EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD

Fifty-second report of the Joint FAO/WHO Expert Committee on Food Additives

World Health Organization
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Recommendations on compounds on the agenda and further information required
Joint FAO/WHO Expert Committee on Food Additives
Rome, 2–11 February 1999

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


Residues monographs are issued separately by FAO under the title:

Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/12, 2000.

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 Organization, 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.
1. **Introduction**

A meeting of the Joint FAO/WHO Expert Committee on Food Additives was held at FAO Headquarters, Rome, from 2 to 11 February 1999. The meeting was opened by Mr J. Lupien, Director, Food and Nutrition Division, FAO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization.

Mr Lupien noted that some compounds on the agenda were used both as pesticides and as veterinary drugs. Use of compounds as pesticides is evaluated by the Joint FAO/WHO Meeting on Pesticide Residues, while use as veterinary drugs is evaluated by the Joint FAO/WHO Expert Committee on Food Additives. As different approaches may be used by the two expert groups, the value for a Maximum Residue Limit (MRL) or the way in which it is expressed for the same compound may differ. Mr Lupien informed the Committee that the relevant committees of the Codex Alimentarius Commission had asked both the Joint FAO/WHO Expert Committee on Food Additives and the Joint FAO/WHO Meeting on Pesticide Residues to discuss the issue and to explore possible avenues for harmonization. He noted that a meeting of representatives of the two expert groups had recently been held on this topic and that the Committee would have the opportunity to discuss the results of that meeting during the present session. As harmonization is an important issue to the Codex Alimentarius Commission, he urged the Committee to give it every consideration.

Eleven previous meetings of the Committee had been held to consider veterinary drug residues in food (Annex 1, references 80, 83, 91, 97, 104, 110, 113, 119, 125, 128 and 134) in response to the recommendations of a Joint FAO/WHO Expert Consultation held in 1984 (1). The present meeting was convened in response to the recommendations made at the fiftieth meeting of the Committee that meetings on this subject should be held annually (Annex 1, reference 134). 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:

- to elaborate further principles for evaluating the safety of residues of veterinary drugs in food and for establishing Acceptable Daily

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1 As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives held in 1956 (FAO Nutrition Meeting Report Series, No. 11, 1956; WHO Technical Report Series, No. 107, 1956), there have been 51 previous meetings of the Joint FAO/WHO Expert Committee on Food Additives (Annex 1).
Intakes (ADIs) and MRLs for such residues when the drugs under consideration are administered to food-producing animals in accordance with good practice in the use of veterinary drugs (see section 2);
— to evaluate the safety of residues of certain veterinary drugs (see section 3 and Annex 2); and
— to discuss matters of interest arising from the report of the Eleventh 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, thirty-fourth, thirty-sixth, thirty-eighth, fortieth, forty-second, forty-third, forty-fifth, forty-seventh, forty-eighth and fiftieth reports of the Committee (Annex 1, references 80, 85, 91, 97, 104, 110, 113, 119, 125, 128 and 134) and in the report of the Joint FAO/WHO Expert Consultation on Residues of Veterinary Drugs in Food (1).

2.2 Modification of the agenda

Cyhalothrin, metrifonate, permethrin, temefos and tilmicosin were removed from the agenda, and their evaluation was postponed to a future meeting, since no data had been submitted. Neomycin was added to the agenda at the request of a sponsor so that new data on residues in cattle liver and kidney could be evaluated. Carazolol was added to the agenda for toxicological re-evaluation.

Abamectin had been referred to the Committee by the Joint FAO/WHO Meeting on Pesticide Residues with a request to suggest that a residue level in cattle meat be proposed. Since this level depended on the definition of residues, the issue was resolved at the joint meeting on harmonization of MRLs (see section 2.9). Abamectin was therefore removed from the agenda of the present meeting.

2.3 Evaluation of antimicrobial agents

The microbiological risk associated with residues of antimicrobial agents in food resulting from their use in animals was addressed at the thirty-second, thirty-sixth, forty-second, forty-fifth and forty-seventh meetings of the Committee (Annex 1, references 80, 91, 110, 119 and
In view of the continued interest in evaluating the effects on public health of the use of antimicrobial agents in food-producing animals, the Committee prepared a systematic approach to assessing the safety of antimicrobial drug residues and their effects on the human intestinal microflora (3).

2.3.1 **Recommendations for the evaluation of the effects of veterinary drug residues on the human intestinal microflora**

In the absence of validated models to predict the effects of veterinary antimicrobial agents on the human intestinal flora, the Committee proposed a comprehensive decision-tree for determining an ADI that takes account of all relevant data from model in vitro and in vivo test systems and includes minimum inhibitory concentrations (MICs). Use of the decision-tree will allow systematic evaluation of the safety of residues of veterinary antimicrobial drugs in food (see Figure 1). The following recommendations were made:

1. Additional microbiological data are not required if there is evidence for at least one of the following:

   (a) The veterinary drug and its residues in milk and edible tissues do not have antimicrobial properties.

   (b) The ingested residues do not enter the lower bowel. Data on the excretion of oral doses and pharmacokinetics and metabolism may be useful.

   (c) The ingested residues are transformed to inactive metabolites before entering the lower bowel. Data on pharmacokinetics and metabolism may be useful.

   (d) The ingested residues are transformed quantitatively to microbiologically inactive metabolites or bound quantitatively to the colon contents, resulting in inactivation of the drug soon after its entry into the lower bowel.

   (e) Data on the effects of the veterinary drug on gastrointestinal microflora in vitro and in vivo provide a basis for concluding that the ADI derived from the toxicological data is sufficiently low to protect the intestinal microflora. Examples of relevant data would include data on changes in the predominant colonic populations, changes in the barrier to colonization, changes in resistant bacterial populations in continuous culture, the effects of the drug on the predominant gastrointestinal microbial flora of animals or humans in a model of the barrier to colonization, or on changes in the incidence of antimicrobial resistance in the gut microflora.

   (f) Clinical data show that the incidence of toxicological effects after therapeutic use of the drug in humans is substantially
Figure 1
Decision-tree for determining the potential adverse effects of residues of veterinary antimicrobial drugs on the human intestinal microflora

Assess the effects of veterinary drug residues, including metabolites, on the human intestinal microflora.

Yes

Does the ingested residue have antimicrobial properties (recommendation 1(a))? Yes

Conclude that the drug residue will not affect the intestinal microflora and use toxicological data to derive the ADI (recommendation 1(a)).

No

Does the drug residue enter the lower bowel (e.g., with the food bolus, by biliary circulation and/or by mucosal secretion) (recommendation 1(b))? Yes

Conclude that the drug residue will not affect the intestinal microflora and use toxicological data to derive the ADI (recommendation 1(b)).

No

Conclude that the drug residue will not affect the intestinal microflora and use toxicological data to derive the ADI (recommendation 1(b)).

Is the ingested residue transformed irreversibly to inactive metabolites by chemical transformation, metabolism mediated by the host or by the intestinal microflora in the bowel and/or by binding to intestinal contents (recommendations 1(b)–1(d))? Yes

Conclude that the drug residue will not affect the intestinal microflora and use toxicological data to derive the ADI (recommendations 1(c) and 1(d)).

No

Do data on the effects of the drug on the colonic microflora provide a basis to conclude that the ADI derived from toxicological data is sufficiently low to protect the intestinal microflora (recommendation 1(c))? Yes

Conclude that the drug residue will not affect the gastrointestinal microflora and use toxicological data to derive the ADI (recommendation 1(c)).

No

Do clinical data from therapeutic use of the class of drugs in humans or data from in vitro or in vivo model systems indicate that effects could occur in the gastrointestinal microflora (recommendation 1(d))? Yes

Determine the most sensitive adverse effect(s) of the drug on the human intestinal microflora, including selection of drug-resistant populations, disruption of the barrier to colonization or changes in the metabolic activity of the microflora in the gastrointestinal tract that have been specifically linked to adverse effects on human health.

No

Conclude that the drug residue will not affect the gastrointestinal microflora and use toxicological data to derive the ADI.
If emergence of antimicrobial resistance is the concern, conduct tests in vitro (continuous culture of faecal inocula) or in vivo (rodents inoculated with human flora); challenge the model system with an antibiotic-resistant species and determine the concentration of the drug that does not select for resistance or the antibiotic-resistant strain when compared with absence of the drug. Use the dose of the drug that has no effect to derive an ADI (recommendation 2(d)).

If disruption of the colonization barrier is the concern, determine the MIC of the drug against 100 strains of the predominant intestinal flora (Table 1) and take the geometric mean MIC of the most sensitive genus or genera to derive an ADI using the equation* developed at the forty-seventh meeting of the Committee (Annex 1, reference 125). Other model systems may be used to establish a no-observed-effect concentration (NOEC) to derive an ADI (recommendation 2(b)). A more realistic ADI can be derived by conducting tests in vitro (continuous culture of faecal inocula) or in vivo (rodents inoculated with human flora). Challenge the model systems with appropriate species (e.g., Clostridium difficile, Salmonella spp., Enterococcus spp., Escherichia coli) and determine the concentration of the drug that does not alter the shedding characteristics of the organisms when compared with absence of the drug. Use the dose of the drug that has no effect to derive an ADI (recommendation 2(c)).

If the concern is change in a specific enzymatic activity that is directly linked to adverse effects on human health, conduct tests in vitro (continuous culture of faecal inocula) or in vivo (rodents inoculated with human flora) to determine the concentration of the drug that does not alter that specific enzymatic activity when compared with absence of the drug. Use the dose of the drug that has no effect to derive an ADI (recommendation 2(e)).

*The equation is as follows:

Upper limit of ADI (µg/kg of body weight) = \( \frac{MIC_{90} (µg/g) \times \text{Mass of colonic contents (g)}}{\text{Fraction of oral dose bioavailable} \times \text{Safety factor} \times \text{Weight of human (kg)}} \)

where:

MIC_{90} = Minimum concentration of an antimicrobial drug completely inhibiting the growth of 50% of the cultures of a particular microorganism, as judged by the naked eye, after a given period of incubation. For the purpose of the evaluation, the MIC_{90} value is the mean MIC_{90} for the strain(s) of the relevant species tested. Alternatively, the lowest MIC_{90} value for the most sensitive species can be used.

Although MIC_{90} values are usually expressed in µg/ml, they are expressed as µg/g in this equation so that the ADI will be in µg/kg. When the MIC_{90} value is converted to these units, it is assumed that the density of the experimental medium is 1 g/ml.

A value of 220 g is used for the mass of the colonic contents and a value of 60 kg is used for the weight of an adult. The safety factor used to take account of uncertainty about the amount and relevance of data available for review may range from 1 to 10. A value of 1 is used when extensive relevant microbiological data are provided.
higher than that of any gastrointestinal side-effects due to disruption of the microflora. Thus, the ADI would be based on toxicological data rather than microbiological data.

2. If none of the above can be demonstrated, the following studies are proposed for establishing an ADI (see Figure 1):

(a) The class of drug should be considered in order to determine whether the main concern is emergence of resistance or disruption of the intestinal microflora.

(b) If effects on the barrier to colonization are a concern, in the absence of other relevant data, the MIC of the veterinary drug against 100 bacterial strains, comprising 10 isolates of organisms that are representative of relevant genera of the microflora in the gastrointestinal tract of healthy individuals, can be used as the basis for a conservative estimate of the ADI. Examples of relevant genera include *Bifidobacterium*, *Bacteroides*, *Clostridium*, *Eubacterium*, *Fusobacterium*, *Enterococcus*, *Lactobacillus*, *Peptostreptococcus*, *Peptococcus* and *Ruminococcus* (Table 1). *Escherichia coli* should not be considered one of the most relevant species of intestinal microflora for screening the effects of antimicrobial compounds, as it is the twenty-second most abundant species commonly detected and represents 1.2% of the isolates from the human gastrointestinal tract (4). An increase in the number of enterobacteria can, however, be used as an indicator of resistance to colonization in the gastrointestinal tract (11), since these bacteria are easy to culture and an increase in their number may suggest the potential for infection by pathogens.

Standardized MIC tests based on methods of the United States National Committee for Clinical Laboratory Standards (12, 13) or those of an equivalent body should be used with inoculum concentrations of 107 and 108 colony-forming units per ml. When more than one strain of the same species is studied, the convention is to quote MIC50 and MIC90 values, the MICs necessary to inhibit 50% and 90%, respectively, of the tested strains of a given species. Since 10 species (or more than one strain of several species within a particular genus) may be tested for estimating an ADI, the MIC50 and the geometric mean in this context are the summary values calculated for each bacterial genus. In practice, if 100 relevant strains are tested, as noted above, and the overall geometric mean value for the most sensitive bacterial genus or genera is used, it is not necessary to use a safety factor. To ensure the greatest margin
Table 1
Predominant bacterial species isolated from the human gastrointestinal tract

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides</td>
<td>B. vulgatus</td>
<td>Peptococcus</td>
<td>Various</td>
</tr>
<tr>
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<td>B. uniformis</td>
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<tr>
<td></td>
<td>B. stercoris</td>
<td>Peptostreptococcus</td>
<td>P. anaerobius</td>
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<td>B. fragilis</td>
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<td>P. productus</td>
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<td>B. ovatus</td>
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<td>P. parvulus</td>
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<tr>
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<td>B. caccae</td>
<td></td>
<td>P. micros</td>
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<tr>
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<td>B. distasonis</td>
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<td>P. prevoti</td>
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<td>B. thetaiotaomicron</td>
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<td>B. capillosus</td>
<td>Prevotella</td>
<td>Various</td>
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<td>B. merdae</td>
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<tr>
<td>Fusobacterium</td>
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<td>Ruminococcus</td>
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<td></td>
<td>F. russil</td>
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<td>R. obeum</td>
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<tr>
<td>Bifidobacterium</td>
<td>B. adolescens</td>
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<td>R. torques</td>
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<td>E. lentum</td>
<td>Escherichia</td>
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<td>E. ventriosum</td>
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<td>Clostridium</td>
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<td>C. ramosum</td>
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<td></td>
<td>C. indolis</td>
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* Adapted from references 4–10.
\(^a\) See section 2.3.1.

of safety, the choice of microorganism should be made from among the relevant genera that are usually considered to be most sensitive to the test compound.

If other suitable, scientifically justifiable data are available, the formula used for estimating an ADI based on microbiological data should not be the primary or sole criterion for determining an ADI. For example, a no-observed-effect concentration (NOEC) can be used to establish an ADI if data are available from in vitro models such as the semi-continuous culture system to monitor population changes of target microorganisms...
within the normal microflora, changes in volatile fatty acid composition, bacterial enzymes associated with specific adverse effects in humans, the emergence of resistance, or disruption of the barrier to colonization.

(c) If disruption of the barrier to colonization is the concern and no data are available, information should be provided to show at least one of the following:

i. Addition of the veterinary drug at concentrations covering the range expected in the colon for an ADI does not alter the barrier afforded by continuous culture of faecal inocula against colonization by challenge with a microorganism such as *Clostridium difficile*, zoonotic pathogens, or *Pseudomonas* spp. Since there is no disruption of the barrier to colonization, this concentration would be used as the NOEC.

ii. Oral administration of the veterinary drug to a monogastric animal (e.g., rat, mouse, or rodent inoculated with human flora), at a concentration that would result in the concentrations expected in the human colon at an ADI, shows no effect on the barrier to colonization from challenge with oral doses of *C. difficile* or zoonotic or opportunistic pathogens.

(d) If emergence of antimicrobial resistance due to consumption of residues is the concern, data to show that the expected residue concentrations in the colon do not change the antibiotic resistance of resident populations of *E. coli* or other bacteria appropriate for the drug class should be provided from in vitro studies using a continuous culture of faecal inocula or in vitro studies in mice, rats, rodents inoculated with human flora, or in pigs.

(e) If the concern is change in a specific enzymatic activity that is directly linked to adverse effects on human health, in vitro or in vivo tests should be conducted to determine the concentration of the drug that does not alter that specific enzymatic activity.

2.4 Safety of residues at the injection site

The Committee was asked by the Codex Committee on Residues of Veterinary Drugs in Foods (2) to reconsider the safety of residues of drugs that may be present at the injection site and to consider the establishment of an acute reference dose (acute RD) in such cases. The acute RD has been defined by a Joint FAO/WHO Consultation (14) as “...the estimate of the amount of a substance in food or
drinking-water, expressed on a body-weight basis, that can be ingested over a short period of time, usually during one meal or one day, without appreciable health risk to the consumer on the basis of all the known facts at the time of the evaluation.” It is usually expressed in milligrams of the chemical per kilogram of body weight.

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

2.5 General issues relating to MRLs

At its forty-eighth meeting, the Committee considered several initiatives to make its process for setting MRLs more transparent and consistent (Annex 1, reference 128). These initiatives were discussed at the fiftieth meeting (Annex 1, reference 134), when the Committee drew up a list of general policy issues relating to the recommendation of MRLs for veterinary drug residues in food. Background and working papers on the following items were prepared for consideration at the present meeting.

2.5.1 Guidelines for recommending MRLs for minor animal species

Nearly all of the MRLs for veterinary drug residues established to date by the Committee have been for edible tissues and for milk and eggs of major animal species (i.e. cattle, sheep, pigs and chickens); very few MRLs have been established for minor animal species (such as deer and rabbits). This paper outlines guidelines proposed for use by the Committee in considering MRLs for veterinary drug residues in minor animal species. The FAO Secretariat will forward this paper for consideration at the next session of the Codex Committee on Residues of Veterinary Drugs in Foods, with a request for comments on the approach.
2.5.2 Considerations for recommending MRLs for fish

Several problems are encountered in recommending MRLs for fish, including the definition of edible tissues and the complex pharmacokinetics and metabolism of veterinary drugs in fish. This paper provides a preliminary outline of the issues involved. A more detailed paper will be prepared for consideration at the fifty-fourth meeting of the Committee.

2.5.3 Guidelines for recommending MRLs for honey and other products from bees

Honey and other products from bees do not constitute a significant part of the usual diet; however, when veterinary drugs are used to treat or control diseases in bees, residues may occur in these products. This paper proposes a practical approach to recommending MRLs for such products. The FAO Secretariat will refer this paper to the Codex Committee on Residues of Veterinary Drugs in Foods for review, with a request for comments on the approach.

2.5.4 Guidelines for recommending MRLs for minor animal species where none exists for major species

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

2.5.5 Expression of numerical MRLs

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

2.5.6 Guidelines for identifying target tissues for residue analysis of food animal products in international trade

Since its thirty-eighth meeting, the Committee has, whenever possible, identified two target tissues for recommending MRLs. One is either muscle or fat (for international trade) and the other is either
liver or kidney (commonly used by national authorities). However, some national authorities have expressed the need for MRLs for all tissues of imported animal food products. This paper proposes guidance on this issue. The FAO Secretariat will refer this paper to the Codex Committee on Residues of Veterinary Drugs in Foods for review, with a request for comments.

2.6 Statistical approaches for recommending MRLs

In order to improve the transparency and scientific basis for recommending MRLs, the Committee has prepared two statistical approaches. These approaches are complementary but can be used separately and are dependent on the quality and quantity of the residue data that are provided. When the data on a veterinary drug with a long history of use are limited (e.g. the available studies included only three animals per group), the Committee has agreed in principle to use mean values and to consider incorporating three standard deviations for determining the upper limits of residue concentrations at a particular time. This approach was used for two compounds at the present meeting.

