dogs, at approximately 20 mg per kg of body weight per day, caused skeletal and visceral malformations in the pups. The Committee noted that this study was difficult to evaluate since it was poorly reported.

In a carcinogenicity study in mice and a similar study in rats, in which oxytetracycline was administered in the diet at dose levels up to 1372 and 150 mg per kg of body weight per day respectively, there was no evidence of an increase in the incidence of tumours. In a second study, rats were fed diets containing up to 2000 mg of oxytetracycline per kg of body weight per day for 103 weeks. A dose-related increase in the incidence of benign phaeochromocytomas was observed in males, but because the survival rate of male rats in the control group was low, this increase was not considered to be significant. Although there was an increase in the incidence of benign neoplasms of the pituitary gland in female rats in the highest-dose group, there was a lower incidence of pituitary-gland hyperplasia than in controls. The Committee concluded that there was no evidence of a carcinogenic effect in rats or mice.

The mutagenic potential of oxytetracycline was investigated in a range of studies. Negative results were recorded in bacterial tests, a chromosomal aberration test, a sister chromatid exchange test (with
and without metabolic activation), and a mouse lymphoma test without metabolic activation. The Committee noted that a positive effect in the mouse lymphoma test with metabolic activation was obtained using dose levels close to toxic concentrations and that the positive effect in the in vivo micronucleus assay in mice was not dose-related.

In assessing the microbiological effects of oxytetracycline, the Committee considered the results of studies on the induction of drug-resistant organisms in dogs and humans. In a 6-week study in dogs, which received oxytetracycline at 2 mg/kg in the diet (equivalent to 50 μg per kg of body weight per day), there was no increase in the level of resistant faecal coliforms. In humans receiving oral treatment with oxytetracycline at 2 g, 20 mg, or 2 mg per day for 7 consecutive days, there was no evidence of resistant bacteria of the family Enterobacteriaceae in the faeces at the lowest dose. The data on the induction of bacterial resistance in dogs, when recalculated on the basis of a 60-kg person, yielded a similar no-effect dose of 3 mg per day.

In view of the results of studies on the toxicological and microbiological effects of oxytetracycline, the Committee concluded that information about the induction of resistant coliforms in the human intestine was most appropriate for the safety assessment of oxytetracycline. The Committee adopted this conservative approach, although it recognized that the no-effect levels in toxicological studies were 18 mg per kg of body weight per day or higher.

An ADI of 0–0.003 mg per kg of body weight was established for oxytetracycline, based on a no-observed-effect level of 2 mg per person per day from the study with human volunteers and a safety factor of 10. It should be noted that the next dose tested was 20 mg per person per day, so the true no-effect level may be significantly higher than suggested by this study. A repeat of the study using doses between these values may result in a higher no-observed-effect level.

The safety factor was selected by the Committee to account for the variation in the intestinal microbial flora among humans. This factor was viewed as being conservative because the people in the study were selected because they had an oxytetracycline-sensitive microbial flora. Furthermore, the MRLs derived from the established ADI would be similar to those derived from in vitro 50% minimal inhibitory concentration data, for many species of microorganisms.
Residue data

The results of studies on the metabolism of the tetracyclines suggest that minimal, if any, metabolism of these compounds occurs in humans, dogs, and rats. Limited information is available on the metabolism of oxytetracycline in humans and rats. Nevertheless, data available from studies involving tetracyclines with structures similar to oxytetracycline indicated that oxytetracycline is unlikely to be metabolized in animals. Hence, the Committee concluded that determination of oxytetracycline by either a microbiological or a chemical method would provide a suitable measure of the total residues of oxytetracycline in the edible tissues, milk, and eggs of food-producing animals.

Extensive studies were available on the depletion of residues of oxytetracycline in food-producing animals. In general, the concentrations of residues of oxytetracycline were highest in the kidney and liver, and lowest in fat. The ADI that has been established makes it difficult to recommend MRLs for edible tissues, milk, and eggs that can be monitored with currently available microbiological methods of analysis. This situation is due to the large contribution of milk to the total daily diet and the large difference between the no-effect level of 2 mg per day in the human study and the next-highest level tested, which was 20 mg per day.