When a large amount of highly reliable data that permits a comprehensive statistical analysis is available, a more refined statistical approach may be used. This approach was employed at the fiftieth meeting of the Committee in recommending MRLs for eprinomectin (Annex 1, reference 134).

The FAO Secretariat will prepare summaries of these two approaches and refer them to the Codex Committee on Residues of Veterinary Drugs in Foods for review, with a request for comments.

The Committee will continue to evaluate other approaches for recommending the most suitable MRLs for specific substances; however, it will continue to recommend MRLs for individual substances on a case-by-case basis.

2.7 Policies for evaluating residues of veterinary drugs in food

The Committee considered a document which summarized all of the policies for establishing ADIs and recommending MRLs for residues of veterinary drugs in foods since its thirty-second meeting in 1987, the first that dealt exclusively with veterinary drugs. The Committee requested that the document be published by FAO in a form that can be updated when necessary. The Committee also recommended that the document be made available as soon as possible on the FAO and WHO web pages in either printed document format (PDF) or hypertext mark-up language (HTML) format. The FAO Secretariat
will forward the document to the Codex Committee on Residues of Veterinary Drugs in Foods for review, with a request for comments.

2.8 Requirements for validation of analytical methods

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

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

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

Some substances are used both as a veterinary drug and as a pesticide. Use of such substances as pesticides may result in residues in the edible tissues of food-producing animals if treated plant commodities are used as animal feed. Residues may also arise in animal tissues from the use of the same substances as veterinary drugs. Because of differences in the evaluation processes used by the Committee and the Joint FAO/WHO Meeting on Pesticide Residues, different MRLs have sometimes been recommended and, to a lesser extent, different ADIs have sometimes been established for the same substance by the two expert groups.

At its Thirtieth Session in 1998, the Codex Committee on Pesticide Residues recommended that the Joint FAO/WHO Meeting on Pesticide Residues (15) and the Committee harmonize their definitions of residues, sampling procedures and other factors that are taken into consideration in recommending MRLs. At its Eleventh Session in 1998, the Codex Committee on Residues of Veterinary
Drugs in Foods recommended that the secretaries of the Joint FAO/WHO Meeting on Pesticide Residues and the Committee convene an informal meeting of experts from both groups to address the issues (2). This informal meeting was held prior to the present meeting of the Committee.

The informal meeting based its deliberations on papers prepared by invited experts. These papers addressed approaches to the harmonization of recommendations on MRLs for lipid-soluble compounds in animal tissues and milk, the definition of residues in products collected for analysis, sampling procedures, estimation of dietary intake and risk assessment procedures used for determining MRLs. A list of 24 recommendations was drawn up for consideration by the Committee and the Joint FAO/WHO Meeting on Pesticide Residues and the above-mentioned Codex committees. The report of the meeting will be forwarded to the Codex committees for their consideration.

3. Comments on residues of specific veterinary drugs

The Committee considered for the first time two insecticides and one production aid. It reconsidered one β-adrenoceptor-blocking agent, one anthelminthic agent, four antimicrobial agents, three production aids and one tranquilizing agent. The recommendations made with regard to these substances and details of further information required are summarized in Annex 2.

3.1 β-Adrenoceptor-blocking agent

3.1.1 Carazolol

Carazolol is a nonspecific β-adrenoceptor-blocking agent that is used primarily in pigs to prevent sudden death due to stress during transport. It was reviewed previously at the thirty-eighth and forty-third meetings of the Committee (Annex 1, references 97 and 113). At its forty-third meeting, the Committee established an ADI of 0–0.1 µg/kg of body weight and recommended the following MRLs for pigs: 5 µg/kg for muscle and fat/skin and 25 µg/kg for liver and kidney, expressed as parent drug.

The Tenth Session of the Codex Committee on Residues of Veterinary Drugs in Foods (16) reviewed the issue of the safety of residues of pharmacologically active drugs at injection sites. In particular, it noted that the goal should be to ensure that the presence of residues at injection sites did not pose a risk to human health. It also noted that the “calculation of risk” due to consumption of residues at
the injection site should be based on an acute RfD. The no-observed-effect level (NOEL) for the acute RfD would be based on the effects of a single dose that was of toxicological and/or pharmacological relevance.

At its present meeting, the Committee considered the β-adrenoceptor-blocking activity of carazolol to be the most relevant biological effect and concluded that the establishment of an acute RfD was appropriate in this case.

Toxicological data
The Committee had previously reviewed data on the β-adrenoceptor-blocking activity of carazolol in rabbits and in volunteers. In the case of volunteers, studies were conducted in healthy subjects and in patients suffering from either chronic bronchitis or asthma. In a study of physical exercise capacity in 12 healthy volunteers, cardiac function was determined after administration of a single oral dose of 5 or 7.5 mg of carazolol per person. A NOEL of 10 μg/kg of body weight was extrapolated from the dose–response curve. A second study, involving groups of five patients suffering from either chronic bronchitis or asthma, was also reviewed by the Committee. Patients who received a single oral dose of 0.1 or 0.7 mg of carazolol had reduced respiratory function, measured as vital capacity and forced expiratory volume, 2 hours later. The overall NOEL in this study was calculated by extrapolation to be 0.5 μg/kg of body weight. The Committee established an acute RfD of 0–0.1 μg/kg of body weight, based on the reduction in respiratory function in compromised subjects and a safety factor of 5. This safety factor was used because the NOEL was from a study in highly sensitive individuals with chronic bronchitis or asthma, who form a substantial part of the general population. The acute RfD provides a margin of safety of 100 in healthy subjects, and the Committee concluded that it therefore made adequate allowance for variations among individuals in the population. The Committee noted that the value of the acute RfD was the same as that for the ADI established at its forty-third meeting.

Residue data
At its forty-third meeting, the Committee evaluated the results of a residue-depletion study of carazolol in pigs. Sixteen pigs were given carazolol at 10 μg/kg of body weight in the neck by intramuscular injection and were then slaughtered in groups of four at 2, 12, 18 and 24 hours after treatment. The concentration of residues at the injection site 2 hours after treatment was 57 μg/kg (range, 31–83 μg/kg) and below the limit of detection at 12 hours. As there were no data on residue concentrations between 2 and 12 hours after treatment, the
Committee used the data at 2 hours to estimate the intake of residues at the injection site. If the concentration of residues at the injection site was 60μg/kg and 300g of injection-site muscle were consumed, the dietary intake of parent carazolol would be 18μg, or three times the acute RfD.

Residues of carazolol at the injection site 2 hours after treatment could result in an intake that exceeds the acute RfD. Therefore, unless appropriate measures can be taken to ensure that the concentrations of residues at the injection site do not result in intake exceeding the acute RfD, use of carazolol during the transport of animals to slaughter is not consistent with safe use of the drug.

3.2 Anthelmintic agent

3.2.1 Doramectin

The use of doramectin as an ecto- and endoparasiticide for cattle was assessed at the forty-fifth meeting of the Committee (Annex 1, reference 119), when an ADI of 0–0.5μg/kg of body weight per day was established, based on a NOEL of 0.1mg/kg of body weight per day for mydriasis in a 3-month gavage study in dogs and a safety factor of 200. At that meeting, the Committee recommended the following MRLs for cattle: 10μg/kg for muscle, 100μg/kg for liver, 30μg/kg for kidney and 150μg/kg for fat, expressed as parent drug. At its present meeting, the Committee reviewed studies of doramectin in pigs.

Pharmacokinetic and metabolic data

The kinetics of doramectin in plasma were determined in eight pigs dosed intramuscularly in the neck with 3H-labelled doramectin at 0.3mg/kg of body weight. The plasma concentration of total residues was determined by liquid scintillation counting, and chromatographic techniques were used to quantify unchanged doramectin. Peak concentrations of 26μg of total residues per litre and 17μg of doramectin per litre were observed about 3 days after injection. The apparent terminal half-lives of elimination from plasma of total 3H-labelled residues and unchanged doramectin were 7.7 and 6.4 days, respectively.

The main route of excretion in pigs is in the faeces, as in cattle, dogs and rats. In pigs 61% of an intramuscular dose of 0.3mg/kg of body weight was excreted in the faeces within 21 days and <1% of the dose was excreted into the urine.

Studies on the biotransformation of doramectin in rats, dogs and cattle were reviewed at the forty-fifth meeting of the Committee. In pigs, the highest concentrations of total residues were found in liver and fat, and only traces were detected in muscle and kidney; therefore,
studies to identify metabolites in pigs were limited to the liver. In studies in pigs and cattle, the doramectin metabolites were similar. The metabolites resulted from O-demethylation in the distal saccharide ring (3'-O-desmethyl doramectin), hydroxylation of the 24-methyl group (24-hydroxymethyl doramectin) and a combination of these biotransformations (24-hydroxymethyl-3'-O-desmethyl doramectin).

The metabolites of doramectin in four pigs treated with ³H-labelled doramectin were determined after subcutaneous administration of a single dose of 0.3 mg/kg of body weight. In one pig that was killed 3 days after treatment, 39% of the total radiolabel was extracted from liver, and 28% of the total residues were identified as the parent drug. The remaining animals were placed in metabolism cages and their faeces and urine were collected at 24-hour intervals for 7 days. The urine and faecal samples containing the highest concentrations of radiolabel were selected for identification of metabolites. The concentrations of the major radiolabelled components were confirmed in a subsequent study in pigs by examination of the liver and faecal samples collected from four animals killed 7 days after intramuscular injection with ³H-labelled doramectin at 0.3 mg/kg of body weight. The faecal samples were collected at 24-hour intervals for 7 days. More residue was extracted from liver than in the earlier experiment (89% of the total radiolabel), and 71% of the total residues were identified as doramectin. The parent drug was the major component, as in all the other species studied, and bound residues constituted only a small fraction of the total. 3'-O-Desmethyl doramectin was detected in the liver and faeces, and 24-hydroxymethyl doramectin was detected only in faeces. 24-Hydroxymethyl-3'-O-desmethyl doramectin was not detected in any sample, even though this metabolite had been reported in cattle treated with doramectin. These data show that the metabolism of doramectin in pigs is generally similar to that in rats, dogs and cattle.

Residue data
Studies with radiolabelled drug. The studies were conducted in compliance with good laboratory practice. Pigs weighing approximately 40 kg received single intramuscular doses of 0.3 mg/kg of body weight of ³H-labelled doramectin into the neck. Two pigs of each sex were slaughtered 7, 14, 21 and 28 days after administration of the dose, and samples of muscle, liver, kidney and fat were collected for analysis of total radiolabel and unchanged doramectin. Samples of injection-site tissue (250 g) were also collected from each pig. The concentrations of residues and unchanged doramectin were higher in liver and fat than in muscle and kidney. The concentrations of total residues were high-
est at 7 days, with 186 µg/kg in liver and 412 µg/kg in fat, and decreased to 37 µg/kg in liver and 58 µg/kg in fat by 28 days. The concentration of doramectin was highest at 7 days, with 66 µg/kg in liver and 242 µg/kg in fat, and decreased to <7 µg/kg in liver and 30 µg/kg in fat by 28 days. The concentrations of residues in kidney and muscle were lower at all sampling times and close to the limit of quantification by day 28.

The ratio of unchanged drug to total residues in each tissue was generally constant over the measured withdrawal periods. When the data for all withdrawal times (days 7, 14, 21 and 28) were combined for each tissue, the unchanged drug accounted for 20% of the total residues in muscle, 30% of the total residues in liver and kidney, and 50% of the total residues in fat. After extraction of the liver samples by a more rigorous method, 71% of the total residues were found to be parent drug. The residues at the injection site persisted for at least 21 days, but by day 28 the concentrations had fallen to 118 µg/kg for total residues and 35 µg/kg for parent drug.

Studies with unlabelled drug. The depletion of doramectin from edible tissues was evaluated in a study in which pigs received single intramuscular injections of doramectin of 375 µg/kg of body weight (1.25 times the recommended dose). Samples of the edible tissues from three male and three female animals were collected 7, 14, 21, 28 and 35 days after dosing. The injection site was excised to provide samples of 500 g, and a 300-g sample of the inner core of the injection site was also taken. The highest concentrations were found 7 days after dosing and were 40 µg/kg in muscle, 160 µg/kg in liver, 80 µg/kg in kidney and 470 µg/kg in fat. The residue concentrations in edible tissues decreased in a linear, logarithmic manner, with half-lives of 7.6–9.5 days. At 35 days after dosing, the residue concentrations were <6 µg/kg in muscle, 18 µg/kg in liver, <7 µg/kg in kidney and 50 µg/kg in fat. The concentrations were highest in the liver and fat and at the injection site at all sampling times. There was close agreement between the mean values for residues in the 300-g and 500-g samples taken at the injection site, indicating that the drug was dispersed evenly throughout the 500-g sample. However, residue data indicate that the residues at the injection site are probably dispersed throughout a larger volume than the samples taken in this study. The mean concentration of doramectin residues at the injection site at 28 days (about 750 µg/kg) was much higher than those observed in the study in which radiolabelled doramectin was given at 300 µg/kg of body weight (35 µg/kg for doramectin and 118 µg/kg for total residues in a 250-g core sample). This difference was not due to the fact that the dose used in the study with unlabelled drug was 25% higher than that in the study with radiolabelled drug, because the concentration of residues of
unlabelled drug at the injection site decreased in a linear and relatively rapid manner, with a mean half-life of approximately 4 days.

Residue-depletion studies in pigs indicate that doramectin is the marker residue and that liver and fat are the appropriate target tissues for determining doramectin residues in the edible tissues of pigs. Muscle and kidney were not considered to be suitable target tissues since the concentrations of both total and unchanged residues were much lower and were near the limit of quantification by 28 days after treatment.

Bound residues. The results of the studies of the metabolism and depletion of total residues conducted with \(^3\text{H}\)-labelled doramectin indicate that doramectin residues are not tightly bound to tissues. When total residues in the liver of a pig killed 7 days after intramuscular dosing with radiolabelled doramectin were extracted using the proposed regulatory method, 93% of the radiolabel was recovered. Unchanged doramectin accounted for the majority (up to 71%) of the extractable residues.

Analytical methods

Doramectin residues in pig tissues were determined by a high-performance liquid chromatography (HPLC) method after extraction from tissue homogenate or fat and conversion to a chemically stable, aromatic, fluorescent derivative. The methods used for the analysis of doramectin in pig tissues were largely the same as those used for the quantification and confirmation of doramectin residues in cattle tissues, which were reviewed at the forty-fifth meeting of the Committee. The HPLC method was specific for doramectin and allowed good chromatographic separation from other avermectins and milbemycins, including ivermectin, abamectin and moxidectin. The proposed method for residue control was different from those used for determining residues of unchanged drug in the residue-depletion studies using radiolabelled drug. In particular, the proposed regulatory method used a more rigorous and efficient extraction procedure.

In the first HPLC method, samples were extracted by incubation at 55°C in hexane, homogenized and repartitioned into acetonitrile. The extracted residues were derivatized to yield a fluorescent compound. The doramectin residues were separated on a reverse-phase HPLC C18 column and quantified alone and in comparison with an internal standard. The assay showed good sensitivity, with a limit of quantification of 2.5μg/kg and a linear range to at least 400μg/kg in liver, kidney, muscle and injection-site tissue, and a limit of quantification of 5μg/kg and a linear range to at least 400μg/kg in fat. More than 80% of the dose of doramectin was recovered from liver and fat and
the accuracy of the method was >90%. The precision, expressed as the coefficient of variation, was ≤10%. The presence of doramectin in pig liver and fat can be confirmed at trace (µg/kg) levels by HPLC–mass spectrometric techniques.

A different method was used to determine the depletion of unchanged radiolabelled drug, involving twice the amount of tissue and a much milder extraction procedure. The sponsor’s claim that similar amounts of doramectin were recovered from muscle, kidney and fat by the two analytical methods was not substantiated experimentally. As the sponsors did not analyse the tissues incurred with residues by both methods, an accurate figure could not be derived for the ratio of unchanged drug to total residues in the liver.

**Maximum Residue Limits**

The ADI is 0–0.5 µg/kg, which is equivalent to a maximum ADI of 30 µg for a 60-kg person.

From the upper 99% confidence interval of the mean concentration of total radiolabelled residues, the theoretical maximum daily intake at day 28 is 22 µg, which is 70% of the ADI. Thus, the study of depletion of radiolabelled drug provides reassurance that the concentration of total residues does not exceed the ADI at day 28. The ratio of marker residue to total residues must be estimated because different results are reported from two studies in the dossier. In the studies with the radiolabelled drug, the ratio is low because the marker residue was incompletely extracted, but a much larger proportion of marker residue was extracted from tissues with the method used for residue control.

In recommending MRLs for doramectin, the Committee took account of the following:

- The drug is intended for use only in pigs and non-lactating cattle.
- The marker residue is the parent drug.
- The target tissues are fat and liver.
- The total residues do not exceed the ADI 28 days after dosing.
- At the forty-fifth meeting, the marker residue was estimated to account for 70% of the total residues in muscle, 55% in liver, 75% in kidney and 80% in fat of cattle. At its present meeting, the Committee recommended that the same values be used for pigs and that the values for the MRLs be harmonized.
- Bound residues account for <10% of the total residues.
- The drug is administered as a single dose.
- The limits of quantification of the analytical methods are 2.5 µg/kg for muscle, liver and kidney and 5 µg/kg for fat.
Table 2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Recommended MRL (µg/kg)</th>
<th>Estimate of total residues (µg/kg)</th>
<th>Theoretical maximum daily intake&lt;sup&gt;a&lt;/sup&gt; (µg doramectin equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>5</td>
<td>7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>Liver</td>
<td>100</td>
<td>182&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18</td>
</tr>
<tr>
<td>Kidney</td>
<td>30</td>
<td>40&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2</td>
</tr>
<tr>
<td>Fat</td>
<td>150</td>
<td>188&lt;sup&gt;e&lt;/sup&gt;</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>29</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed as parent drug.

<sup>b</sup> Based on a daily intake of 0.5 kg of meat made up of 300 g of muscle, 100 g of liver, and 50 g each of kidney and fat.

<sup>c</sup> The marker residue accounted for 70% of the total residues in muscle. However, any residues present in muscle were below the limit of quantification. Therefore, residues from muscle were disregarded in calculating the theoretical maximum daily intake of residues from tissues.

<sup>d</sup> The marker residue accounted for 55% of the total residues in liver.

<sup>e</sup> The marker residue accounted for 75% of the total residues in kidney.

<sup>f</sup> The marker residue accounted for 80% of the total residues in fat.

• Any residues present in muscle were below the limit of quantification after 28 days; therefore, the MRL for muscle was set at twice the limit of quantification, which is considered only as a “guidance” MRL. Residues from muscle were disregarded in the calculation of the theoretical maximum daily intake of doramectin from tissues.

The Committee recommended MRLs in pigs of 5 µg/kg for muscle, 100 µg/kg for liver, 30 µg/kg for kidney and 150 µg/kg for fat, expressed as parent drug. From these values for the MRLs, the theoretical maximum daily intake of doramectin residues from pig tissues would be 29 µg (Table 2).

For the purpose of estimating the theoretical maximum daily intake of doramectin, the MRLs for cattle tissues recommended at the forty-fifth meeting will apply, which provide a theoretical maximum daily intake of 33 µg.

The Committee drew attention to the high concentrations of doramectin residues at the injection site.

3.3 Antimicrobial agents

3.3.1 Dihydrostreptomycin and streptomycin

The Committee previously evaluated dihydrostreptomycin at the twelfth, forty-third and forty-eighth meetings of the Committee (Annex 1, references 17, 113 and 128). A group ADI of 0–50 µg/kg of body weight for the combined residues of dihydrostreptomycin and streptomycin was established at the forty-eighth meeting.

At its forty-third meeting, the Committee requested the following information on residues for evaluation in 1997:
1. An evaluation report or results of experimental studies on the metabolism of dihydrostreptomycin and streptomycin.
2. Data on residues of dihydrostreptomycin and streptomycin.
3. Results of studies to determine the relationship between the antimicrobial activity of the residues and their concentration, as measured by specific chemical methods.

At the forty-eighth meeting, the sponsors provided an evaluation report in response to point 1 but did not supply new data on residues in eggs, as the drug had not been and was not intended to be used in laying birds. The sponsors also provided information to support their analytical methods and additional data in response to point 3.

Residue data
At its present meeting, the Committee reviewed three new residue-depletion studies of animals treated with proprietary preparations of dihydrostreptomycin and streptomycin according to the recommended treatment regimen. The animals were killed 2 days after drug withdrawal and their tissues were collected for analysis using an antimicrobial method (a bioassay) or HPLC. In the first study, cattle were dosed intramuscularly with a combination of streptomycin sulfate (10mg/kg of body weight) and procaine penicillin (8mg/kg of body weight). In the second study, sheep were dosed intramuscularly with streptomycin sulfate (10mg/kg of body weight) and in the third, pigs were dosed intramuscularly with a combination of dihydrostreptomycin sulfate (5mg/kg of body weight) and streptomycin sulfate (5mg/kg of body weight). The tissues with the incurred residues were used for method validation studies.

The results are shown in Table 3. Although the 2-day withdrawal time was short, there were no measurable residues in any of the fat or muscle tissues (excluding the injection-site tissues). Residues were found in liver, kidney and injection-site tissue. There was close agreement between the residue values for both methods.

Analytical methods
At the present meeting, the Committee considered descriptions of two different analytical methods used to measure residues of dihydrostreptomycin and streptomycin. The first is a bioassay method, which does not distinguish between the two compounds. The second method is an HPLC assay for measuring the compounds independently and in combination. Data were submitted to allow evaluation of the characteristics of the two methods.

A specific requirement of the Committee at its forty-eighth meeting was that the analytical methods allow determination of the relationship between the antimicrobial activity of the residues using the
Table 3

Total residues of dihydrostreptomycin and streptomycin in tissues of cattle, sheep and pigs 2 days after a single intramuscular injection of streptomycin sulfate, alone or in combination with other drugs

<table>
<thead>
<tr>
<th>Species</th>
<th>Analytical method</th>
<th>Total residues (mg/kg) (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
<td>Liver</td>
</tr>
<tr>
<td>Cattle</td>
<td>Bioassay</td>
<td>&lt;0.3 (± 0.03)</td>
</tr>
<tr>
<td></td>
<td>HPLC</td>
<td>1.70 (± 0.86)</td>
</tr>
<tr>
<td>Sheep</td>
<td>Bioassay</td>
<td>&lt;0.3 (± 0.03)</td>
</tr>
<tr>
<td></td>
<td>HPLC</td>
<td>1.23 (± 0.47)</td>
</tr>
<tr>
<td>Pigs</td>
<td>Bioassay</td>
<td>&lt;0.3 (± 0.03)</td>
</tr>
<tr>
<td></td>
<td>HPLC</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: not detected; SD: standard deviation.

* Cattle received a combination of streptomycin sulfate (10mg/kg of body weight) and procaine penicillin (8mg/kg of body weight), while sheep received the same dose of streptomycin sulfate alone and pigs were given a combination of streptomycin sulfate (5mg/kg of body weight) and dihydrostreptomycin sulfate (5mg/kg of body weight).

* All values are means for four animals.

* Injection site.

bioassay method and their concentration, as measured by specific chemical methods. Measurements should be made in edible tissues fortified with dihydrostreptomycin and streptomycin and in tissues sampled from animals treated with these compounds. The studies in animals, including the sampling and analytical procedures, were conducted in accordance with requirements for good laboratory practice. The analytical methods were evaluated at the sponsor’s laboratory and did not appear to have any other accreditation.

All of the animal tissue samples weighed at least 200g; they were homogenized and divided before storage at -20°C.

Bioassay method. The sponsor provided the standard operating procedure for the bioassay and its validation in bovine, ovine and porcine tissues. Muscle, liver, kidney and fat tissues were homogenized in phosphate buffer at pH 2.0. A standard curve was obtained using samples fortified with dihydrostreptomycin and streptomycin at concentrations of 300–8000μg/kg. The supernatant obtained after centrifugation was used for the bioassay. *Bacillus subtilis* ATCC 6633 was used as the test organism. No cross-reaction with penicillins was observed when penicillinase was added. The characteristics of the assay are summarized in Table 4.
**Table 4**  
**Characteristics of the two analytical methods submitted for the determination of dihydrostreptomycin and streptomycin residues in animal tissues**

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Bioassay</th>
<th>HPLC assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality assurance</td>
<td>Performed in sponsor’s laboratory</td>
<td>Performed in sponsor’s laboratory</td>
</tr>
<tr>
<td>Matrices</td>
<td>Muscle (at the injection site and from elsewhere on the animal), liver, kidney, fat (cattle and sheep) and fat/skin (pigs)</td>
<td>Muscle (at the injection site and from elsewhere on the animal), liver, kidney, fat (cattle and sheep) and fat/skin (pigs)</td>
</tr>
<tr>
<td>Accuracy&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CV 0.8–11.4%</td>
<td>CV 0–13.4%</td>
</tr>
<tr>
<td>Recovery</td>
<td>Determined from a standard curve prepared using fortified samples</td>
<td>Determined from a standard curve prepared using fortified samples</td>
</tr>
<tr>
<td>Linearity</td>
<td>$r^2 \geq 0.991$</td>
<td>$r^2 \geq 0.980$</td>
</tr>
<tr>
<td>Limit of determination</td>
<td>200µg/kg for liver, 300µg/kg for all other tissues</td>
<td>12–153µg/kg for all tissues</td>
</tr>
<tr>
<td>Limit of quantification</td>
<td>300µg/kg for all tissues</td>
<td>400µg/kg for dihydrostreptomycin, 200µg/kg for streptomycin</td>
</tr>
<tr>
<td>Specificity in blank</td>
<td>Good</td>
<td>Good at limit of quantification</td>
</tr>
<tr>
<td>Specificity in relation to other antimicrobials</td>
<td>Poor</td>
<td>No interference with benzylpenicillin, gentamicin or lincomycin</td>
</tr>
</tbody>
</table>

CV: coefficient of variation.  
<sup>a</sup> At the limit of quantification (see Table 3).  

**HPLC assay.** The sponsor provided the standard operating procedure for an HPLC method for measuring dihydrostreptomycin and streptomycin in muscle, liver, kidney and fat of cattle and sheep and in muscle, liver, kidney and fat with skin of pigs. Fortified tissue samples for the calibration curves and validation procedures were prepared using 10-g samples of tissues from control animals by adding dihydrostreptomycin at concentrations of 400–6000µg/kg and streptomycin at concentrations of 200–3000µg/kg. The tissue samples were deproteinized with perchloric acid and centrifuged at 4600g for 5 minutes. The liquid extracts were placed onto a solid-phase sulfonic acid column, washed with water, and the dihydrostreptomycin and streptomycin were eluted with phosphate buffer at pH 7.5. Water, perchloric acid and ion-pair concentrate were added to the eluate,
which was then analysed by HPLC with post-column derivatization and fluorescence detection. The accuracy of the method was measured using fortified samples containing dihydrostreptomycin at concentrations of 400 and 5000 µg/kg. There was no interference with benzylpenicillin, gentamicin or lincomycin. The limit of quantification was found to be 400 µg/kg for dihydrostreptomycin and 200 µg/kg for streptomycin in all tissues. No correction for recovery was necessary because the standard calibration curve was constructed on the basis of the results for fortified material. The standard curve was not compared with a standard curve based on pure standards. The characteristics of the assay are summarized in Table 4.

Both methods were suitable for measuring residues of dihydrostreptomycin and streptomycin in fortified samples of muscle, liver, kidney and fat tissue from cattle, sheep and pigs. The bioassay was suitable for measuring the sum of the antimicrobial activity of the two drugs in tissues. Although penicillins did not interfere in the bioassay, other antimicrobials may do so. The bioassay is best suited to the measurement of residues of the two drugs in animals known to have been treated. If the bioassay were to be used for regulatory control purposes, samples found to be positive would have to be examined by more specific methods, such as the HPLC method.

Correlation between the bioassay and the HPLC method. In the initial study, reported at the forty-eighth meeting, the results obtained with the two assays for sheep liver and kidney tissues fortified with dihydrostreptomycin at 500 or 1200 µg/kg were compared. The study confirmed that the results were equivalent. In the new studies, the results for fortified blank tissue samples from cattle, sheep and pigs were used to derive calibration curves for each assay. The curves for both assays showed a linear response over a wide range of concentrations, with acceptable correlation coefficients (>0.992 for the bioassay and >0.980 for the HPLC method). The accuracy of the bioassay was 89–103% when samples fortified with dihydrostreptomycin and streptomycin at 300 µg/kg were examined; for the HPLC method, the accuracy was 85–113% for streptomycin and 88–118% for dihydrostreptomycin when samples fortified at 200 µg/kg were tested. The coefficients of variation at the limit of quantification were usually <10% but occasionally 10–15%.

Maximum Residue Limits
The ADI of 0–50 µg/kg of body weight is equivalent to a maximum ADI of 3000 µg for a 60-kg person.

At its forty-third meeting, the Committee recommended the following temporary MRLs for dihydrostreptomycin and streptomycin in
cattle, pigs, sheep and chickens: 500μg/kg for muscle, liver and fat, and 1000μg/kg for kidney. The Committee also recommended a temporary MRL of 200μg/kg for cows’ milk.

At its present meeting, the Committee considered that the sponsors had provided satisfactory answers to all of its requests made at the forty-third meeting. It therefore decided to delete the temporary status of the MRLs, except that for milk. The temporary MRL for milk was retained, pending the receipt of information on a validated analytical method to quantify low concentrations of dihydrostreptomycin and streptomycin in milk. This information is required for evaluation in 2001. The MRLs for muscle, liver and fat were raised to 600μg/kg, to take into account the twofold higher limit of quantification of the bioassay method compared with the HPLC method. The recommended MRL for kidney was 1000μg/kg in cattle, pigs, sheep and chickens. The MRLs were expressed as the sum of the concentrations of dihydrostreptomycin and streptomycin.

From the above MRL values, the theoretical maximum daily intake of dihydrostreptomycin and streptomycin residues is 620μg (equivalent to 21% of the maximum ADI), based on a daily food intake of 300g of muscle, 100g of liver, 50g each of liver and fat, and 1.5 kg of milk (Annex 1, reference 85). If milk is excluded, the theoretical maximum daily intake is 320μg or 11% of the ADI.

The Committee was aware of the existence of more sensitive analytical methods for dihydrostreptomycin and streptomycin in edible tissues and requested that descriptions of these methods be made available to the next session of the Codex Committee on Residues of Veterinary Drugs in Foods.

3.3.2 Neomycin

Neomycin is an aminoglycoside antibiotic. It was previously evaluated by the Committee at its forty-third and forty-seventh meetings (Annex 1, references 113 and 125).

At its forty-third meeting, the Committee established a temporary ADI of 0–30μg/kg of body weight for neomycin. Both toxicological and microbiological data were evaluated, and the toxicological data were considered to provide the most appropriate end-point; however, the ADI was made temporary in view of deficiencies in the data on genotoxicity. Temporary MRLs were recommended for cattle, pigs, sheep, goats, turkeys, ducks and chickens: 500μg/kg for muscle, liver and fat and 5000μg/kg for kidney, expressed as the parent drug. The temporary MRLs recommended for chickens’ eggs and cows’ milk were 500μg/kg, also expressed as the parent drug.
At its forty-seventh meeting, the Committee evaluated new data on
genotoxicity and established an ADI of 0–60μg/kg of body weight,
based on the NOEL of 6mg/kg of body weight per day for ototoxicity
in the guinea-pig and a safety factor of 100. Although no new residue-
depletion studies were submitted, the Committee recommended that
the MRL for kidney be increased from 5000μg/kg to 10000μg/kg to
permit practical withdrawal times for all target animal species. The
Committee noted that a withdrawal period of approximately 30 days
is required for residue levels to fall below the MRL in young calves.
The Committee also recommended that the temporary designation
for the MRLs in pigs, sheep, goats, turkeys, ducks and chickens be
removed.

Residue data
In the residue-depletion studies with neomycin considered previ-
ously, the oral route of administration had been used. At its present
meeting, the Committee evaluated a residue-depletion study con-
ducted according to good laboratory practice in which cattle were
treated by intramuscular injection. The concentrations of neomycin
residues were determined by a new HPLC method with fluorimetric
detection that had a limit of quantification one-fifth of that of the anti-
microbial method used in many of the earlier studies.
Twenty-four ruminating beef cattle, aged approximately 6 months,
were treated by intramuscular injection for 5 consecutive days with
a combination containing neomycin (12mg/kg of body weight),
procaine benzylpenicillin (21mg/kg of body weight), procaine hydro-
chloride (3mg/kg of body weight) and methylprednisolone
(0.41mg/kg of body weight). The animals were allocated randomly
into six groups, each consisting of two males and two females. Two
additional animals, one male and one female, served as controls. The
treated cattle were slaughtered 7, 14, 21, 30, 45 or 60 days after the last
injection; the control animals were slaughtered at the same time as the
animals in the group killed 7 days after drug withdrawal. Samples of
muscle, liver, kidney, fat and muscle tissue from the site of the last
injection were collected at each withdrawal time. Neomycin residues
were determined in the tissues by an HPLC method with fluorimetric
detection. The method was acceptable, on the basis of its specificity,
recovery after extraction, linearity, precision and accuracy. The limit
of quantification of the method was 100μg/kg for muscle, liver, kidney
and fat.

Neomycin was detected in kidney, liver, fat, injection-site muscle and
muscle from elsewhere on the animal. Kidney tissue had the highest
concentration of residues followed by liver, injection-site muscle, fat
and muscle from elsewhere on the animal, respectively (see Table 5).
Table 5
Mean residue concentrations of neomycin in tissues of cattle given an intramuscular injection of neomycin in combination with other drugs* on 5 consecutive days

<table>
<thead>
<tr>
<th>Withdrawal time (days)</th>
<th>Mean residue concentration (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>7</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>1400&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>14</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td></td>
<td>380&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>21</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td></td>
<td>610&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td></td>
<td>350&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>45</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td></td>
<td>340&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td></td>
<td>210&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

LOQ: limit of quantification (100 µg/kg).
* The animals received a combination of neomycin (12 mg/kg of body weight), procaine benzylpenicillin (21 mg/kg of body weight), procaine hydrochloride (3 mg/kg of body weight) and methylprednisolone (0.41 mg/kg of body weight).
<sup>a</sup> Injection site.

Analytical methods
The HPLC method with fluorimetric detection used to determine the concentration of neomycin in tissues consists of four stages: homogenization, precipitation of proteins with 5% trichloroacetic acid, purification on an ion-exchange column and HPLC analysis using post-column derivatization. The absolute recoveries of residues from muscle, liver, kidney and fat were 66%, 57%, 59% and 58%, respectively. The concentrations shown in Table 5 were corrected for recovery by comparison with a standard curve prepared from data for the respective tissue matrix.

Maximum Residue Limits
The ADI is 0–60 µg/kg of body weight, which is equivalent to a maximum ADI of 3600 µg for a 60-kg individual. The previously recommended MRLs of 500 µg/kg for eggs and milk account for 800 µg of the ADI, leaving 2800 µg to be distributed between the four edible tissues. The MRLs recommended for liver and kidney in cattle were 15 000 and 20 000 µg/kg, respectively, on the basis of the upper 99% confidence limit of the mean concentration of residues in animals killed at 30 or 45 days after the last injection. The recommended MRLs for neomycin in cattle, pigs, sheep, goats, turkeys, ducks and chickens are summarized in Table 6.
Table 6
Recommended MRLs for neomycin in cattle, pigs, sheep, goats, turkeys, ducks and chickens

<table>
<thead>
<tr>
<th>Species</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
<th>Milk</th>
<th>Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>500</td>
<td>15000</td>
<td>20000</td>
<td>500</td>
<td>500</td>
<td>—</td>
</tr>
<tr>
<td>Pigs</td>
<td>500</td>
<td>500</td>
<td>10000</td>
<td>500</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sheep</td>
<td>500</td>
<td>500</td>
<td>10000</td>
<td>500</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Goats</td>
<td>500</td>
<td>500</td>
<td>10000</td>
<td>500</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Turkeys</td>
<td>500</td>
<td>500</td>
<td>10000</td>
<td>500</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ducks</td>
<td>500</td>
<td>500</td>
<td>10000</td>
<td>500</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Chickens</td>
<td>500</td>
<td>500</td>
<td>10000</td>
<td>500</td>
<td>—</td>
<td>500</td>
</tr>
</tbody>
</table>

From the MRL values for the edible tissues and milk of cattle and eggs of chickens, the calculated theoretical maximum daily intake of neomycin residues is 3475 µg, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat, 100 g of eggs and 1.5 kg of milk (Annex 1, reference 85).

3.3.3 Thiamphenicol

Thiamphenicol is an antimicrobial agent that is given orally to non-ruminating cattle, pigs, poultry and fish for the treatment of certain bacterial diseases. As oral thiamphenicol is not suitable for treating ruminants, parenteral formulations of thiamphenicol glycine hydrochloride are used.

Thiamphenicol was previously evaluated by the Committee at its forty-seventh meeting (Annex 1, reference J25). At that time, a temporary ADI of 0–6 µg/kg of body weight was established on the basis of the NOEL of 1.25 mg/kg of body weight per day for maternal toxicity in a developmental toxicity study in rabbits and a safety factor of 200. The ADI was designated as "temporary" since only a summary report of the carcinogenicity study in rats was available. For further evaluation of the carcinogenic potential of thiamphenicol, the Committee requested detailed reports on the carcinogenicity study in rats and on the range-finding study used to establish dose levels in that study.

At its forty-seventh meeting, the Committee also recommended temporary MRLs for thiamphenicol of 40 µg/kg for muscle, liver, kidney and fat, expressed as the parent drug, in poultry and ruminating cattle. MRLs were not recommended for eggs because they were found to contain unacceptably high concentrations of thiamphenicol residues. No MRLs were proposed for cows' milk or the edible tissues of pigs because no data were provided on total residues in milk and the data
supplied for pigs were considered inadequate to form a basis for MRLs. The Committee requested the results of residue-depletion studies with radiolabelled and unlabelled thiamphenicol for the identification of the marker residue and target tissues in poultry, pigs and young (non-ruminant) calves for evaluation in 1999.

At the present meeting, data were submitted by the sponsor that partly addressed the Committee’s request. The sponsor also provided information on the pharmacokinetics of thiamphenicol and on residue depletion in fish and sheep. The sponsor did not, however, submit studies with radiolabelled compound, as had been requested by the Committee.