The Committee recommended MRLs for milk, muscle, fat, and eggs at the detection level of the microbiological method: 0.1, 0.1. 0.01, and 0.2 mg/kg, respectively. The Committee further recommended MRLs of 0.3 mg/kg for liver and 0.6 mg/kg for kidney, in all species. The last two recommendations reflect the typical residue distribution of oxytetracycline in these tissues.

Therefore, the estimated maximum daily intake of oxytetracycline is 150 µg in milk, 30 µg in muscle, 0.5 µg in fat, 20 µg in eggs, 30 µg in liver, and 30 µg in kidney (taking into account the food intake data on page 9), yielding a total of approximately 260 µg. This value slightly exceeds the ADI of 200 µg per person (one-tenth of the no-observed-effect level of 2 mg per day). Since the ADI was derived from a conservative value of 2 mg per person per day and the consumption data are at the upper limit of the range for individual intake of animal products, the Committee concluded that the recommended MRLs are conservative and do not present a risk for the consumer.

The Committee concluded that no further studies on residues of oxytetracycline were required.
3.3 Growth promoters

Two quinoxaline-1,4-dioxide compounds, carbadox and olaquinodox, were considered by the Committee. They are similar in structure, as shown in Fig. 4.

![Structural formulae of carbadox and olaquinodox](image)

Although their mode of action as growth promoters is not fully understood, they are known to inhibit bacterial DNA synthesis and to produce bacterial DNA damage in vitro, possibly by a free-radical mechanism involving reduction of the parent drug. It is thought that this may account for their antimicrobial properties (22). The compounds were evaluated individually.

3.3.1 Carbadox

This is the first occasion on which carbadox has been reviewed by the Committee. Carbadox is an antimicrobial drug used to promote growth in pigs and to control swine dysentery and bacterial swine enteritis. The commercial product is intended for use in starter and/or grower rations but not in finisher rations. It is usually administered in the feed at 50 mg/kg and may be given to pigs up to four months of age.

Toxicological data

The Committee considered data from short-term, long-term and "relay" toxicity studies, and from studies on carcinogenicity, mutagenicity, and reproduction with carbadox, together with data from mutagenicity and long-term toxicity studies of its metabolites.

The metabolism of carbadox has been studied in rats, monkeys and pigs using $^{14}$C carbadox, labelled in either the phenyl ring or the carbonyl group of the side-chain. The metabolism of carbadox
was characterized by the rapid reduction of the N-oxide groups, the cleavage of the methyl carbazate side-chain, and the liberation of respired CO₂. The primary metabolite in the urine was quinoxaline-2-carboxylic acid, which was also excreted in conjugated form. The detectable residues in tissues, up to 24 hours after drug withdrawal, were carbadox, desoxycarbadox, quinoxaline-1,4-di-N-oxide-2-carboxaldehyde, and quinoxaline-2-carboxylic acid. Hydrazine was a minor metabolite but would be expected to be present only for a short time before undergoing further metabolism.

The concentration of carbadox and desoxycarbadox in the tissues declined rapidly (half-life about 6 hours), and after 3 days, was less than 5 µg/kg. In a study in pigs in which carbonyl-labelled [¹⁴C]carbadox was administered by gavage, the concentration of total residues in liver at 5 days withdrawal time corresponded to 0.12 mg/kg methyl carbazate equivalent. However, some of this radioactivity was due to the presence of amino acids, which had been labelled by incorporation of ¹⁴CO₂. When ring-labelled [¹⁴C]carbadox was administered to pigs, extremely low levels of unidentified metabolites remained in the liver at withdrawal periods longer than 7 days. These residues were partially released and converted to quinoxaline-2-carboxylic acid by alkaline digestion of the liver.

The Committee also reviewed recent reports of the effect of carbadox on adrenal function in pigs, although the information did not affect the outcome of the evaluation.

Several long-term feeding studies in rats were evaluated which demonstrated dose-related increases in the incidence of benign and malignant liver tumours at doses of carbadox above 1.0 mg per kg of body weight per day. Doses above 25 mg per kg of body weight per day produced pronounced toxic effects, which precluded chronic treatment. Data from a variety of mammalian and non-mammalian genotoxicity studies were also reviewed. Positive findings were reported in 14 of the 15 tests carried out. The Committee concluded that carbadox appears to be both genotoxic and carcinogenic.