Toxicological data
At its present meeting, the Committee considered information on the short-term toxicity and carcinogenicity of thiamphenicol in Fischer 344 rats. The studies did not conform to good laboratory practice, but were carried out in accordance with appropriate standards for study protocol and conduct.

In a pilot study to determine the range of doses to be used in a carcinogenicity study, thiamphenicol was administered in the drinking-water to groups of 12 Fischer 344 (F344) rats at a dose of 0, 125, 250 or 500 mg/l (equal to 0, 9, 17 and 36 mg/kg of body weight per day in males and to 0, 12, 21 and 39 mg/kg of body weight per day in females) for 13 weeks. No deaths were observed. At 250 and 500 mg/l in drinking-water, numerous adverse effects were reported, including dose-dependent suppression of body-weight gain, a slight reduction in erythropoiesis in bone marrow, reduced erythrocyte and platelet counts, decreased relative thymus weight, increased relative liver and kidney weights, testicular lesions with degeneration of the seminiferous tubules, reduced sperm counts and spermatogranulomas in the epididymis. In addition, minor changes were observed in serum biochemical parameters, including reduced concentrations of total serum protein and cholesterol, and an increased albumin:globulin ratio. In all treated animals, thiamphenicol caused dose-related enlargement of the caecum, which is a common side-effect of long-term oral antimicrobial treatment in rodents. At the lowest dose, no other relevant treatment-related adverse effect was seen in tissues or organs or on haematological or serum biochemical parameters. The results indicated that a dose of 250 mg/l (corresponding to 17 mg/kg of body weight per day) was slightly toxic, whereas 125 mg/l (equal to 9 mg/kg of body weight per day) was the NOEL.

On the basis of the findings in the pilot study, doses of thiamphenicol of 0, 125 and 250 mg/l in drinking-water (equal to 0, 5 and 11 mg/kg
of body weight per day in males and 0, 7 and 14 mg/kg of body weight per day in females) were selected for the 2-year study of carcinogenicity in F344 rats. The highest dose caused a reduction in body-weight gain, whereas food and water consumption remained unchanged. The mean survival time of thiamphenicol-treated animals was not different from that of controls. The incidence and severity of non-neoplastic lesions were similar in all groups and were considered to be spontaneous and typical for aged F344 rats. The weight of the pituitary gland was increased in females. The incidence of malignant tumours was not significantly increased in treated animals over that in controls, and in all groups the distribution, histological characteristics and frequency of the tumours observed were similar to those reported to occur spontaneously in this strain of rats. The incidences of tumours at some sites were slightly increased in animals at the highest dose, but did not exceed the reported spontaneous incidence in historical controls. The increased incidence of pituitary adenomas reached significance only in females and was not associated with any increase in the incidence of preneoplastic lesions or differences in induction time.

On the basis of these findings, the Committee concluded that there was no evidence for the carcinogenicity of thiamphenicol in the 2-year study in rats. This conclusion was further supported by the absence of genotoxic effects of thiamphenicol in five in vitro genotoxicity tests and in an in vivo test for micronucleus formation in mice evaluated by the Committee at its forty-seventh meeting. At its present meeting, the Committee noted that a histopathological survey had shown no evidence of toxic effects of thiamphenicol on target tissues such as bone marrow and testicular tissue. Furthermore, no changes were reported that reflected the slight, biologically insignificant alterations in some of the haematological and biochemical parameters observed at the lowest dose in the 13-week toxicity study in rats. The NOEL in the 2-year carcinogenicity study was therefore 5 mg/kg of body weight per day.

The Committee reconsidered the developmental toxicity study in rabbits that was used at the forty-seventh meeting to establish the ADI, on the basis of the NOEL of 1.25 mg/kg of body weight per day for maternal toxicity. The Committee considered that this effect was not an appropriate toxicological end-point because of the known sensitivity of rabbits to orally administered antimicrobial agents. The Committee considered that the NOEL of 5 mg/kg of body weight per day in the 2-year carcinogenicity study in rats was the most relevant toxicological end-point.
Microbiological data

The Committee further reconsidered the microbiological data on thiamphenicol that were reviewed at its forty-seventh meeting. At that time, the mean MIC_{90} of all 261 strains of 16 bacterial species of human origin that had been studied was used to calculate the ADI. The most recent approach of the Committee for assessing the microbiological risks of residues of antimicrobial substances is described in section 2.3 of this report. At its present meeting, the Committee calculated an ADI for thiamphenicol on the basis of antimicrobial activity from the formula described in section 2.3.1, using the MIC_{90} of the most sensitive, relevant bacterial species of the human gut flora, *Fusobacterium* spp., which was 0.5 µg/ml:

\[
\text{Upper limit of ADI} = \frac{0.5 \, \mu g/g \times 220 \, \mu g}{0.4 \times 1 \times 60 \, \text{kg}} = 4.58 \, \mu g/kg \text{ of body weight}
\]

The Committee established an ADI of 0–5 µg/kg of body weight for thiamphenicol, based on its antimicrobial activity against *Fusobacterium* spp. This ADI is one-tenth of the toxicological ADI of 0–50 µg/kg of body weight that would be derived on the basis of the NOEL of 5 mg/kg of body weight per day in the 2-year carcinogenicity study in rats and a safety factor of 100.

An addendum to the toxicological monograph was prepared.

Metabolic and pharmacokinetic data

**Pigs.** In a study that complied with good laboratory practice, 16 pigs weighing 15–22 kg and aged about 7 weeks were fed thiamphenicol at a dose equal to approximately 30 mg/kg of body weight in the diet twice daily for 5 consecutive days. Blood samples were taken immediately before dosing and once daily for the next 10 days. A maximum mean plasma concentration of 1.3 mg/l was attained 8 hours after the first dose. The plasma concentrations declined after withdrawal of the drug and were close to or below the limit of detection of the analytical method (21 µg/kg) at 5 days after withdrawal.

In a more recent study that was also in conformity with the requirements for good laboratory practice, four barrows (castrated male pigs) weighing 100–120 kg were given thiamphenicol orally at 30 mg/kg of body weight or intravenously at 10 mg/kg of body weight. Blood

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* For the purpose of this evaluation, the MIC_{90} value is the mean MIC_{90} for thiamphenicol against the 20 strains of the most sensitive relevant genus isolates from the human gastrointestinal tract, in this case *Fusobacterium* spp.

* The mass of the colonic contents.

* The fraction of thiamphenicol available to the gut microflora.

* A safety factor of 1 was used because extensive relevant microbiological data were available.

* The weight of an adult person.
and urine samples were collected at 0.25, 0.5, 1, 2, 3, 4, 6, 8 and 24 hours after dosing: a further urine sample was collected at 48 hours after dosing. Thiampenicol was measured before and after enzymatic hydrolysis with β-glucuronidase. Following oral administration, the half-life of free thiamphenicol was 3.9 hours and the integrated area under the concentration–time curve (AUC) was 24 mg/l × h; for the sum of thiamphenicol and thiamphenicol glucuronide the half-life was 4.6 hours and the AUC was 91 mg/l × h. After intravenous administration, the half-life of free thiamphenicol was 3.4 hours and the AUC was 12 mg/l × h; for the sum of thiamphenicol and thiamphenicol glucuronide the half-life was 4.6 hours and the AUC was 23 mg/l × h. In urine, the highest concentrations of thiamphenicol and thiamphenicol glucuronide (combined) were found 4 hours after either oral (610–720 mg/l) or intravenous dosing (360–1100 mg/l). The mean plasma thiamphenicol glucuronide concentrations were higher than the concentrations of free thiamphenicol at all times after the last oral dose. The results of this study show that glucuronide formation is an important route of elimination of thiamphenicol in pigs.

Sheep. The pharmacokinetics of thiamphenicol was investigated in 12 cross-bred sheep, aged from 9 months to 1 year and weighing 30–35 kg, which were given thiamphenicol glycinate intramuscularly at a dose of 20 mg/kg of body weight every 8 hours for 24 hours. Blood samples were collected at 10, 15 and 30 minutes and 1, 2, 4, 6 and 8 hours after the first three doses and analysed by an HPLC method with a limit of detection of 100 μg/l. Thiampenicol was rapidly absorbed from the injection site, to give a peak plasma concentration of 20 mg/l within 30 minutes. The plasma concentration then declined, with an elimination half-life of 1.5 hours and a mean residence time of 2.0 hours. The drug could not be detected in plasma after 24 hours.

Fish. Yellowtail fish (Seriola quinqueradiata) weighing 190 g and reared at 28 °C received thiamphenicol as a single dose equivalent to 100 mg/kg of body weight mixed in their feed. Blood was collected from seven fish after 3, 6, 12, 24 and 48 hours. The concentration of thiamphenicol, measured by a colorimetric method, peaked at 9.4–12 mg/l between 3 and 6 hours after dosing. No drug could be detected in blood after 48 hours but high levels were detected in bile at 24 hours, providing support for the hypothesis that biliary excretion is the major pathway of elimination in fish. Metabolites were not investigated.

European seabass (Dicentrarchus labrax) reared at 15 °C received thiamphenicol at a dose of 15 or 30 mg/kg of feed, either as a single dose by gavage or as five doses in their diet on consecutive days. The drug was absorbed more slowly from feed than from gavage, and absorption was incomplete (AUC0–72 hours 200 mg/l after a single dose of
30 mg/kg of feed by gavage and 39 mg/l × h after five doses of 15 or 30 mg/kg of feed in the diet). The concentrations of thiamphenicol in blood declined rapidly and were below the limit of quantification of the analytical method (50 μg/l) 7 days after the last treatment. No metabolites were reported.

Residue data
At its forty-seventh meeting, the Committee had requested new residue-depletion studies with radiolabelled and unlabelled thiamphenicol in young (non-ruminant) calves, pigs and poultry. The data provided to the present meeting were only for pigs. Thiamphenicol was not identified unambiguously as the correct marker residue in all pig tissues, nor was any information given on the ratio of thiamphenicol to total residues in pig tissues at any time, even though studies of pharmacokinetics have shown that the thiamphenicol glucuronide conjugate is the predominant form of thiamphenicol in plasma and a significant metabolite in the urine of pigs after oral dosing.

Pigs. In a recent residue-depletion study that complied with good laboratory practice, 25 cross-bred pigs weighing 25–30 kg each were divided randomly into five equal groups. Four groups were fed a diet containing thiamphenicol at a dose equivalent to 30 mg/kg of body weight per day for 5 days, while the fifth group received standard, non-medicated diet. The pigs in the four treatment groups were slaughtered at 5, 10, 16 and 18 days after withdrawal of the drug, respectively. The groups were treated at different times so that all animals were killed on the same day. Their tissues were analysed for thiamphenicol but not for the glucuronide metabolite. Thiamphenicol was identified in muscle only in two animals killed at day 10, at a concentration of 28 μg/kg in one animal and below the limit of quantification (21 μg/kg) in the other. Between days 5 and 18, the mean concentrations of residues of thiamphenicol declined from 58 μg/kg to 27 μg/kg in liver, from 540 μg/kg to 39 μg/kg in kidney and from 230 μg/kg to 95 μg/kg in skin. The coefficients of variation were very large at several times, particularly for the values in kidney.

In a second residue-depletion study, which also complied with good laboratory practice, 32 cross-bred pigs weighing 45–65 kg each were divided into eight groups of two males and two females. Animals in seven groups were dosed intramuscularly with thiamphenicol at 30 mg/kg of body weight per day for 5 days, and the eighth group was untreated. The pigs in the seven treatment groups were slaughtered 8 hours and 1, 4, 8, 15, 21 and 28 days after the last dose of the drug, respectively. Samples of muscle at the site of injection, muscle from
elsewhere on the animal, liver, kidney, visceral fat and skin/fat were
taken from each animal and analysed by HPLC for thiamphenicol but
not for the glucuronide metabolite. The concentrations of thiam-
phenicol had decreased to <50μg/kg in all tissues investigated 8 days
after withdrawal of the drug. After 28 days, thiamphenicol residues
were detected at concentrations above the limit of quantification
(20μg/kg) in muscle at the site of injection only.

**Sheep.** In a residue-depletion study that did not comply with good
laboratory practice, 16 cross-bred sheep (males weighing 55–74kg
and females weighing 39–56kg) were divided into four groups of two
males and two females. Animals in each group received intramuscular
injections of thiamphenicol at 30mg/kg of body weight per day for 5
consecutive days. The sheep in the four groups were slaughtered 4, 8,
12 and 16 days after the last dose of the drug, respectively. Samples of
muscle at the site of injection, muscle from elsewhere on the animal,
liver, kidney and abdominal fat were taken from each animal and
analysed for thiamphenicol but not for thiamphenicol glucuronide. At
day 4, thiamphenicol was not found in muscle but was found at a
concentration of 43μg/kg in kidney and 340μg/kg in skin. No residues
were detected in any other tissues after that time.

**Fish.** In a study that did not comply with good laboratory practice,
yellowtail fish (*Seriola quinqueradiata*) weighing 210g and reared at
23.0–27.5°C received thiamphenicol in their feed at a concentration
equivalent to 45mg/kg of body weight per day (recommended dose)
or 90mg/kg of body weight per day for 14 days. Samples of muscle,
liver, kidney and skin with fat were collected on day 10 of administra-
tion and 0, 3, 7, 10, 14, 21 and 28 days after the end of treatment. The
concentrations of residues of free thiamphenicol were highest in liver,
followed by kidney and skin, respectively; the concentrations in
muscle were very low. In all tissues, the concentrations fell below the
limit of detection of the analytical method (20μg/kg) 3 days after
cessation of treatment.

In a recent study, which was also not performed according to good
laboratory practice, European seabass (*Dicentrarchus labrax*) weighing
140–150g and reared at 18–28°C received thiamphenicol in their
feed at a concentration equivalent to 40mg/kg of body weight per day
for 5 days (the recommended dose and duration of treatment).
Samples of muscle, liver, skin and vertebrae were collected from
groups of 10 fish on days 2 and 4 of dosing and 1, 2, 3, 5, 7 and 10
days after the end of treatment. The concentrations of thiamphenicol resi-
dues were determined by an HPLC method. The concentrations of
free thiamphenicol 2 days after withdrawal of the drug were 60μg/kg
in muscle, 1500μg/kg in liver and 90μg/kg in skin. Five days after
withdrawal, the concentrations of residues in all tissues were below the limit of detection of the HPLC method (20–100μg/kg, depending on the tissue).

In a related study, which was also not performed according to good laboratory practice, gilthead seabream (Sparus aurata) weighing 100–120 g received thiamphenicol in their feed at a concentration equivalent to 40 mg/kg of body weight per day for 5 days. Samples of muscle, liver, skin and vertebrae were collected from groups of 10 fish on days 2 and 4 of administration and 1, 2, 3 and 5 days after cessation of treatment, and analysed for thiamphenicol by an HPLC method. The concentrations of free thiamphenicol 2 days after withdrawal of the drug were 240 μg/kg in muscle, 380 μg/kg in liver and 180 μg/kg in skin. Residues fell below the limit of detection of the HPLC method (20–100 μg/kg, depending on the tissue) 5 days after cessation of treatment.

**Analytical methods**

Several methods for the analysis of thiamphenicol residues have been reported which were not reviewed by the Committee at its forty-seventh meeting.

An HPLC method that meets the requirements for good laboratory practice has been developed for the detection and quantification of residues of free thiamphenicol in the plasma, urine and edible tissues of pigs. After a simple purification procedure, thiamphenicol is determined by reverse-phase HPLC with detection by ultraviolet spectroscopy at 224 nm. For kidney and urine samples, an additional purification step and a different elution solvent are required. The limit of quantification of the method is 20 μg/kg and the limit of detection is 5 μg/kg in all tissue matrices. The coefficient of variation is <5% for all matrices except liver, for which it is 8%. Concentrations of thiamphenicol well below the stated limit of quantification and even below the stated limit of detection have been reported in studies in which this method has been used. The accuracy and precision of the method were not stated, although both the limit of quantification and the coefficients of variation were cited to two decimal places. The same HPLC procedure has also been tested for the determination of thiamphenicol in samples of plasma, urine and tissue from sheep. The method has also been used, after the introduction of an enzymatic hydrolysis step, to determine the sum of the concentrations of thiamphenicol and thiamphenicol glucuronide in plasma and urine samples. This modified method was not used to determine thiamphenicol glucuronide in tissue samples in the studies reviewed at the present meeting.
An HPLC procedure for the determination of thiamphenicol residues in fish tissues has been reported, but it has not been tested in accordance with the requirements for good laboratory practice. The limits of quantification were 20μg/kg in muscle, 100μg/kg in liver and 50μg/kg in skin and vertebrae.

**Maximum Residue Limits**

The Committee considered the following factors in recommending MRLs for thiamphenicol:

- An ADI of 0–5μg/kg of body weight, based on a microbiological end-point, was established by the Committee. This corresponds to a maximum ADI of 300μg for a 60-kg person.
- The parent drug is the marker residue currently recommended for monitoring compliance with the temporary MRLs. The limits of quantification and detection of the available analytical methods for free thiamphenicol in pigs are 20μg/kg and 5μg/kg, respectively. No validated analytical methods are available for measuring the sum of the concentrations of thiamphenicol and thiamphenicol conjugates in the edible tissues.
- The parent drug is the predominant substance in the plasma and urine of cattle and poultry, whereas the glucuronide conjugate is the major metabolite in the plasma and urine of pigs. The major metabolites of thiamphenicol in the tissues of food animals and fish species are unknown, and the occurrence of conjugated metabolites in the edible tissues has not been investigated. The possibility that thiamphenicol undergoes extensive metabolism in liver cannot be discounted; however, no data were available to determine the proportion of the total residues represented by the marker residue in any species. The Committee recognized that thiamphenicol glucuronide is not microbiologically active but could be converted in humans to the microbiologically active parent drug after ingestion.
- The Committee was concerned that the residues of thiamphenicol might be bound in tissues, as drugs with a similar structure are known to yield bound residues.
- The sponsor has not provided data on the use of thiamphenicol in non-ruminating calves and poultry, as requested by the Committee at its forty-seventh meeting. However, the results of new studies in pigs and fish were supplied, in which thiamphenicol was used as the marker residue.

The Committee recommended temporary MRLs in pigs of 50μg/kg for muscle and fat, 100μg/kg for liver, and 500μg/kg for kidney. A temporary MRL of 50μg/kg was recommended for fish muscle with naturally adhering skin.
From these MRLs, the theoretical maximum daily intake of thiamphenicol would be 53 μg, based on a daily intake of 300 g of muscle, 100 g of liver, and 50 g each of kidney and fat (Annex 1, reference 85).

Owing to the lack of information on tissue metabolites, the Committee recommended that the marker residues for thiamphenicol should be the sum of thiamphenicol and thiamphenicol conjugates, measured as thiamphenicol. This marker residue would apply to the temporary MRLs until further work has been conducted to establish the distribution of metabolites in edible tissues.

Because the data requested by the Committee at its forty-seventh meeting to support the use of thiamphenicol in cattle and chickens were not provided, the temporary MRLs previously recommended for these species were withdrawn.

The following information is required for evaluation in 2002:

1. The results of a residue-depletion study in pigs given radiolabelled drug to determine the proportions of the total residues accounted for by free thiamphenicol and thiamphenicol conjugates in all tissues.

2. A validated analytical method for use with all animal tissues, which includes an enzymatic hydrolysis step to allow determination of the sum of thiamphenicol and thiamphenicol conjugates as free thiamphenicol.