The Committee evaluated a "relay" toxicity study in which pigs were fed carbadox at up to 200 mg/kg in the diet for 30 days and killed at zero withdrawal time; their livers were then fed to rats as a 10% dietary component for 2 years. Although this study was conducted satisfactorily and no treatment-related effects were reported, the Committee did not use it for assessing the carcinogenicity of carbadox residues because of the low number of
animals used and because only small quantities of residues can be consumed by rats in this type of study.

In a long-term study in rats, desoxycarbadox was reported to produce an increase in the incidence of tumours. All rats that received 25 mg per kg of body weight desoxycarbadox daily for 10 months in the diet developed hepatic tumours. The incidence of tumours was increased in all treated groups, at doses of 5–25 mg per kg of body weight per day. While the most pronounced change occurred in the liver, tumour incidence was also elevated at other sites, including the skin and mammary glands.

The mutagenic potential of desoxycarbadox was investigated in a range of studies. The Committee noted that, while desoxycarbadox produced negative results in most mutagenicity test systems, positive findings were recorded in the cell transformation test and the Ames test using liver microsomes from rats treated with polychlorinated biphenyls.

The Committee noted that the tumorigenic potential of desoxycarbadox was apparently greater than that of the parent compound, and that desoxycarbadox would therefore probably make a significant contribution to the tumorigenic activity of carbadox in rats. However, the Committee also noted that the metabolism data for carbadox supported the conclusion that desoxycarbadox was a relatively short-lived intermediate between carbadox and quinoxaline-2carboxylic acid.

The Committee also considered information on the carbadox side-chain metabolite, methyl carbazate. This information consisted of results from two long-term studies in rats and a number of mutagenicity assays. In the more informative long-term study (in which more animals per dose and concurrent controls were used), rats received methyl carbazate in the diet for 710 days. There was no increase in the incidence of tumours and no other treatment-related effects were observed at 10 mg per kg of body weight per day, which was the highest dose tested. The lack of effect limited the usefulness of this study. The no-observed-effect level for the study was 10 mg per kg of body weight per day. The useful mutagenicity studies were negative.

The results of studies on the pharmacokinetics, mutagenicity, and carcinogenicity of hydrazine were also considered by the Committee. Pharmacokinetic data from rats and rabbits as well as hydrazine's known chemical reactivity suggest that it should be rapidly eliminated. The structure of carbadox and its known metabolic
pathways indicate that hydrazine is probably a further metabolite of methyl carbazate. Hydrazine showed mutagenic and carcinogenic potential.

In evaluating the potential toxicity of residues of carbadox, the Committee also considered toxicity data on the carbadox metabolite quinoxaline-2-carboxylic acid to be useful. In a carcinogenicity study in mice in which quinoxaline-2-carboxylic acid was administered in the diet for 19 months, no treatment-related increases in tumour incidence or any other effects on the animals were reported. The no-observed-effect level was 100 mg per kg of body weight per day.

Several chronic toxicity and carcinogenicity studies in which quinoxaline-2-carboxylic acid was administered for 24 months were conducted in rats. There were no effects on incidence of tumours, the only findings being a slight reduction in weight gain at 100 mg per kg of body weight per day. The no-observed-effect level was 50 mg per kg of body weight per day.

In a three-generation reproduction study in rats, quinoxaline-2-carboxylic acid was given in the diet at levels of up to 100 mg per kg of body weight per day. No treatment-related effects were observed. Developmental studies were conducted in rats and rabbits in which the compound was administered by gavage at levels of up to 100 mg per kg of body weight per day. There was no evidence of maternal toxicity, embryotoxicity, or teratogenicity in either species. The no-observed-effect levels in these studies were 100 mg per kg of body weight per day.

With current analytical procedures, quinoxaline-2-carboxylic acid is the only carbadox metabolite that can be identified in liver from pigs treated according to good practice in the use of veterinary drugs. However, uncertainty remains because of the presence of unidentified residues in liver. Because of the genotoxic and carcinogenic nature of carbadox and some of its metabolites, the Committee was not able to establish an ADI.