3.4 Insecticides

3.4.1 Deltamethrin

Deltamethrin is a synthetic pyrethroid insecticide used for the control of Diptera and Mallophaga in animals and for insect control in plants. It is applied topically as a dip, spray or pour-on preparation to cattle, sheep, pigs, poultry and salmon.

Deltamethrin has not been evaluated previously by the Committee. The Joint FAO/WHO Meeting on Pesticide Residues evaluated deltamethrin toxicologically in 1980, 1981 and 1982 (17–19). An ADI of 0–10 μg/kg of body weight was established at the 1982 meeting. MRLs were recommended for veterinary use in 1990 and for use as a pesticide for plant protection purposes in 1982.

Pharmacokinetic data

The Committee noted the importance of determining the pharmacokinetics of the drug in laboratory animals after oral administration, as this is the route of human exposure. The pharmacokinetics of the drug in target animals following topical application for the treatment of ectoparasite infestations was also reviewed. The Committee noted,
however, that the target animals are also exposed to deltamethrin when it occurs as a contaminant on or in plant foods. This source of exposure was reviewed by the 1990 Joint FAO/WHO Meeting on Pesticide Residues (20).

The deltamethrin molecule has eight stereo-isomers. The active stereo-isomer is a pure (>99%) single cis-isomer consisting of two subunits joined by an ester bridge, which is readily hydrolysed. In all of the studies described below, the metabolic fate of the two resulting components was determined by administering deltamethrin labelled with $^{14}$C on one side of the ester bridge ($[^{14}\text{C}]$gem-dimethyl-deltamethrin) or the other ($[^{14}\text{C}]$benzyl-deltamethrin). A third $^{14}$C-labelled compound, deltamethrin labelled on the cyanide (–CN) group, which may be labile, was tested only in rats.

**Cattle.** $[^{14}\text{C}]$Deltamethrin was applied topically as a single dose of 0.21 g to the hide of one cow. Blood and milk were collected over 10 days, and hair samples were taken for 45 days after dosing; excreta were not tested. The concentrations of radiolabel in blood and whole milk were similar, reaching peak values within 2.5 days and declining to <1 µg/kg in about 9 weeks; the half-lives were 4.3 days in milk and 4.4 days in butterfat. The majority (95%) of the radiolabel was found in milk fat. The concentration of radiolabel remained >1 mg/kg for approximately 75 days in the hair around the head and for about 100 days in the body hair. The mechanism of absorption from the hide was not clear.

A 2.0% deltamethrin pour-on formulation for cattle containing either $[^{14}\text{C}]$gem-dimethyl-deltamethrin or $[^{14}\text{C}]$benzyl-deltamethrin was applied dermally at about four times the recommended dose to two dairy cows for 3 consecutive days. The following values for total residues were obtained with the two radiolabelled compounds, respectively: total recovery of radiolabel, 85% and 80% of the administered dose; recovery in washings and wipe samples of skin, 36% and 48%; recovery in the treatment enclosure, 37% and 19%; and recovery from the skin at the site of administration, 10% of the administered dose for both preparations. Excretion of radiolabel in the faeces accounted for 0.6% of the administered dose, and that in the urine accounted for 0.3%. Less than 0.01% of the administered dose was recovered in milk, bile and tissues collected from either cow. At least 11% of the radioactive dose was absorbed, and about 70% of the administered dose remained at the site of application. Essentially all of the radiolabel (>95%) found in skin from the site of application was unchanged $[^{14}\text{C}]$deltamethrin. The concentrations of radiolabelled material in blood indicated that it was rapidly absorbed and transported systemically; however, the concentrations
remained low (1 and 4 µg/l at 1 hour after dosing and <1 µg/l at 12 hours) throughout the study. The concentrations of radiolabelled residues were low in all of the tissues analysed, ranging from 1 µg/kg in muscle to 13 µg/kg in liver. No radiolabel was detected in blood collected at slaughter. Milk contained up to 2 µg of deltamethrin equivalents per litre and the radiolabel was located in the milk fat.

**Chickens.** In a study performed in accordance with good laboratory practice, laying hens received [14C]gem-dimethyl-deltamethrin or [14C]benzyl-deltamethrin topically once daily for 3 consecutive days at a nominal dose of 0.15 mg/kg of body weight. The birds were killed about 23 hours after the final dose. The following values for total residues were obtained with [14C]gem-dimethyl-deltamethrin and [14C]benzyl-deltamethrin, respectively: recovery from feathers at the site of application, 32–62% and 41–53% of the administered dose; recovery from dressings at the site of application, 3–13% and 2–8%; recovery from excreta, 1–4% and 1–3%. The concentrations of residues were <1 µg/kg in eggs and <2.5 µg/kg in muscle. The concentrations were highest in liver (up to 18 µg/kg) and were detectable in skin/fat and whole blood.

**Fish.** Thirteen salmon weighing 174 g (mean) and maintained at 12°C were injected in the caudal vein with a 1:1 mixture of [14C]gem-dimethyl-deltamethrin and [14C]benzyl-deltamethrin at a dose of 0.25 mg/kg of body weight. The tentative half-life in blood was calculated to be 54 hours.

**Metabolic data**

The metabolism of deltamethrin was studied in cattle and chicken liver preparations incubated in vitro with either [14C]gem-dimethyl-deltamethrin or [14C]benzyl-deltamethrin and shown to be similar. The metabolites that were identified were 3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid, 3-phenoxybenzaldehyde, 3-phenoxybenzoic acid, 3-phenoxybenzyl alcohol, 3-(4-hydroxyphenoxy) benzoic acid and 3-(4-hydroxyphenoxy)benzyl alcohol. The metabolites were formed as a result of cleavage of the ester bridge and oxidation.

**Cattle.** A 2.0% deltamethrin pour-on formulation for cattle containing either [14C]gem-dimethyl-deltamethrin or [14C]benzyl-deltamethrin was applied topically at about twice the recommended dose to two dairy cows for 3 consecutive days. The animals were slaughtered approximately 24 hours after the last dose and the liver, kidney, renal fat and milk fat analysed to determine the nature of the residues. No individual radiolabelled metabolite (or extract) and none of the residual radiolabel accounted for more than 10 µg/kg of deltamethrin.
equivalents. [14C]Deltamethrin was the major radiolabelled component in the renal fat and milk fat, accounting for 59% (7 μg/kg) and 55% (5 μg/kg) of the total residues, respectively, in the case of [14C]gem-dimethyl-deltamethrin, and for 48% (4 μg/kg) and 42% (4 μg/kg) of the total residues, respectively, in the case of [14C]benzyl-deltamethrin. Little, if any, [14C]deltamethrin was found in the liver or kidney, indicating that considerable metabolism had occurred in these tissues. Polar metabolites, including N-(3-phenoxybenzoyl)-L-glutamate, were found in the liver (31% of the total residues) and kidney (33% of the total residues) of the cow treated with [14C]benzyl-deltamethrin, and up to seven metabolites (including trace amounts of 3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid) were found in the liver and kidney of the cow treated with [14C]gem-dimethyl-deltamethrin. Residues that remained in the liver and kidney after extraction with organic solvents were released (solubilized) by hydrolysis with 3 mol/l hydrochloric acid.

**Chickens.** In chickens treated topically with [14C]gem-dimethyl-deltamethrin and [14C]benzyl-deltamethrin, the major metabolites identified in excreta were polar compounds, accounting for only about 0.1% of the administered dose. Deltamethrin was the major component of the residues in excreta, accounting for about 30% of the radiolabel in the sample. Other (minor) components accounted for 1% or less of the radiolabel.

In liver samples from chickens dosed topically with [14C]gem-dimethyl-deltamethrin, polar metabolites and deltamethrin accounted for 1.8% and 1.6% of the total residues, respectively. In liver samples from hens dosed with [14C]benzyl-deltamethrin, polar compounds and deltamethrin were major components, accounting for 5.4% and 11% of the total residues, respectively. The remaining metabolites did not correspond to the available reference compounds. Analysis of skin at the site of application, feathers, occlusive covers and swabs by HPLC and thin-layer chromatography showed that the major component of the total residues was unchanged deltamethrin.

**Residue data**

Studies of the residues of deltamethrin found after topical or parenteral administration to cattle, sheep, pigs and poultry were reviewed by the 1990 Joint FAO/WHO Meeting on Pesticide Residues (20) and are summarized below.

**Cattle.** Three heifers weighing 200–240 kg received a topical application of [14C]deltamethrin at 2 mg/kg of body weight and were killed 3, 7 and 14 days after dosing. Tissue samples were collected for analysis of total residues and parent drug. The concentrations of residues in fat
were 130–190μg/kg and persisted over 14 days. They consisted almost entirely of the parent drug. The parent drug also accounted for a significant proportion of the very low concentrations of residues in muscle. In kidneys, <10% of the total residues was accounted for by the parent drug. The high concentration of residues found in liver (mean value of 310μg/kg at 14 days) persisted, but <20% was extractable, and the concentration of the parent drug was below the limit of quantification of the analytical method.

The residual concentrations of parent drug were reported in at least three studies in which cows were dipped using deltamethrin formulations under field conditions (18). The concentrations of residues were below the limit of quantification in muscle, liver, and kidney, but were up to 70–150μg/kg in fat. In seven studies in which pour-on preparations were used, the maximum concentrations of residues were 30μg/kg in muscle, 5μg/kg in liver, 34μg/kg in kidney and 70–370μg/kg in fat. In three studies in which spray-on preparations were tested, the maximum concentrations of residues were 14μg/kg in muscle, <5μg/kg in liver and kidney, and 360μg/kg in fat.

In a new study, six lactating cows were dosed with a pour-on formulation of unlabelled deltamethrin at 0.4mg/kg of body weight twice, with a 7-day interval. A further group of 12 lactating cows was dosed at 1.6mg/kg of body weight using the same protocol. The cows were milked twice daily until they were slaughtered on days 3, 7 or 14 after the second treatment. Samples of the edible tissues were analysed for total residues of deltamethrin (the sum of α-, R-, cis- and trans-deltamethrin) by gas chromatography with electron capture detection (GC-ECD). The residues in muscle, liver, kidney and whole milk were below the limit of quantification (15μg/kg) in all samples. Some samples of subcutaneous fat collected at day 1 contained residues at up to 110μg/kg, but the concentrations in all other samples were below the limit of quantification (45μg/kg). In renal fat, the concentrations of residues were above the limit of quantification only in samples from cows in the high-dose group; the values ranged from 46 to 90μg/kg, with no depletion over the 14-day period. The range of concentrations in milk fat declined from a high of 110–530μg/kg on day 1 to 100–110μg/kg on day 7 and was below the limit of quantification (75μg/kg) on day 14.

[^14]C gem-Dimethyl-deltamethrin was applied topically at a dose of about 0.55mg/kg of body weight to a lactating dairy cow, and the quantity of radiolabel in whole milk and in butterfat was determined. The ratio of residues in butterfat to that in whole milk remained constant at 20:1, indicating that most of the residues were in the fat. The maximum level in whole milk was 5.7μg/kg and was found 2.5
days after dosing. The terminal half-life of deltamethrin residues in both milk and butterfat was about 4 days.

**Sheep.** No residue-depletion studies conducted with labelled drug were submitted. In one study of 23 sheep, three groups were dipped and three groups received a pour-on preparation of deltamethrin. In sheep that were dipped, the maximum concentrations of residues were reported to be 32 µg/kg in muscle, <30 µg/kg in liver and kidney, and 470 µg/kg in fat. Only one muscle sample contained residues above the limit of quantification of the analytical method (15 µg/kg); this value may be an erroneous result. In sheep that received the pour-on preparation, the concentrations of residues in muscle, liver and kidney were below the limit of quantification (30 µg/kg), and the highest concentration of residues in fat was 80 µg/kg.

**Pigs.** No residue-depletion studies conducted with radiolabelled drug were submitted to the Committee. In two studies reviewed by the 1990 Joint FAO/WHO Meeting on Pesticide Residues (18), in which nine pigs received pour-on preparations of deltamethrin, the maximum concentrations of residues were below the limit of quantification (10 µg/kg in muscle and liver, 7 µg/kg in fat and 200 µg/kg in kidney). The concentration of residues in the skin at the site of application in one pig was 3200 µg/kg 3 days after dosing.

**Chickens.** Groups of hens received topical applications of [14C]gem-dimethyl-deltamethrin or [14C]benzyl-deltamethrin once daily for 3 consecutive days at a dose intended to provide 0.15 mg/kg of body weight. The birds were killed about 23 hours after the final application, and the concentrations of residues were measured in muscle, liver, skin/fat and eggs. The concentrations of residues in eggs and breast muscle and in some of the samples of leg muscle were below the limit of detection (1 µg/kg). The concentrations in liver and fat were 1–18 µg/kg and 1–20 µg/kg, respectively. In liver, the parent drug accounted for 13% of the total residues in the animals treated with [14C]gem-dimethyl-deltamethrin and 21% in those dosed with [14C]benzyl-deltamethrin. When hens were sprayed with a 0.0025% or 0.005% aqueous solution of deltamethrin, the concentrations of residues in muscle, liver, kidney, fat and eggs were all below the limit of quantification (15 µg/kg) at 1, 2, 3, 4 and 8 days after treatment.

**Fish.** Fifty-five Atlantic salmon with a mean weight of 140 g were immersed for 30 minutes in seawater containing 5 µg/l of a 1:1 mixture of [14C]gem-dimethyl-deltamethrin and [14C]benzyl-deltamethrin. The fish were maintained at 12°C and were killed at 10 intervals between 1 hour and 10 days. The highest concentrations of total residues were found during the first 3 days; they were 9 µg/kg in
muscle, 95\(\mu\)g/kg in liver, 41\(\mu\)g/kg in kidney and 13\(\mu\)g/kg in skin. The lowest concentrations of total residues were found during days 7–10; they were 1\(\mu\)g/kg in muscle, 3\(\mu\)g/kg in liver, 2\(\mu\)g/kg in kidney and 3\(\mu\)g/kg in skin.

Two studies were conducted with unlabelled deltamethrin at 8\(^°\)C. In the first study, salmon weighing 199–489g were immersed in seawater containing deltamethrin at a concentration of 10\(\mu\)g/l for 30 minutes, whereas the recommended dose is 3\(\mu\)g/l. Fish were killed at 18 intervals between 3 minutes and 14 days and residues were determined by a GC-ECD method. No residues were found in muscle, liver, skin or plasma (limits of quantification, 15\(\mu\)g/kg for muscle, 14\(\mu\)g/kg for liver and skin, and 14\(\mu\)g/l for plasma). In the second study, salmon with a mean weight of 240g were immersed for 30 minutes in seawater containing deltamethrin at a concentration of 5\(\mu\)g/l and were killed at 11 intervals between 2 hours and 14 days. No residues were found, except in muscle at 2 and 6 hours (16\(\mu\)g/kg) and in skin at 2 hours (25\(\mu\)g/kg). The limits of quantification were the same as in the first study.

**Bound residues.** Fat and muscle of all species and cows’ milk contained <10\% bound residues. The extensive metabolism of deltamethrin in the liver of all species resulted in residues of low relative molecular mass that were either bound or incorporated into hepatocellular components. About 64\% of the total residues in bovine liver and about 32\% of those in kidney could not be extracted. In hens, bound residues accounted for 43–68\% of the total residues in liver. The nature of the bound residues was not characterized, but the radiolabel could be released (solubilized) after hydrolysis of the tissues with 3 mol/l hydrochloric acid.

**Analytical methods**

The 1990 Joint FAO/WHO Meeting on Pesticide Residues considered the analytical methods available for deltamethrin (20). The limits of quantification of some of the methods previously evaluated to determine the concentrations of deltamethrin in tissues were lower than those of the most recent method (see below). Suitable methods tested according to good laboratory practice have been submitted to the Committee since 1990 for measuring deltamethrin residues in muscle, liver, kidney and fat of cattle and chickens, and in cows’ milk and chicken eggs. The methods have not been validated for sheep tissues. A similar method for determining residues in salmon tissues was submitted to the present meeting of the Committee.

Residues of deltamethrin were extracted from tissues, milk and eggs. After purification, deltamethrin was analysed by GC-ECD. The cali-
ibration curve was determined using concentrations from 0.015 to 15μg/l. The correlation coefficient ($r^2$) was 0.997. The proportion of the drug recovered from batch samples of all cattle tissues and milk was 90–101%, and the proportion recovered from chicken muscle, liver, fat and eggs was 89–93%. The limits of quantification were 15μg/kg for muscle, liver, kidney, milk and milk fat and 45μg/kg for fat and eggs. The proportions of the drug recovered from salmon were 82% of a dose of 25μg/kg and 79% of a dose of 250μg/kg in muscle, 73% of a dose of 20μg/kg and 65% of a dose of 500μg/kg in liver, and 80% of a dose of 20μg/kg and 71% of a dose of 500μg/kg in skin. The limits of quantification for salmon tissues were 15μg/kg for muscle, 14μg/kg for skin and liver, and 14μg/l for plasma.

**Maximum Residue Limits**

The 1982 Joint FAO/WHO Meeting on Pesticide Residues established an ADI of 0–10μg/kg of body weight (equivalent to a maximum ADI of 600μg for a 60-kg person) for deltamethrin. The 1990 Joint FAO/WHO Meeting on Pesticide Residues established the following MRLs for deltamethrin as a pesticide, taking into account its use as a veterinary drug: 500μg/kg in meat (fat), 50μg/kg in offal and 20μg/kg in milk. The species to which the MRLs for meat and offal apply were not specified.

In recommending MRLs for deltamethrin, the Committee considered the following factors:

- Studies of metabolism were reported only for cattle and chickens.
- Residue-depletion studies with radiolabelled drug and analytical methods were available for cattle, chickens and salmon.
- Residue-depletion studies with unlabelled drug were available for cattle, chickens, salmon and sheep.
- The drug is applied topically to the skin.
- The parent drug is absorbed, and its residues are distributed predominantly in body fat and milk fat.
- The parent drug undergoes extensive metabolism in the liver and kidney, with rapid excretion of the metabolic products.
- The parent drug is proposed as the marker residue and is a good indicator of residues in body fat and milk fat and at the site of application. Because the drug undergoes extensive metabolism it is difficult to monitor the total residues in liver and kidney.
- As the concentrations of residues of the marker compound in muscle, milk and eggs are very low, the limits of quantification of the analytical methods used should also be low.
- The limits of quantification of the methods used to determine residues in cattle and chicken tissues are 15μg/kg for muscle, liver,
kidney, eggs, milk and milk fat and 45μg/kg for body fat. The limits of quantification of the methods used for salmon muscle and skin are 15 and 14μg/kg, respectively.

- The maximum concentration of total residues of [14C]deltamethrin in cattle and poultry was 37μg (<7% of the ADI).
- The concentrations of total residues and residues of the parent drug in salmon muscle and skin were less than twice the limit of quantification at all times. The Committee extended the MRL to all Salmonidae even though the metabolism of deltamethrin in this family was not investigated.
- The residues other than the parent drug may be assumed not to have the neuronal or toxicological activity of deltamethrin. Many of the residues in liver and kidney are the products of extensive metabolism and a large proportion of those in liver are non-extractable, i.e. bound residues.

The Committee took account of the MRLs recommended by the 1990 Joint FAO/WHO Meeting on Pesticide Residues and recommended the same MRLs for liver, kidney and fat. The Committee noted that the concentrations of residues in muscle, milk and eggs are less than twice the limit of quantification of the analytical methods used and therefore recommended MRLs based on the sensitivity of the methods. These “guidance MRLs” are 30μg/kg for muscle in cattle, sheep, chickens and salmon and for cows’ milk and chickens’ eggs, expressed as parent drug. Since these values are “guidance MRLs”, they should not be used to calculate the theoretical maximum daily intake (see Table 7).

### Table 7

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Recommended MRL (μg/kg)</th>
<th>Estimate of total residues (μg/kg)</th>
<th>Theoretical maximum daily intake (μg deltamethrin equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Liver</td>
<td>50</td>
<td>250–1250&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25–125</td>
</tr>
<tr>
<td>Kidney</td>
<td>50</td>
<td>1667&lt;sup&gt;d&lt;/sup&gt;</td>
<td>53</td>
</tr>
<tr>
<td>Fat</td>
<td>500</td>
<td>833&lt;sup&gt;e&lt;/sup&gt;</td>
<td>42</td>
</tr>
<tr>
<td>Milk</td>
<td>30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Eggs</td>
<td>30&lt;sup&gt;h&lt;/sup&gt;</td>
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<td>NA</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>150–250</td>
</tr>
</tbody>
</table>

NA: Not applicable, as the residue concentrations are too low to be included in the calculation.

<sup>a</sup> Based on a daily intake of 300g of muscle, 100g of liver, 5g each of kidney and fat, 1.5kg of milk and 100g of eggs.

<sup>b</sup> “Guidance MRL”, set at twice the limit of quantification of the analytical method.

<sup>c</sup> The marker residue accounted for 4–20% of the total residues in liver.

<sup>d</sup> The marker residue accounted for 3% of the total residues in kidney.

<sup>e</sup> The marker residue accounted for 60% of the total residues in fat.
From the above recommended MRLs, the theoretical maximum daily intake would be 150–250μg (Table 7), which is equivalent to 25–42% of the maximum ADI.

The MRLs for cattle and chicken tissues were extended to sheep muscle, liver, kidney and fat and salmon muscle.

3.4.2 Phoxim

Phoxim is an organophosphorus insecticide used for topical treatment of cattle, sheep, goats and pigs. It has not previously been evaluated by the Committee. It was evaluated toxicologically by the 1982 and 1984 Joint FAO/WHO Meetings on Pesticide Residues (19, 21). The 1984 Joint Meeting established an ADI of 0–1μg/kg of body weight on the basis of inhibition of plasma cholinesterase activity. Phoxim was evaluated by the Committee at its present meeting at the request of the Codex Committee on Residues of Veterinary Drugs in Foods. MRLs based on horticultural use have been recommended by several Joint FAO/WHO Meetings on Pesticide Residues (21–24); however, as the sponsor has indicated that it does not intend to continue to support horticultural use of phoxim, the MRLs recommended by the Codex Committee on Pesticide Residues for the plant protection uses of phoxim are likely to be withdrawn (15). Data were available on the veterinary use of phoxim in a number of food animal species, including cattle, pigs, sheep, goats and rabbits. Most of the studies, however, were not conducted according to current standards of good laboratory practice. For veterinary use, phoxim is usually administered as a spray or dip at a concentration of 500mg/l or as a pour-on formulation at a concentration of 7.5% (w/v).

Toxicological data

The Committee considered the results of studies on the pharmacokinetics, metabolism, acute, short-term and long-term toxicity, carcinogenicity, genotoxicity, reproductive toxicity and delayed neurotoxicity of phoxim. Although most of the studies did not meet current standards for study protocol and conduct, they did provide satisfactory information for evaluation of the safety of the compound.

After oral administration of radiolabelled phoxim to mice, rats and pigs, the radiolabel was rapidly and almost completely absorbed and was rapidly taken up into the major organs and tissues. The major route of elimination in mice, rats and pigs was the urine, while faecal excretion was of minor importance. In rats, there was also some indication of biliary excretion. In rats and pigs, elimination was rapid (>80% via the urine within 24 hours) and was virtually complete within 2–3 days. In mice, elimination of the radiolabel was somewhat
slower, apparently because of retention of phosphate-derived metabolites in the urinary bladder. Although there were some qualitative and quantitative differences in metabolism between mice, rats and pigs, the main routes of metabolism were the same, involving de-ethylation, hydrolysis of the phosphorus ester bond (either before or after oxidative desulphuration to yield PO-phoxim), and conjugation of the resulting cyanobenzaldehyde. Despite the presence of products that were assumed to have arisen from PO-phoxim, this substance was not detected in mice, rats or pigs. Hence, if formed, it must be a very short-lived intermediate in mammals.

Animal species differ in their sensitivity to single oral doses of phoxim. The LD₅₀ values ranged from 20 to 40 mg/kg of body weight in chickens, from 250 to >1200 mg/kg of body weight in guinea-pigs, rabbits, cats and dogs, and from 1200 to 10000 mg/kg of body weight in mice and rats. Thus, phoxim is of only low to moderate acute oral toxicity in mammalian species.

The short-term toxicity of phoxim was evaluated after oral administration to mice, rats, dogs and rhesus monkeys. In two dose range-finding studies of 6 and 8 weeks’ duration, phoxim was administered to mice in their diet at 0.5–750 mg/kg of feed (equal to 0.28–510 mg/kg of body weight per day). In these studies, phoxim had effects on the liver (increased weight and hepatocyte alterations indicative of an adaptive response) at 30 mg/kg of feed and above, and on the kidney (increased weight) at 150 mg/kg of feed and above. Inhibition of plasma cholinesterase activity was observed at and above 5 mg/kg of feed, while inhibition of erythrocyte and brain cholinesterase activity was observed only at 750 mg/kg of feed. On the basis of a slight increase in liver weight in female mice, the overall NOEL in these studies was 5 mg/kg of feed, equal to 3.1 mg/kg of body weight per day. The Committee did not consider inhibition of plasma cholinesterase activity to be of toxicological significance.

Studies were carried out in rats which received phoxim by gavage at doses of 2–50 mg/kg of body weight per day for 21 or 30 days, or in the diet at 4–500 mg/kg of feed (equivalent to 0.4–50 mg/kg of body weight per day) for 3 months. In two studies in which phoxim was administered by gavage, cholinesterase activity in plasma, erythrocytes and brain tissue was inhibited by ≥20% at doses of 2, 5 and 50 mg/kg of body weight and above, respectively. In the two studies in which phoxim was administered in the diet, signs of cholinergic poisoning were observed at 500 mg/kg of feed, organ weight changes at 120 mg/kg of feed and above, and inhibition of cholinesterase activity in plasma at 15 mg/kg of feed and above and in erythrocytes at 40 mg/kg of feed and above. The overall NOEL in the studies involving dietary
administration was 12 mg/kg of feed, equivalent to 1.2 mg/kg of body weight per day, on the basis of inhibition of cholinesterase activity in erythrocytes (in the absence of data on brain cholinesterase activity).

Three 3-month studies in dogs were reported, in which phoxim was administered in the diet at concentrations ranging from 0.3 to 1000 mg/kg of feed, equivalent to 0.0075 to 25 mg/kg of body weight per day. In these studies, treatment with phoxim at 200 and 1000 mg/kg of feed resulted in changes in the weights of the gonads and liver and in the activities of alkaline phosphatase and lactate dehydrogenase in plasma, in inhibition of erythrocyte cholinesterase activity, in weight loss (females only), and in signs of cholinergic poisoning. Inhibition of plasma cholinesterase activity was observed at 1 mg/kg of feed and above. Brain cholinesterase activity was not determined in these studies. The overall NOEL was 50 mg/kg of feed, equivalent to 1.3 mg/kg of body weight per day, on the basis of inhibition of erythrocyte cholinesterase activity (in the absence of data on brain cholinesterase activity) and increased plasma alkaline phosphatase activity.

Rhesus monkeys received phoxim by gavage for 6 months at a dose of 0.2, 0.65 or 2 mg/kg of body weight per day. Apart from marked inhibition of plasma cholinesterase activity at doses at and above 0.2 mg/kg of body weight per day and very slight inhibition of erythrocyte cholinesterase activity at 2 mg/kg of body weight per day, phoxim had no effect. In the absence of data on brain cholinesterase activity, the NOEL was 0.65 mg/kg of body weight per day on the basis of slight inhibition of erythrocyte cholinesterase activity.

In a 24-month carcinogenicity study, phoxim was administered in the diet at concentrations of 1, 5, 150 or 450 mg/kg of feed (equal to 0.47, 2.4, 67 or 200 mg/kg of body weight per day) to mice of a strain known to be highly susceptible to the development of liver tumours. Plasma, erythrocyte and brain cholinesterase activities were decreased at 150 mg/kg of feed and above. The body weights of males were increased at 150 and 450 mg/kg of feed; females were also affected at the highest dose. Signs of effects on the liver (including changes in weight, in plasma cholesterol and total bilirubin levels, and in plasma alanine aminotransferase and alkaline phosphatase activities) were evident at 450 mg/kg of feed. At this dose, there were also increased incidences of non-neoplastic histological changes in the livers of males and of hepatocellular adenomas in females. At 150 mg/kg of feed, the only effect on the liver was increased plasma cholesterol concentrations in animals of each sex. The NOEL was 5 mg/kg of feed, equal to 2.4 mg/kg of body weight per day, on the basis of increased plasma cholesterol concentrations and inhibition of brain cholinesterase ac-
tivity. The increase in the incidence of adenomas observed in females at the highest dose was thought to be a consequence of the hepatoproliferative effect of the compound.

Long-term toxicity studies were conducted in rats and dogs, which received phoxim in the diet at 15–375 mg/kg of feed (equal to 0.8–27 mg/kg of body weight per day) and 0.1–750 mg/kg of feed (equivalent to 0.0025–19 mg/kg of body weight per day), respectively, for 24 months.

In rats dosed at 375 mg/kg of feed, reductions were seen in food intake in males, in body weight in females, and in plasma glutamate dehydrogenase activity, total bilirubin level, the weights of the heart, lungs, spleen and adrenals, and plasma, erythrocyte and brain cholinesterase activity in animals of each sex. Decreased plasma and erythrocyte cholinesterase activity and adrenal weights were also observed in rats at 75 mg/kg of feed. There were no histopathological differences between control and treated animals, nor was there any difference in the incidence of tumours. The NOEL was 15 mg/kg of feed, equal to 0.8 mg/kg of body weight per day, on the basis of lowered adrenal weights at higher dose levels.

Male and female dogs given phoxim at 750 mg/kg of feed had liver damage, as shown by increased liver weights and plasma alanine aminotransferase and alkaline phosphatase activities, decreased serum cholesterol level, and histopathological alterations in hepatocytes. Plasma, erythrocyte and brain cholinesterase activities were also reduced in animals of each sex at this dose. In addition, male dogs showed clinical signs of toxicity and decreased body-weight gain. Reductions in plasma and erythrocyte cholinesterase activity were also observed in male and female dogs at 15 mg/kg of feed. The NOEL was 15 mg/kg of feed, equivalent to 0.38 mg/kg of body weight per day, on the basis of effects on the liver and inhibition of brain cholinesterase activity.

Phoxim has been tested in vitro for its ability to induce reverse mutations in Salmonella typhimurium and Saccharomyces cerevisiae, DNA damage in Bacillus subtilis, and chromosomal aberrations in human lymphocytes. It has also been tested in vivo for its ability to induce micronucleus formation, chromosomal aberrations and dominant lethal mutations in mice. Cytogenetic alterations were found in human lymphocytes in vitro at a cytotoxic dose in the absence of exogenous metabolic activation. The results of all other tests were negative. On the basis of these data and the results of the long-term assays in rodents, the Committee concluded that phoxim is not genotoxic and is unlikely to have carcinogenic potential in humans.
In a three-generation study of reproductive toxicity, rats were given phoxim in the diet at a concentration of 0, 15, 75 or 375 mg/kg of feed (equivalent to 0, 0.75, 3.8 and 19 mg/kg of body weight per day). The only effect seen was a slight reduction in the number of pups in the second litter of the third generation that survived after 4 weeks’ lactation from dams receiving 375 mg/kg of feed. On the basis of this effect, the NOEL was 75 mg/kg of feed, equivalent to 3.8 mg/kg of body weight per day.

In a study of developmental toxicity in rats given a dose of 0, 30, 100 or 300 mg/kg of body weight per day orally on days 6–15 of gestation, phoxim was toxic to the dams, retarding body-weight gain during treatment at the highest dose. It did not cause embryotoxicity, fetotoxicity or teratogenicity at any dose. The NOEL for maternal toxicity was 100 mg/kg of body weight per day, on the basis of reduced body-weight gain. The NOEL for developmental toxicity was 300 mg/kg of body weight per day, the highest dose tested.

A study of developmental toxicity was conducted in rabbits given phoxim at a dose of 0, 12, 36 or 72 mg/kg of body weight per day orally on days 6–18 of gestation. At the highest dose, phoxim increased the rate of embryonic resorption and decreased fetal body weights. This dose was also toxic to the dams, which showed signs of toxicity and marked decreases in food consumption and in body-weight gain. There was no indication of teratogenicity at any dose. The NOEL for maternal toxicity was 36 mg/kg of body weight per day, on the basis of reduced food consumption and body-weight gain. The NOEL for developmental toxicity was also 36 mg/kg of body weight per day, on the basis of decreased fetal body weight and an increased rate of embryonic resorption.

In a study of delayed neurotoxicity, hens protected by the antidote atropine received phoxim orally at a dose of 50 mg/kg of body weight, which was repeated after 21 days. During the observation period of 42 days, the hens showed only transient signs of toxicity and no abnormal signs or behaviour from day 26 after the second dose. At the end of the study, no paralysis was present, and no histological evidence of peripheral neuropathy or demyelination was observed. The Committee concluded that phoxim does not induce delayed neurotoxicity in hens. Although the effect of phoxim on neuropathy target esterase has not been investigated in hens, the Committee concluded that such a study was not necessary in view of the negative results obtained in adequately conducted assessments of the capacity of phoxim to induce delayed neuropathy.

The Committee established an ADI of 0–4 μg/kg of body weight for phoxim, based on the NOEL of 0.38 mg/kg of body weight per day for
effects on the liver and inhibition of brain acetylcholinesterase activity in the 2-year toxicity study in dogs and a safety factor of 100. This ADI differs from that established by the 1984 Joint FAO/WHO Meeting on Pesticide Residues, as the Committee concluded that inhibition of plasma cholinesterase activity is not a relevant end-point for risk assessment. The Joint Meeting is now of a similar opinion (24).

A toxicological monograph was prepared.

**Pharmacokinetic and metabolic data**

The metabolism and excretion of phoxim-methyl were studied in one lactating dairy cow after oral administration of capsules containing [14C]phoxim-methyl at 0.2 mg/kg of body weight. In the initial study, the cow received a single dose; subsequently, it received the same daily dose on 5 consecutive days. Phoxim-methyl was rapidly absorbed and the radiolabel was excreted mainly in urine. Hippuric acid was the major urinary metabolite, indicating a complex metabolic pathway. Residues in milk accounted for 0.2% of the recovered radiolabel and had declined by 90% 48 hours after treatment.

Two pigs each received a capsule containing [14C]phoxim at a dose of 5 mg/kg of body weight orally. Phoxim was rapidly absorbed. Most of the radiolabel was excreted in urine, as had been observed in laboratory animals, and the major urinary metabolite (almost 90%) was cyanobenzaldoxime, which was excreted as its glucuronic acid conjugate. Values for pharmacokinetic parameters such as total clearance, renal clearance and mean residence time of the parent compound could not be derived from these data.

Tissues from the cow and the pigs in the above studies were analysed for total radiolabelled residues, but the distribution of residues in the various tissues did not reflect that observed in studies in which unlabelled drug was applied topically. These data were therefore not summarized.

**Residue data**

Residue-depletion studies with [14C]phoxim were conducted in cattle and pigs, but were of limited value as the veterinary formulations and routes of administration were not used. In one study in cattle, the methyl analogue was used in which the oxygen-bonded ethyl groups in phoxim were replaced by methyl groups.

**Cattle.** The depletion of phoxim in cattle was evaluated in a study that did not conform to good laboratory practice, in which four yearling cattle weighing 250–370 kg were sprayed twice with a formulation containing phoxim at 1000 mg/l (twice the recommended concentration), 8 days apart. Two cattle were slaughtered at 14 days and two at
28 days after the second treatment. Insufficient data were provided in the report to allow calculation of the dose rate in milligrams per kilogram of body weight. The highest concentrations of residues were found in fat samples 14 days after the second treatment (0.32 and 0.37 mg/kg, respectively); after 28 days the concentration had declined to 0.02 mg/kg in both animals. No residues were detected in liver, kidney or muscle samples.

Three studies that did not conform to the principles of good laboratory practice were conducted in lactating cows to assess the concentrations of residues of phoxim in milk. In the first study, three dairy cows each received two treatments, 8 days apart, with a spray formulation containing phoxim at 1000 mg/l (twice the recommended concentration). The concentrations of residues in milk were 0.22–0.42 mg/kg 12 hours after the second treatment and below the limit of detection (0.002 mg/kg) after 7 days. In the second study, three lactating cows were each sprayed twice at an 8-day interval with a dip formulation containing phoxim at 500 mg/l (the recommended concentration). The concentrations of residues in milk after the second treatment depleted rapidly, from 0.08–0.21 mg/kg at 12 hours to 0.004–0.010 mg/kg by day 3. In the third study, in which five cows each received a single treatment with a spray containing phoxim at 500 mg/l, the concentrations of residues in milk declined rapidly, from 0.04–0.08 mg/kg at 12 hours to below the limit of detection (0.002 mg/kg) at day 2 and in subsequent milkings. The same cows were then given two treatments with the same formulation at an interval of 6 days. The concentrations of residues in milk were 0.002–0.004 mg/kg 12 hours after the second treatment and were below the limit of detection on day 3 and in samples taken subsequently.

**Pigs.** In a study conducted according to good laboratory practice, 22 pigs were treated twice with a pour-on formulation of phoxim at 30 mg/kg of body weight (the recommended dose), 14 days apart. Tissue samples were collected 7, 14, 21, 28 and 35 days after the second treatment and were analysed by an HPLC method with a limit of detection of 0.002–0.003 mg/kg, depending on the tissue. No residues of phoxim were detected in muscle, kidney or liver at day 7. The concentrations of residues were highest in fat and were similar in samples from the site of application and abdomen (0.49 and 0.51 mg/kg, respectively, on day 7 and 0.22 mg/kg in both tissues on day 14). After 35 days, phoxim residues were slightly more persistent in fat from the site of application than in fat from other body areas, although the concentrations in all samples were near or below the limit of quantification (0.01 mg/kg). In skin samples taken from the site of application, the concentrations of phoxim residues declined
from 0.35 mg/kg at day 7 to 0.05 mg/kg at day 21 and were near or below the limit of detection at day 35.