On the basis of data from studies on the toxicity of quinoxaline-2-carboxylic acid, and on the metabolism and depletion of carbadox, and the nature of the compounds released from the bound residues, the Committee concluded that residues resulting from the use of carbadox in pigs were acceptable, provided that the MRLs recommended on page 50 were not exceeded.

48
Residue data

A pivotal study was conducted in which swine were given unrestricted access to feed containing 55 mg/kg \(^{14}C\)carbadox (uniformly labelled in the phenyl ring) for 5 consecutive days and killed at 30 days (4 animals), 45 days (3 animals) or 70 days (3 animals) after treatment. Two untreated swine were killed for control purposes. Samples of the four principal edible tissues were collected and subsequently assayed for total radioactivity.

The radioactive tracer used in this study had a specific activity of 8.4 \(\mu\)Ci/mg and an overall purity of more than 99% as determined by thin-layer chromatography and high-pressure liquid chromatography. The detection limit of the assay method was reported to be 1 \(\mu\)g/kg. The concentration of total residues in the tissues is shown in Table 5.

Table 5. Concentration of total residues in pigs after administration of carbadox in feed

<table>
<thead>
<tr>
<th>Withdrawal time (days)</th>
<th>Concentration of total residues ((\mu)g/kg of parent drug equivalent)*</th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>74* (50-117)</td>
<td>15*</td>
<td>10-21</td>
<td>5* (3-6)</td>
<td>2* (1-3)</td>
</tr>
<tr>
<td>45</td>
<td>20* (17-21)</td>
<td>5* (4-6)</td>
<td>3* (2-4)</td>
<td>1*</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>13* (13-14)</td>
<td>4* (3-5)</td>
<td>2* (2-3)</td>
<td>&lt;1*</td>
<td></td>
</tr>
</tbody>
</table>

*From the data obtained in a study in which pigs were given 55 mg of \(^{14}C\)carbadox per kg of feed for five days.
Numbers in parentheses refer to the range of values obtained.
These figures are based on data from four pigs.
These figures are based on data from three pigs.

In a similar study, except for a decreased level of feed consumption, the tissues were assayed for extractable and bound radioactivity, following sequential extraction with methanol, acetone and n-hexane. The results showed that more than 90% of the total residues in tissues at 30 and 45 days withdrawal time were non-extractable.

The liver tissue was assayed for quinoxaline-2-carboxylic acid (as measured by methyl quinoxaline-2-carboxylate) by a method involving alkaline digestion, thin-layer chromatography, gas–liquid chromatography, and reverse-isotope dilution. The concentration of the residue was 18.9 \(\mu\)g/kg at 30 days withdrawal time, decreasing to 5.5 \(\mu\)g/kg at 45 days, and 1.3 \(\mu\)g/kg at 70 days.

A study aimed at characterizing the bound residue was conducted using \(^{14}C\)carbadox (labelled in the phenyl ring). Liver samples were
collected from swine at approximately one week withdrawal and extracted with enzymes under acidic, neutral, and alkaline conditions. However, no more than 19% of the radioactivity was extractable and no major metabolites could be identified. Bioavailability studies of the bound residues by in vivo methods were not considered feasible because of the low level of residues in the liver tissues at withdrawal periods of four weeks. The Committee concluded that the bound residues in swine liver at 28 days after treatment would not represent a risk for consumers.

Studies with [14C]carbadox and unlabelled carbadox demonstrated that the concentration of quinoxaline-2-carboxylic acid extracted by alkaline hydrolysis was less than 30 µg/kg after 28 days withdrawal. Practical analytical methods are available for measuring quinoxaline-2-carboxylic acid to 30 µg/kg in liver. Methods have been reported for detecting quinoxaline-2-carboxylic acid in muscle at 5 µg/kg. Therefore, the Committee recommended MRLs of 0.03 mg/kg in liver and 0.005 mg/kg in muscle of pigs, based on the levels of, and expressed as, quinoxaline-2-carboxylic acid.