Three residue-depletion studies that were not conducted according to good laboratory practice were carried out in pigs. In the first study, four pigs were sprayed twice with a 1000 mg/l solution of phoxim (twice the recommended concentration), 8 days apart. Two pigs were slaughtered at 14 days and two at 28 days after the second treatment. The concentrations of residues in fat were 0.04 and 0.05 mg/kg at day 14 and <0.01 and 0.13 mg/kg at day 28, respectively. No residues were detectable in liver, kidney or muscle samples (limit of detection, 0.01 mg/kg). In the second study, six pigs received a spray application of phoxim at 500 mg/l (the recommended concentration), which was repeated after 1 week. Tissue samples were collected 7 and 14 days after the second application. No phoxim residues were detected in fat samples (limit of detection, 0.05 mg/kg). In the third study, nine pigs received a pour-on application of phoxim along the dorsal midline at the recommended dose of 30 mg/kg of body weight, which was repeated after 14 days. Tissue samples were collected 7, 14 and 28 days after the second application. No residues were detectable in liver, kidney or muscle samples (limit of detection, 0.01 mg/kg). In fat samples, the concentrations declined from 0.3–1.1 mg/kg at day 7 to 0.06–0.09 mg/kg at day 28.

**Sheep.** Five studies that did not comply with good laboratory practice were reported in which sheep were treated with spray or “plunge-dip” veterinary formulations of unlabelled phoxim.

In one study, nine sheep were sprayed twice at an interval of 8 days with a solution containing phoxim at 1000 mg/l (twice the recommended concentration). Samples of muscle, liver, kidney and fat were collected 7, 14 and 21 days after the second treatment and analysed by a gas chromatographic method with a limit of detection of 0.01 mg/kg. The highest concentrations of residues were found in fat, declining from 2.4–2.8 mg/kg at day 7 to 0.11–0.62 mg/kg at day 21. In muscle, the concentrations were 0.03–0.07 mg/kg at day 7 and below or near the limit of detection in subsequent samples (<0.01–0.04 mg/kg). In kidney, the concentrations were <0.01–0.07 mg/kg at day 7 and below the limit of detection at day 21. No residues were detected in liver samples.

In a second study, nine sheep were sprayed twice at an interval of 8 days with a solution containing phoxim at 500 mg/l (the recommended concentration) or 1000 mg/l. Tissue samples were collected 14 and 21 days after the second treatment with the recommended dose and 21 days after the second treatment with the higher dose. No residues were detected in muscle, liver or kidney from treated sheep. In fat
samples, the concentrations were 0.17–0.66 mg/kg at 14 days and 0.03–
0.17 mg/kg at 21 days after treatment with 500 mg/l and 0.20–0.52 mg/
kg at 21 days after treatment with 1000 mg/l.

In a larger study, three groups of six ewes weighing 35–47 kg were
plunged for 1 minute into tanks containing solutions of phoxim at
1000, 2000 or 3000 mg/l (twice, four times and six times the recom-
mended concentration, respectively). Tissue samples were collected
from all the treated animals 21 and 45 days after treatment. No
residues of phoxim were found in muscle, liver or kidney samples
from any of the treated animals (limit of detection, 0.05 mg/kg).
At day 45, residues were not detected in fat after treatment with
1000 mg/l, but concentrations of <0.05–0.6 mg/kg were found after
treatment with 2000 mg/l and <0.05–1.0 mg/kg after 3000 mg/l.

In another study, 12 sheep were plunged into a dip containing a
500 mg/l solution of phoxim (the recommended concentration) or
were sprayed twice at an interval of 7 days with a 1000 mg/l solution.
Samples of perirenal and omental fat were collected 28 and 35 days
after the dip or the second spray treatment and analysed for both
phoxim and its expected oxygen metabolite (limit of detection,
0.05 mg/kg for both compounds). No traces of the metabolite were
found in any samples. At day 35, the concentrations of phoxim resi-
dues in fat were below the limit of detection in the sheep that had
been dipped, but were <0.05–0.10 mg/kg in the animals treated with
the spray.

In a study in which six lactating ewes were plunged into a dip contain-
ing phoxim at 500 mg/l, no residues were detected in milk samples
taken on 3 consecutive days after exposure and analysed by liquid
chromatography (limit of detection, 0.03 mg/kg).

Goats. In a study that did not comply with good laboratory practice,
six male and three female goats weighing 22–45 kg were sprayed
twice, at an interval of 8 days, with a solution containing unlabelled
phoxim at 1000 mg/l (twice the recommended concentration).
Samples of muscle, liver, kidney and fat were collected 7, 14 and 21
days after the second treatment from the nine goats and one un-
treated control animal and analysed by an HPLC method (limit of
detection, 0.01 mg/kg). The highest concentrations were found in fat
samples, declining from 0.53–0.85 mg/kg at day 7 to 0.08–0.10 mg/kg at
day 21. In muscle, the concentrations declined from 0.01–0.05 mg/kg
at day 7 to <0.01–0.03 mg/kg at day 21. Phoxim was detected in liver
and kidney from one goat and in kidney from a second goat at the
limit of detection on day 7; no residues could be detected in liver and
kidney samples from other goats at this sampling time or in these
tissues at 14 or 21 days.
Rabbits. In a study that was not conducted according to good laboratory practice, two groups of 16 rabbits weighing 3 kg (mean) were treated on the ear with a lotion containing 0.1% unlabelled phoxim or were sprayed with a solution providing an estimated daily dose of 1 mg of phoxim per rabbit for 7 days. Two animals from each group were killed at 1, 3, 5, 7, 10, 15, 21 and 28 days and their tissues were collected for residue analysis. The concentrations of residues depleted rapidly after either treatment to 0.008–0.014 mg/kg in liver, kidney and fat. The residues in muscle were below the limit of detection of the analytical method (0.005 mg/kg) at day 7.

Analytical methods
Phoxim residues can be analysed by gas chromatography with a phosphorus-specific detector (alkali flame or flame photometric), with limits of detection of 0.01–0.05 mg/kg for all tissues in the studies reported. Full data on validation of this method were not available, but it has been used successfully in residue-depletion studies in several laboratories. Greater detail was provided on the validation of an HPLC method for analysis of porcine tissues, which has a limit of quantification of 0.01 mg/kg. Most of the HPLC methods reported involve tissue homogenization, extraction with acetonitrile (hexane is used for fat), acetonitrile–hexane partitioning, purification with alumina or silica gel solid-phase extraction and chromatographic analysis. The range of analytical recoveries reported is 70–100% for all species and tissues studied. The methods appear to meet regulatory needs as the results were reproducible in several studies carried out in various laboratories by different analysts and have adequate sensitivity and recovery. The instrumentation and techniques required for the methods are common in most laboratories for residue analysis.

Maximum Residue Limits
In recommending MRLs for veterinary use of phoxim, the Committee considered the following factors:

- The Committee has established an ADI of 0–4 μg/kg of body weight, which is equivalent to a maximum ADI of 240 μg for a 60-kg person.
- As phoxim is not currently proposed for use in crop protection, the entire ADI is available for veterinary use.
- The proportion of the total residues accounted for by the marker residue could not be determined from the available studies, and the data were inadequate for a complete assessment of the toxicity of the metabolites of phoxim. As no other residues were detected or identified in the residue-depletion studies, the marker residue is assumed to be the parent drug.
• Residue-depletion studies with unlabelled drug indicate that fat is the target tissue in all species.
• The 1983 Joint FAO/WHO Meeting on Pesticide Residues recommended an MRL of 0.2 mg/kg for phoxim in cattle meat (fat). MRLs of 0.01 mg/kg for cows' milk and 0.05 mg/kg for sheep meat (fat) were recommended in 1984 and 1988, respectively.
• Residue-depletion studies demonstrate that the concentrations of residues in treated animals are very low immediately after treatment and subsequently in all tissues except fat. The MRLs recommended for muscle, liver and kidney are based on twice the limit of quantification of the available analytical methods (0.01 mg/kg).
• The MRLs for fat are based on the study carried out in pigs according to good laboratory practice, using an estimate based on the upper 99% confidence limit of the mean. They are extended to other species in which similar results were obtained in residue-depletion studies.
• The MRLs for sheep can be extended to goats, as similar results were obtained in studies in the two species.
• Suitable analytical methods are available for determining phoxim at the recommended MRLs in edible tissues.

On the basis of the maximum observed concentrations of residues in the edible tissues of cattle, pigs, sheep and goats, and in milk from cows treated with phoxim by the recommended topical routes of administration, the Committee recommended the following temporary MRLs: 50 μg/kg for muscle, liver and kidney and 400 μg/kg for fat, expressed as parent compound, in cattle, pigs, sheep and goats; 10 μg/kg for whole cows’ milk, expressed as parent compound.

From the above MRLs, the theoretical maximum daily intake of residues of phoxim is 58 μg, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat, and 1.5 kg of milk (Annex 1, reference 85).

The Committee requires the following information for evaluation in 2002:

1. The results of studies using radiolabelled phoxim to determine the proportion of the total residues accounted for by the marker residue in ruminants and pigs following topical application of the formulated product.
2. The results of residue-depletion studies conducted in accordance with good laboratory practice in cattle and sheep given the currently recommended treatments.
3. Validation of the available analytical methods for the detection of phoxim residues in tissues of cattle, sheep and goats, and in cows’ milk.

3.5 Production aids

3.5.1 Estradiol-17β, progesterone and testosterone

Estradiol-17β and progesterone are hormones produced primarily by the developing follicle and corpus luteum, respectively, of the ovary in adult females. Testosterone is a hormone produced primarily by the testes. Estradiol-17β in combination with progesterone or testosterone propionate is administered to cattle to increase their rate of weight gain and to improve their efficiency of feed conversion into edible tissues. Estradiol-17β, progesterone and testosterone were reviewed by the Committee at its thirty-second meeting (Annex 1, reference 80), when establishment of an ADI and MRLs was considered “unnecessary”. Estradiol-17β, progesterone and testosterone were re-evaluated at the present meeting to take into consideration any data that had been generated since their previous review and to make a quantitative estimate of the amounts that could be consumed safely.

Toxicological data

Estradiol-17β. The Committee considered published data from studies on the oral bioavailability, metabolism, short-term toxicity, reproductive toxicity, genotoxicity and long-term toxicity/carcinogenicity of exogenous estrogens. Numerous reports on studies of the use of exogenous estrogens in women were considered, as were studies in experimental animals on the mechanisms of action of estradiol-17β. The extensive database derived from the results of epidemiological studies of women taking oral contraceptive preparations containing estrogens or postmenopausal estrogen replacement therapy was also used to evaluate the safety of estradiol-17β.

Estradiol-17β is an 18-carbon steroid and the most potent of the natural estrogens. Estradiol-17β exerts its biological effects largely by receptor-mediated mechanisms, as it binds with high affinity and high specificity to intracellular receptors. Binding of estradiol-17β directly affects the growth and development of the reproductive tract and breasts, and the appearance of secondary sex characteristics. In non-pregnant females, estradiol-17β acts synergistically with progesterone during the luteal phase of the menstrual cycle to initiate events leading to a new cycle. Continued estradiol-17β production is essential for the normal growth and development of the fetus. In addition to its effects on reproductive tissues, estradiol-17β is an important metabolic hormone, particularly because of its effects on the cardiovascular, skeletal and gastrointestinal systems.
In general, estradiol-17β is inactive when given orally because it is inactivated in the gastrointestinal tract and liver. However, fine-particle formulations of estradiol-17β are effective when given orally and are used therapeutically. The bioavailability of a single 4-mg dose of fine-particle estradiol-17β administered orally to 14 young women was 5% of that of a dose administered intravenously. At least 60% of the absorbed dose appeared in the serum as estrone and estrone sulfate and was available as part of the endogenous pool.

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

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

In studies of developmental toxicity in rats, all embryos were re-absorbed in dams which received estradiol-17β subcutaneously at a dose equivalent to 25 mg/kg of body weight on day 10 of pregnancy. In an interim report of a multigeneration study of reproductive toxicity in female rats, estradiol-17β was administered in the feed at a dose of 0.003, 0.17, 0.69 or 4.1 mg/kg of body weight per day. No viable pups were observed at 0.69 or 4.1 mg/kg of body weight. As the concentration of progesterone was altered at various times in all treated groups, a NOEL was not determined in this study.

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

A normal biological function of estrogens is to increase the number of proliferating cells in the endometrium and breast. This effect is exerted by binding with high affinity to estrogen receptors. These receptors, of which there are several forms, are found in many tissues. In cultured human breast cancer cells containing estrogen receptors, estradiol-17β stimulated growth when added at concentrations of 0.001 nmol and above, with a maximal response at about 0.1 nmol. Estradiol-17β does not stimulate the growth of cultured human breast
cancer cells that do not contain estrogen receptors. At higher concentrations, estrogens also stimulate cell proliferation in rat liver in vivo and in vitro. Any factor that increases mitotic activity reduces the time available for repair of DNA damage before the next cell division. An agent that causes cell proliferation may not, however, induce the mutagenic events that are required for neoplasia. If receptor-mediated stimulation of cell growth is an important mechanism in the induction of neoplasia by estradiol-17β, late-stage carcinogenic activity would be expected to predominate. Experimental studies in rodents in which estradiol-17β was administered in conjunction with known carcinogens support this mechanism, as do observations of an increased incidence of cancer among women taking postmenopausal hormone replacement therapy. In long-term studies of carcinogenicity in animals, reviewed at the thirty-second meeting (Annex 1, reference 80), oral and parenteral administration of estradiol-17β increased the incidence of tumours only in hormone-dependent tissues, including the kidneys of male Syrian hamsters. The Committee concluded that the carcinogenicity of estradiol-17β is most probably a result of its interaction with hormonal receptors.

The most common uses of estrogens in humans are for oral contraception and postmenopausal replacement therapy. For oral contraception, the xenobiotic estrogen ethinylestradiol is usually used. Fine-particle estradiol-17β and conjugated equine estrogen preparations are commonly used in postmenopausal hormone replacement therapy. In a study involving 23 healthy postmenopausal women given four courses of 0.3, 0.62, 1.2 or 2.5 mg of conjugated equine estrogens per day for 2 weeks followed by no medication for 3 weeks, the NOEL was 0.3 mg/day on the basis of changes in the serum concentrations of corticosteroid-binding globulin (CBG; also known as transcortin). These results indicate that there is a threshold level for estrogen administered orally, below which there is no increase in serum concentrations of CBG. In a second study involving 23 healthy postmenopausal women receiving various estrogen preparations orally, 0.3 mg/day of conjugated equine estrogens or fine-particle estradiol-17β had no effect on the serum concentrations of follicle-stimulating hormone, angiotensinogen, sex-hormone-binding globulin or CBG. Thus, 0.3 mg/day, equivalent to 5 μg/kg of body weight per day, was the NOEL for these hormonal effects of estradiol-17β. A further indication that this is the NOEL is that 0.3 mg/day of estradiol-17β administered orally to women did not relieve symptoms of the menopause.

A study of approximately 7700 infants whose mothers had reported taking oral contraceptives while pregnant showed no evidence that estrogens present a teratogenic hazard.
Because of differences in the pharmacokinetics and pharmacodynamics of natural and xenobiotic estrogen preparations, the Committee concluded that data on the use of estrogens for postmenopausal replacement therapy are more appropriate for evaluating the safety of estradiol-17β than data on their use for oral contraception.

Epidemiological studies on women who took estrogens, either alone or in combination with progestogens and androgens, showed that the risks for cancers at most sites were unaffected; however, the risks for cancers of the endometrium and breast were increased. In a meta-analysis of 30 epidemiological studies of women who had at any time used postmenopausal estrogen-only therapy, the relative risk for endometrial cancer was 2.3 (95% confidence interval, 2.1–2.5). The relative risk for endometrial cancer among women who had taken postmenopausal estrogen-only therapy for more than 10 years was 9.5 (95% confidence interval, 7.4–12). The addition of progestogens to postmenopausal estrogen therapy reduces the excess risk substantially, although it may not be eliminated completely. In a review of 51 epidemiological studies of women taking hormonal replacement therapy, the relative risk for breast cancer was increased by a factor of 1.023 (95% confidence interval, 1.011–1.036) for each year of use. These estimates of relative risk are based on the results of studies of women who used postmenopausal estrogen replacement therapy preparations containing either conjugated equine estrogens (average dose, 0.625 mg/day) or estradiol-17β (1–2 mg/day). Overall, the available data suggest that the increased incidence of cancers of the breast and endometrium observed among women receiving postmenopausal estrogen replacement therapy is due to the hormonal effects of estrogens.

The Committee established an ADI of 0–0.05 µg/kg of body weight on the basis of the NOEL of 0.3 mg/day (equivalent to 5 µg/kg of body weight per day) in studies of changes in several hormone-dependent parameters in postmenopausal women. A safety factor of 10 was used to account for normal variation among individuals, and an additional factor of 10 was added to protect sensitive populations.

Progesterone. The Committee considered published data from studies on the oral bioavailability, metabolism, short-term toxicity, reproductive toxicity, genotoxicity, and long-term toxicity/carcinogenicity of progesterone. Numerous reports of studies on progesterone in humans were considered. In addition, the extensive database derived from studies of women taking progestogens as a component of oral contraception, as injectable progestogen-only contraception, and in postmenopausal hormone replacement therapy was used to support the safety evaluation.
Progesterone is a 21-carbon steroid that is the only important natural progestogen. Its normal role is to prepare the uterus for implantation and to maintain pregnancy. Continued production of progesterone is necessary to maintain pregnancy. The production of progesterone by the corpus luteum is controlled by the release of luteinizing hormone from the pituitary gland. In non-pregnant females, an elevated concentration of progesterone inhibits the cyclic release of luteinizing hormone, and higher levels inhibit the production of follicle-stimulating hormone. Progesterone opposes some of the effects of estrogens, but prior stimulation with estrogens is essential for progesterone to elicit biological responses. It exerts its biological effects through receptor-mediated mechanisms, as it binds with high affinity and high specificity to an intracellular receptor protein. Binding of progesterone activates the receptor, resulting in the activation of specific genes.

Less than 10% of progesterone is bioavailable after oral administration, as it is inactivated in the gastrointestinal tract and/or liver.

The acute toxicity of progesterone after oral administration is low.

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

Mouse pups given subcutaneous injections of 100 µg of progesterone per day for 5 consecutive days (equivalent to 200 mg/kg of body weight per day), beginning 36 hours after birth and observed for up to 1 year had an increased incidence of mammary gland tumours. Female rabbits given an average dose of 8 mg/kg of body weight intramuscularly every second week for 2 years developed endometrial cysts, which were sometimes associated with atypical hyperplasia, but no significant changes were observed in other tissues.

No multigeneration study of the reproductive toxicity of progesterone was available. Developmental toxicity was not seen in studies in rats and rhesus monkeys. Rats dosed at 5–25 mg/kg of body weight on days 14–19 of gestation delivered pups that showed no evidence of masculinization. Rhesus monkeys given progesterone at 5 mg/kg of
body weight intramuscularly on 5 days per week beginning at 1 month of gestation and continuing to birth delivered healthy offspring with no evidence of abnormalities. The Committee noted that exogenous progesterone has been used to maintain pregnancy, with no evidence of toxicity and with no effect on the normal conclusion of pregnancy.

The most common uses of progestogens in humans are for contraception and postmenopausal hormone replacement therapy. For hormone replacement therapy, synthetic progestogens are usually used either alone or in combination with estrogens. In a study designed to explore anti-proliferatory and secretory end-points in the endometrium, women received fine-particle progesterone at 300 or 600 mg/day (300 mg twice a day) orally for 2 weeks after pretreatment with estrogens for 30 days. The group receiving 300 mg/day showed incomplete conversion of the uterus to full secretory activity, while the group receiving 600 mg/day showed full secretory activity in the uterus. Mitotic activity in the uterus was suppressed in women in the high-dose group. In studies in which women were given progesterone orally at 200 or 300 mg/day for 1 or 5 years, there was no evidence of endometrial hyperplasia or carcinoma. Oral administration of a single dose of 200 mg of fine-particle progesterone (equivalent to 3.3 mg/kg of body weight) to women provided concentrations in blood similar to those found during the luteal phase of the ovulatory cycle. This dose was considered to be the lowest-observed-effect level (LOEL) in humans.