3.3.2 Olaquindox

This is the first occasion on which olaquindox has been reviewed by the Committee. Olaquindox is an antimicrobial drug which is used to promote growth in pigs. The commercial product is intended for use in starter and/or grower rations but not in finisher rations. It is usually administered in the feed at doses of 25–100 mg/kg and may be used in pigs up to four months of age.

Toxicological data

The toxicological data considered by the Committee included the results of acute and subchronic studies, together with the results of studies on mutagenicity, carcinogenicity, and effects on reproduction and development. Olaquindox is almost completely absorbed from the gastrointestinal tract in rats, dogs, and pigs. In studies using radiolabelled olaquindox, the radioactivity was shown to be widely distributed in the tissues, with residues in the liver being the most persistent. The compound was primarily eliminated in the urine, with lesser amounts being excreted in the faeces and expired air of the animals. In the pig elimination was virtually complete by 48 hours after a
single intragastric dose. Any remaining radioactivity was then
eliminated with a half-life of 5–9 days, far in excess of that of
olaquindox (about 3–5 hours). The parent compound accounted for
70% of the radioactivity in the urine, up to 24 hours after
administration. There were approximately 16 metabolites detected
in the urine; the six major metabolites have been fully characterized.

In acute and short-term toxicity studies in rats and mice, rats were
about twice as sensitive as mice. In a 90-day study in rats, effects
were observed in the testes, ovaries, thyroid, and adrenal cortex at
a dose of 5 mg per kg of body weight per day and above. These
lesions were described histopathologically as atrophy, and in the
adrenal glands the effect was greatest in the zona glomerulosa. No
treatment-related abnormalities of the pituitary gland were reported.
A 90-day study in beagle dogs and a 19-week study in rhesus
monkeys also produced evidence of toxic effects on the endocrine
glands, liver, and kidney. Of five female monkeys dosed at 40 mg per
kg of body weight per day, one died during treatment and two died
during the planned 17-week recovery period. The two survivors
failed to re-establish normal ovarian cycles during the recovery
period.

In a 20-week study, groups of five male and five female pigs were
fed up to 250 mg of olaquindox per kg of diet. The plasma concen-
trations of urea and creatinine were elevated at 160 and 250 mg/kg
diet, which suggested an effect on the kidney. All of the pigs in the
highest-dose group that survived the study period continued to show
evidence of this effect during a 16-day recovery period. Plasma
sodium, potassium and chloride concentrations were altered during
the study, but did not return to normal during the recovery period.
Histopathological changes were found at necropsy in kidney and
adrenal tissues from both groups. The manufacturers reported a no-
observed-effect level of 100 mg/kg in feed for this study.

However, in other studies in piglets in which olaquindox was fed
at 25, 50, 100 or 200 mg/kg diet for 6 weeks, a dose-dependent fall
in plasma aldosterone concentration together with hyponatraemia,
hypochloraemia, and hyperkalaemia occurred in all groups by the
end of the study. Hydropic degeneration of adrenal cortex cells was
recorded. There were no effects on weight gain or clinical signs.

Developmental studies in which the compound was administered
orally were conducted in mice and rats. In mice, fetal weights were
reduced at 180 mg per kg of body weight per day, but malformations
were absent. In rats, malformations occurred at 180 mg per kg of
body weight per day, and reductions in maternal weight gain, litter size, and fetal weight were also observed at this dose.

A three-generation reproduction study in which olaquindox was administered in the diet was conducted in rats. No malformations were observed, the only findings being reductions in fertility rate and litter size in the second and third generations at the highest dose of 25 mg per kg of body weight per day.

The genotoxicity of olaquindox was investigated in a range of in vitro and in vivo studies. Positive findings were reported in assays for point mutation and for DNA damage in bacteria, sister-chromatid exchange in Chinese hamster V-79 cells, and chromosome damage in human lymphocytes in vitro and mammalian bone-marrow cells in vivo, and in several micronucleus tests. Two dominant lethal assays in male mice were negative, but a third gave a weak positive response. Two dominant lethal assays in female mice gave positive results, but this may have been partly due to the toxicity of the drug. A weak positive result was obtained in an in vivo cytogenicity assay using Chinese hamster spermatogonia. Olaquindox did not bind to rat DNA in vivo.

The Committee considered data from six long-term studies in rodents, but because of poor survival and deficiencies in experimental design and reporting, only two carcinogenicity studies, in mice and rats, were evaluated.

In the study in mice, doses of olaquindox up to an equivalent of 54 mg per kg of body weight per day were administered in the diet for life (up to 635 days). An increase in the incidence of benign adrenal cortical adenomas and benign proliferative lesions (nodular hyperplasia and adenoma) in the lung was observed in male mice at the highest dose level. There was no effect on the incidence of malignant tumours.

In the rat carcinogenicity study, which included fetal exposure to the drug in utero, olaquindox was administered in the diet at levels up to an equivalent of 30 mg per kg of body weight per day until 80% of the male and female controls had died (about three years). Survival was decreased at 30 mg per kg of body weight per day in both sexes, but there was no increase in the incidence of benign or malignant tumours.

The Committee considered olaquindox to be a genotoxic agent. There was some evidence to suggest that olaquindox was a germ-line mutagen, but more extensive testing in appropriate mammalian studies will be required to resolve this issue. In the carcinogenicity
studies only the mouse showed an increase in the incidence of
tumours, and these were benign. Because of doubts over the
mechanism of this effect and the results of the genotoxicity studies
the Committee was unable to establish an ADI for olaquindox.
However, the Committee concluded that residues resulting from the
use of olaquindox in food-producing animals under conditions of
good practice in the use of veterinary drugs were temporarily
acceptable.

Residue data

As stated earlier, olaquindox is rapidly absorbed from the gut of
pigs, rats, and dogs and the major route of excretion is the urine. A
radiometric study was carried out in which five female pigs were
treated with a single oral dose of [14C]olaquindox (labelled on C-3
of the quinoxaline ring) at 2 mg per kg of body weight. One male pig
was similarly dosed with uniformly ring-labelled [14C]olaquindox.
The results showed that the parent drug accounted for 70% of the
total residues in urine. Five other metabolites were identified in
which the side-chain was oxidized to the acid and/or one or both of
the N-oxide groups were reduced. No data on the characterization
of the metabolites in tissues were available. The female pigs were
killed 2, 4, 8, 14, and 28 days after dosing. The concentration of total
14C-labelled residues in muscle, liver, and kidney is presented in
Table 6. Further characterization of the residues was not achieved
and no marker residue was identified.

Table 6. Concentration of total 14C-labelled residues (μg/kg) in female pigs after
oral administration of olaquindox.*

<table>
<thead>
<tr>
<th>Withdrawal time (days)</th>
<th>Concentration of total 14C-labelled residues (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>28</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

*From the data obtained in a study in which female pigs were given a single oral dose of 2 mg of
14C olaquindox per kg of body weight.

Other studies in pigs were reported in which olaquindox was
administered in the feed. The tissue residues were measured by
analytical methods, involving an extraction procedure, reduction of
the N-oxide group(s) and then determination of the concentration of the parent drug and one other metabolite of interest possessing the same alcohol side-chain as the parent drug. Until further information is available on the nature of the tissue residues, these methods are inadequate for the purposes of accurately measuring the levels of tissue residues. For example, in studies in pigs no residues were detected in tissues at 48 hours withdrawal time using the chemical assay although they were clearly present in the study using $[^1^4]$Colaquindox.

Before the Committee can establish MRLs for olaquindox, the results of a tissue depletion study designed to characterize the nature and availability of residues of olaquindox in tissues and to identify a suitable marker compound will be needed. The Committee was aware that these data were already being generated by the manufacturer of olaquindox. The results of these studies, and the results of studies designed to provide an indication of the toxic potential of the residues, are required by 1993.

Depending upon the results of these studies, the following additional information may be needed:

1. Data to assess the genotoxic potential of olaquindox on germ-line cells, which would, at a minimum, necessitate a repeat of the Chinese hamster spermatogonia study.
2. Studies designed to assess the effects of olaquindox on adrenal function (including sensitive parameters such as plasma adrenal hormones and electrolyte levels), sperm morphology, and fertility in rats, so that a no-observed-effect level can be determined for each of these indicators.
3. Information on the binding of olaquindox or its metabolites to structural proteins such as tubulin, or to enzymes or proteins involved in DNA synthesis or repair. (Such binding may help explain why, despite its obvious genotoxic potential, olaquindox does not bind to DNA and has given equivocal results in carcinogenicity studies.)

4. RECOMMENDATIONS

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 for this purpose.
2. The Committee has previously recognized that certain groups of individuals, such as farm workers and veterinarians, may be exposed to high concentrations of veterinary drugs during the administration of formulated drugs to food-producing animals. In view of the concerns of the Committee regarding the genotoxicity/carcinogenicity of olaquindox and carbarsone, the potential health implications of such exposure should be drawn to the attention of the appropriate authorities.

3. The Committee recognizes that widespread exposure of non-target organisms to ivermectin may result from the use of this drug as an antiparasitic agent in food-producing animals. The potential ecological implications of such exposure should be drawn to the attention of the appropriate authorities for further investigation.

4. The Committee recognizes that the transfer of resistant pathogenic organisms to humans from foodstuffs may be associated with the use of antimicrobials in food-producing animals, and recommends that the consequences of such transfer be drawn to the attention of the appropriate authorities for further investigation.

5. Since data have been generated on the pharmacokinetics, metabolism, mechanism of action, and adverse reactions resulting from human therapy with drugs that are also used in food-producing animals, the Committee requests that increased efforts should be made to provide such data to future meetings. The Committee believes that these data may be extremely useful in the interpretation of animal experiments and for establishing ADIs.

REFERENCES


Annex 1

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


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).


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


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.


## RECOMMENDATIONS ON COMPOUNDS ON THE AGENDA

<table>
<thead>
<tr>
<th>Substance</th>
<th>Acceptable Daily Intake (ADI) for humans and other toxicological recommendations</th>
<th>Recommended Maximum Residue Limit (MRL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthelmintic drugs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Closantel</td>
<td>0–0.03 mg per kg of body weight</td>
<td>Edible tissues of sheep: 1.5 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bovine tissues: muscle: 0.5 mg/kg¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>kidney: 2 mg/kg²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>liver: 1 mg/kg²</td>
</tr>
<tr>
<td>Ivermectin</td>
<td>0–0.0002 mg per kg of body weight</td>
<td>Liver (all species): 0.015 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fat (all species): 0.02 mg/kg</td>
</tr>
<tr>
<td>Levamisole</td>
<td>0–0.003 mg per kg of body weight¹</td>
<td>Edible tissues and milk (all species): 0.01 mg/kg¹</td>
</tr>
<tr>
<td><strong>Antimicrobial agents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzylpenicillin</td>
<td>0.03 mg per person per day²</td>
<td>Liver, kidney, and muscle (all species): 0.05 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Milk: 0.004 mg/kg</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>0–0.003 mg per kg of body weight</td>
<td>All species: muscle: 0.1 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>liver: 0.3 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>kidney: 0.8 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>fat: 0.01 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>milk: 0.1 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>eggs: 0.2 mg/kg</td>
</tr>
<tr>
<td><strong>Growth promoters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbadox</td>
<td>Limited acceptance of residues¹</td>
<td>Swine liver: 0.03 mg/kg⁴</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Swine muscle: 0.005 mg/kg⁴</td>
</tr>
<tr>
<td>Olaquindox</td>
<td>Limited acceptance of residues¹</td>
<td>No MRLs allocated⁷</td>
</tr>
</tbody>
</table>

**Notes to Annex 2**

1. Temporary MRL (see Annex 3).
2. Temporary acceptance (see Annexes 3 and 4).
3. Daily intake of the parent drug should be kept below this level.
4. Insufficient information was available to establish an ADI. The Committee concluded that residues resulting from the use of carbadox in pigs were acceptable, provided that the recommended MRLs are not exceeded.
5. Residues are based on the levels of, and expressed as, quinoxaline-2-carboxylic acid.
6. Insufficient information was available to establish an ADI. The Committee concluded that residues resulting from the use of olaquindox in food-producing
animals under conditions of good practice in the use of veterinary drugs were temporarily acceptable (see Annex 3).

7. MRLs were not established because insufficient information was available (see Annex 3).
FURTHER TOXICOLOGICAL STUDIES AND OTHER INFORMATION REQUIRED OR DESIRED

Anthelminthic drugs

Closantel

The Committee requires results of the following studies by 1992:

1. A metabolism study in cattle that characterizes the total residue, in particular to show the relationship between the concentration of closantel and total residues at various times.
2. A study of total residue depletion in cattle using $^{14}$Cclosantel at the maximum use level.
3. Adequate studies of residue depletion in cattle using unlabelled closantel at the maximum use levels to assess the concentrations of residues that occur under field conditions.

Levamisole

The Committee requires the following by 1994:

1. A comprehensive assessment of the incidence of granulocytopenia and agranulocytosis, thrombocytopenia, and haemolytic anaemia in humans receiving levamisole, together with dose-response information.
2. The results of studies that demonstrate that the mechanisms for the production of haemolytic anaemia in dogs and neutropenia or agranulocytosis in humans are related phenomena, such as experiments in which the specificity of the antibody response and of the target cells is studied.
3. A comparison of the metabolites of levamisole produced in humans, laboratory animals, and food-producing animals; if differences in major metabolites are demonstrated, evidence should be obtained on the potential of the metabolites that are produced in food-producing animals to induce haematological effects.
4. The results of a metabolism study in food-producing animals that characterizes the total residues, which should include data on the
relationship between a suitable marker compound and the total residues after different withdrawal periods.

5. The results of radiometric studies on the depletion of total residues in food-producing animals.

6. The results of depletion studies using the maximum recommended dose levels of unlabelled levamisole, with sufficient animals to permit an assessment of the residues that occur under field conditions.

**Antimicrobial agent**

*Benzylopenicillin*

The Committee recommends:

1. The provision of further information and the results of new studies on the depletion of residues of benzylpenicillin from the edible tissues of food-producing animals.
2. Investigation of the accuracy and precision of the assays used to measure residues of benzylpenicillin.
3. The development of more sensitive chemical assays for benzylpenicillin.

**Growth promoter**

*Olaquindox*

Before the Committee can establish MRLs for olaquindox, the results of a tissue depletion study designed to characterize the nature and availability of residues of olaquindox in tissues and to identify a suitable marker compound will be needed.

The results of these studies, and the results of studies designed to provide an indication of the toxic potential of the residues, are required by 1993.

Depending upon the results of these studies, the following additional information may be needed:

1. Data to assess the genotoxic potential of olaquindox on germ-line cells, which would, at a minimum, necessitate a repeat of the Chinese hamster spermatogonia study.
2. Studies designed to assess the effects of olaquindox on adrenal function (including sensitive parameters such as plasma adrenal
hormones and electrolyte levels), sperm morphology, and fertility in rats, so that a no-observed-effect level can be determined for each of these indicators.

3. Information on the binding of olaquindox or its metabolites to structural proteins such as tubulin, or to enzymes or proteins involved in DNA synthesis or repair.
DIVERGENT OPINION ON LEVAMISOLE
(Professor A. Somogyi)

"I disagree with the decision of the Committee to establish a temporary ADI based on what I regard as an irrelevant end-point determined in an inadequate study performed on three dogs each per sex and dose. According to my best scientific judgement, the data available for evaluation by the Committee are insufficient to reach a meaningful conclusion as to a safe level for residues of levamisole in food of animal origin.

My dissension emanates in particular from the following facts:

1. Data derived from clinical observations on patients treated with levamisole show that one of the adverse effects of this drug in humans is a mostly reversible but occasionally fatal agranulocytosis.

2. A threshold dose for the induction of this haematological disorder is not known.

3. In none of the numerous studies performed on experimental animals could agranulocytosis be reproduced; hence, no animal model exists for this effect of levamisole in humans.

4. Neither the identity nor the biological actions of 95% of levamisole residues are known.

Therefore, results obtained in animal tests, including the study on which the temporary ADI is based, cannot provide an acceptable basis for the human safety evaluation of levamisole residues in food of animal origin. Rather, until proven otherwise, it has to be assumed that there is no safe level for levamisole residues in food. Irrespective of its size, no safety factor applied to an inappropriate parameter can, in a scientifically meaningful way, ever be regarded as a substitute for pertinent data suitable to resolve the open issues enumerated above."

68