Extensive literature exists on the use of synthetic progestogens in combination with estrogens for oral contraception. While synthetic progestogens differ from natural products in their pharmacokinetics, pharmacodynamics, tissue specificity, and potency, a prominent feature of the synthetic agents is a protective effect against the untoward effects of estrogens. No increase in the risk for cancer at any site was found in women taking only progesterone or other progestogens orally for contraception. When used in combination with estrogens in postmenopausal replacement therapy, progesterone reduced the excess risk for endometrial cancer found with estrogen alone but did not alter the increased risk for breast cancer.

The Committee established an ADI of 0–30 μg/kg of body weight for progesterone on the basis of the LOEL of 200 mg (equivalent to 3.3 mg/kg of body weight) for changes in the uterus. A safety factor of 100 was used to allow for extrapolation from a LOEL to a NOEL and to account for normal variation among individuals.

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

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

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

Testosterone has low bioavailability when given by the oral route, as it is inactivated in the gastrointestinal tract and liver. After oral administration of 25 mg of testosterone to young women, approximately 4% of the dose was found to be bioavailable in plasma.

The acute toxicity of testosterone after oral administration is very low. Few studies have been conducted of the toxicity of testosterone in animals treated by the oral route. Short- and long-term studies in animals demonstrate that its adverse effects are due to its hormonal activity. Therefore, the most sensitive toxicological targets are hormone-sensitive tissues, such as the prostate gland. Six adult male baboons received intramuscular injections of testosterone enanthate to provide a dose equivalent to 8 mg/kg of body weight each week for up to 28 weeks. At the end of the study, histological evidence of non-neoplastic alterations was found in the prostate gland. Female rabbits given an average dose of testosterone equivalent to 6 mg/kg of body weight intramuscularly every second week for 2 years developed endometrial cysts and secretions from the mammary gland. No significant changes were found in tissues from organs other than those of the reproductive system.

In studies of developmental toxicity, testosterone was embryotoxic; in rats, a dose of testosterone equivalent to 25 mg/kg of body weight
administered as subcutaneous implants on day 10 of pregnancy resulted in resorption of all embryos. No multigeneration studies of reproductive toxicity of testosterone have been conducted, as elevated levels of testosterone interfere with normal reproductive function in both males and females.

In mammalian cells, no chromosomal aberrations, mutations or DNA adducts were found following treatment with testosterone alone. The Committee concluded that testosterone has no genotoxic potential. No studies to examine the carcinogenicity of testosterone in experimental animals have become available since the previous evaluation. An increased rate of prostatic cancer has been detected in testosterone-treated rats, which is consistent with the hormonally mediated effects of the drug and its metabolites.

In men, the physiological concentrations of circulating testosterone are 3–10 ng/ml. In women, the concentrations of circulating testosterone are below 1 ng/ml, and most of the circulating testosterone is derived from conversion of dehydroepiandrosterone and androstenedione from adrenal and ovarian sources. Androgens are used therapeutically in men with deficient testicular function to restore normal testosterone levels. The effects of excess androgens, particularly in young boys, include deepened voice, acne and growth of facial hair, while in women hair loss and menstrual irregularities may be seen. In a clinical trial involving five eunuchs, an oral dose of 100 mg/day of a fine-particle formulation of testosterone had no effect on sexual function, while an oral dose of 400 mg/day was effective in restoring full sexual function. Thus, an oral dose of 100 mg/day (equivalent to 1.7 mg/kg of body weight per day) was the NOEL in this study.

In studies in which postmenopausal women received the testosterone analogue, methyltestosterone, alone or in combination with estrogens, oral doses of up to 10 mg/day for over 6 months induced virilizing signs (e.g. acne and hirsutism). The effects were dose- and time-dependent. The Committee noted that methyltestosterone is more potent than testosterone when given orally.

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

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

A combined toxicological monograph on estradiol-17β, progesterone and testosterone was prepared.

**Residue data**

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

The present report is based on data obtained using the products described in Table 8. Most of the studies were conducted to demonstrate the safety of these products when used according to their approved uses. The Committee also reviewed reports of several investigations in which fixed combinations of trenbolone acetate and estradiol-17β were implanted as experimental products.

Since most of the products evaluated were combinations and as the concentrations found in tissues reflect specific kinetic and dynamic properties of the individual products (e.g. negative feedback of endogenous production of certain hormones), the Committee decided to evaluate residues of the administered products rather than the individual hormonal substances (e.g. estradiol).

**Statistical evaluations and intake calculations.** The results of the residue-depletion studies were evaluated statistically. The distribution of residues was described by a number of characteristics, including the mean, standard deviation, geometric mean and median. The Committee noted that it could not be assumed that the hormone concentrations had a normal distribution and that the tissue concentrations were sometimes below the limits of detection or quantification of the analytical methods. It therefore considered the median to be the most stable and convenient parameter on which to base an estimate of the central tendency of the data without excluding any individual result or making specific assumptions about substitute values for results such as “below the limit of quantification”. Appropriate median values (see below) were also used as the basis for calculating theoretical maximum daily intakes.

The objective of the intake calculations was to obtain conservative estimates of the theoretically possible excess dietary intake of persons
Table 8
Composition of hormonal implants used for growth promotion in cattle

<table>
<thead>
<tr>
<th>Product</th>
<th>Target animals</th>
<th>Estradiol-17β</th>
<th>Estradiol benzoate</th>
<th>Testosterone</th>
<th>Testosterone propionate</th>
<th>Progesterone</th>
<th>Trenbolone acetate</th>
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<td>Compudose®</td>
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* Concomitant or sequential use of Implix®/Revalor® is not approved.
* Use of Torelor® is not approved.
who consume large amounts of meat (e.g. 500 g/day) which could be attributed to approved uses of the products reviewed. The calculations were performed in the following stepwise manner:

- The median concentrations of hormone found in the edible tissues of control animals and of treated animals at every given time point of each study were multiplied by the respective values for daily consumption of "meat" that are conventionally used by the Committee in calculating the theoretical maximum daily intake of substances. These values are 300 g of muscle, 100 g of liver, and 50 g each of kidney and fat. The median value of a residue or contaminant in food is the appropriate value to be used if lifetime dietary intake is to be assessed. The procedure used here was chosen because no numerical MRLs were recommended by the Committee.

- The results obtained in this way for muscle, liver, kidney and fat were added to derive a figure for the total intake of residues of estradiol-17β, progesterone and testosterone from 500 g of "meat".

- When data for several times after implantation of the hormones were available, the highest values were used to account for the fact that withdrawal periods have not been established for the use of any of the approved products. When the highest values were not found at the same times with all three hormones, the time at which the highest intake of estrogen was recorded was selected. This is a conservative approach, since the ADI for estradiol-17β is lower than those for progesterone and testosterone. The effect of this selection on the estimates for the other two hormones was negligible.

- In order to estimate the excess intake defined above, the theoretical maximum daily intake calculated for a concurrent untreated control population was subtracted from the figure obtained for treated animals.

The results of all the relevant intake calculations are summarized in Table 9, which also provides information on excess intakes of estrogens, the most relevant group of residues. The degree of underestimation of the "true" theoretical maximum daily intake was determined from information in a study with implants containing 14C-labelled hormones and hormone esters in the same proportions as in the commercial products. On the basis of the total radiolabel found in tissues of animals slaughtered 15 days after implantation of the hormone and the fractions of total residues identified as conjugates, the individual contributions of free and conjugated hormones present in muscle, liver, kidney and fat to the theoretical maximum daily intake can be calculated, as shown in Table 10. These data indicate that the estimated theoretical maximum daily intakes resulting from
<table>
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<tr>
<th>Product</th>
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<th>Treatment group</th>
<th>Theoretical maximum daily intake (ng/person per day)</th>
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<td>Controls, slaughtered on day 360</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Animals implanted on days 0, 118 or 240; slaughtered on day 301</td>
<td>3.7</td>
<td>11</td>
</tr>
<tr>
<td>Product*</td>
<td>Target animals</td>
<td>Treatment group</td>
<td>Theoretical maximum daily intake (ng/person per day)</td>
<td>Notes</td>
</tr>
<tr>
<td>----------</td>
<td>----------------</td>
<td>-----------------</td>
<td>-----------------------------------------------------</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>$E_1$</td>
<td>$E_2$-$17\alpha$</td>
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<tr>
<td>Synovex® H</td>
<td>Heifer calves</td>
<td>Controls, slaughtered on day 61</td>
<td>1.1</td>
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<tr>
<td></td>
<td></td>
<td>Controls, slaughtered on day 119</td>
<td>1.2</td>
<td>2.2</td>
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<tr>
<td></td>
<td></td>
<td>Controls, slaughtered on day 240</td>
<td>0.8</td>
<td>1.7</td>
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<tr>
<td></td>
<td></td>
<td>Controls, slaughtered on day 301</td>
<td>1.4</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls, slaughtered on day 329</td>
<td>0.7</td>
<td>2.1</td>
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<tr>
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<td></td>
<td></td>
<td>Implanted animals slaughtered on day 119</td>
<td>3.0</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>Heifers</td>
<td>Controls</td>
<td>1.4</td>
<td>1.5</td>
</tr>
<tr>
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<td></td>
<td>Implanted animals slaughtered on day 15</td>
<td>3.9</td>
<td>15</td>
</tr>
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<td></td>
<td>Pregnant heifers</td>
<td>120 days' pregnant, unsynchronized controls</td>
<td>93</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120 days' pregnant, synchronized controls</td>
<td>113</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120 days' pregnant, implanted for 61 days</td>
<td>34</td>
<td>15</td>
</tr>
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<td></td>
<td>180 days' pregnant, synchronized controls</td>
<td>280</td>
<td>48</td>
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<tr>
<td></td>
<td></td>
<td>180 days' pregnant, implanted for 61 days</td>
<td>107</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>240 days' pregnant, synchronized controls</td>
<td>326</td>
<td>139</td>
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<tr>
<td></td>
<td></td>
<td>240 days' pregnant, implanted for 61 days</td>
<td>377</td>
<td>49</td>
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<td>Synovex® S</td>
<td>Steers</td>
<td>Controls</td>
<td>Implied animals slaughtered on day 15</td>
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<td>-----------</td>
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</tr>
<tr>
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<td></td>
<td>1.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>6.3</td>
<td></td>
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<td></td>
<td></td>
<td>190</td>
<td>6.8</td>
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<table>
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<tr>
<th>Torelorm</th>
<th>Steers</th>
<th>Controls</th>
<th>Animals implanted on day 0; slaughtered on day 30</th>
<th>Animals implanted on days 0 or 60; slaughtered on day 90</th>
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<tbody>
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<td></td>
<td></td>
<td>3.1</td>
<td>19</td>
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</tr>
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<td>11</td>
<td>66</td>
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<td>54.9</td>
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<td>7.3</td>
<td>89</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>74.2</td>
<td></td>
</tr>
</tbody>
</table>

E1: estrone; E2: estradiol; P: progesterone; T: testosterone.

* For the composition of the products, see Table 8.

Notes to Table 9

1. As the method used includes extraction and deconjugation of conjugates, the figures for estimated intake represent total parent compound and can be used as given.
2. Free and conjugated fractions of estradiol-17β were determined in all tissues; however, estrone (free fraction only) was determined only in liver and fat. The "true" theoretical maximum daily intakes for estrogens, therefore, could be 50% higher than the values given. No data were available to make a precise estimate of a correction factor. The study with Torelorm was an experimental investigation and does not reflect approved use.
3. Calculations of intake based on determinations of concentrations of free hormones in muscle and fat. The values are probably underestimates of the "true" theoretical maximum daily intakes. No information was available to correct these values.
4. The analytical method was not described in the report. The values for estradiol-17β are based on concentrations in the liver only. The study was an experimental investigation and does not reflect approved use.
5. The method does not allow determination of conjugates. In view of the effects of trenbolone-estradiol combinations on estrogen concentrations in other studies, the theoretical maximum daily intake may be a significant underestimate of the "true" value.
6. Calculations of intake based on determinations of concentrations of free hormones in muscle, liver, kidney and fat. The fractions of conjugated hormones were not determined. Data from a study in animals slaughtered 15 days after implantation of the radiolabelled hormone (see pages 67 and 73 of the main text and Table 10) indicate that the estimated theoretical maximum daily intakes for consumption of residues can be multiplied by a factor of 2.
7. Calculations of intake based on determinations of concentrations of free hormones in muscle, liver, kidney and fat. The fractions of conjugated hormones were not determined. The data could not be corrected owing to lack of relevant information; however, in view of the well-established significant reduction in the theoretical maximum daily intake as a consequence of implantation of pregnant heifers, such a correction is apparently unnecessary.
Table 10
Contributions (%) of free and conjugated hormones in edible tissues of cattle to the theoretical maximum daily intake of humans

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Testosterone propionate</th>
<th>Estradiol benzoate</th>
<th>Progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heifers</td>
<td>Heifers</td>
<td>Steers</td>
</tr>
<tr>
<td></td>
<td>Free</td>
<td>Conjugated</td>
<td>Free</td>
</tr>
<tr>
<td>Muscle</td>
<td>6.7</td>
<td>5.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Liver</td>
<td>29.9</td>
<td>38.1</td>
<td>36.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.8</td>
<td>4.2</td>
<td>9.9</td>
</tr>
<tr>
<td>Fat</td>
<td>11.0</td>
<td>1.0</td>
<td>12.2</td>
</tr>
<tr>
<td>Total</td>
<td>51.4</td>
<td>48.6</td>
<td>63.9</td>
</tr>
</tbody>
</table>
consumption of the edible tissues of steers or heifers given Synovex® S or Synovex® H implants, respectively, can be multiplied by a factor of 2. This correction is probably not relevant in the case of progesterone, as its tentatively identified conjugated metabolites have no hormonal properties.

For total estrogens, the highest excess intakes calculated in this way were 30–50 ng/person per day (see Table 9, Synovex® H in conjunction with note 6 and Finaplex-S® in heifers in conjunction with note 2). This range of excess intakes is less than 2% of the maximum ADI for estradiol-17β (3000 ng for a 60-kg person). In studies conducted with experimental combinations (e.g. Implix® BF/Revalor® and Implix® BM/Revalor®), the resulting excess intakes were more than twice as high (about 4% of the ADI) as those seen with the approved formulations.

For progesterone, the highest excess intake of the parent compound, which is the only relevant, hormonally active residue, was approximately 500 ng/person per day for its approved uses (see Table 9, Synovex® H in heifer calves). This excess intake corresponds to approximately 0.03% of the maximum ADI for progesterone (1800 μg for a 60-kg person).

For testosterone, the highest excess intake of the free hormone was about 60 ng/person per day for all approved uses (see Table 9, Synovex® H in heifers). This intake corresponds to approximately 0.05% of the maximum ADI for testosterone (120 μg for a 60-kg person). The intake of other possibly relevant metabolites, which have not been identified, could theoretically be of the same order of magnitude.

Analytical methods

The Committee critically examined the available information on both the scope and the performance of the analytical methods used to determine the concentrations of residues of estradiol-17β, progesterone and testosterone. The radioimmunoassays that were developed about 20 years ago have been thoroughly validated and are supported by an excellent database. For example, concentrations of <1 ng of estrogenic hormone per kilogram of meat were quantified in many studies with these methods, whereas equivalent sensitivity was not achieved with certain more recently developed methods, particularly those used to investigate experimental combinations of hormones. All of the methods for estimating specific hormone residues were, however, generally valid. Some of the methods were not designed for determination of both free hormones and their relevant metabolites and conjugates in all relevant edible tissues, although this
was an indispensable requirement for sufficiently accurate analysis of both endogenously produced and exogenously administered estrogenic compounds. Estrone and estradiol-17β are interconvertible, undergo comparable metabolism and have similar estrogenic potency. The bioavailability of the glucuronides and sulfates of estrone and estradiol-17β is also similar.

These differences in the scope and performance of the analytical methods underlined the necessity of reviewing estradiol-17β, progesterone and testosterone concurrently, by product.

**Maximum Residue Limits**

The Committee noted that although the hormone concentrations found in specific populations of treated animals were often statistically significantly higher than the corresponding values for concurrent controls, they were within the physiological range of concentrations of these substances in cattle and that the calculated excess intakes contributed only a small additional amount of hormone to the intakes resulting from consumption of other foods of both animal and plant origin.

On the basis of the available data, the Committee concluded that there would be no need to specify numerical MRLs for estradiol-17β, progesterone and testosterone and recommended MRLs “not specified”1 for the edible tissues of cattle when the products are used according to good practice in the use of veterinary drugs. The Committee recommended, however, that the total intake of estrogenic residues resulting from the use of any approved hormonal product be kept below the calculated excess intake levels shown in Table 9.

3.5.2 **Porcine somatotropins**

Three analogues of native porcine somatotropin (pST) that are produced by recombinant DNA techniques were reviewed for the first time by the Committee. These products2 are used in animal production to increase body-weight gain and the efficiency of feed conversion into edible tissues and to affect carcass composition, resulting in pigs with more protein and less fat. The Committee considered only

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

2 Grolene®, Reporcin® and Somagrepor®; common names were not available for these products.
the safety for consumers of foods containing recombinant porcine somatotropin (rpST) residues.

pST, a single polypeptide chain of 190 amino acids, exists as three recombinant variants comprising either 183, 191 or 193 amino acids. In one product, the amino acid methionine is added at the N-terminus, while in another the first seven amino acid residues from the N-terminus are deleted. The third product has an additional three amino acids at the N-terminus and substitutions at positions 6, 11, 183 and 191. Although the molecular structure of the recombinant products differs from that of native pST, the Committee considered it appropriate to evaluate them together because they all act by binding with high affinity to the pST receptor. The administered compounds are designated as “rpST” below, even though pST elicits the same responses.

pSTs are injectable products administered to pigs during the 30 days before slaughter known as the “finishing phase”. The products are injected as single daily doses of 3–5 mg/animal, starting with 60–70 μg/kg of body weight, or as a sustained-release implant containing 100–150 mg of active ingredient, equivalent to 3.3–5 mg/day over 30 days.

Toxicological data
The Committee considered the results of studies on the pharmacokinetics, acute and short-term toxicity and reproductive toxicity of pST and rpST, and the results of studies on insulin-like growth factor (IGF-I), the production of which is stimulated by somatotropin. All of the pivotal studies of toxicity were performed according to appropriate standards for study protocol and conduct. Most of the short-term studies focused primarily on effects on biological parameters such as body-weight gain, haematology and clinical chemistry after oral or parenteral administration of rpST, and only a few included comprehensive histopathological examinations.

Somatotropin is found in all mammalian species. The structural homology between porcine and human somatotropin is approximately 66%. The differences in amino acid sequences result in the “species specificity” of somatotropins. Pituitary-derived pST is biologically inactive when injected into hypophysectomized rhesus monkeys and in humans who have received pST or plasmin-digested pST by injection. In addition, it has been shown that pST does not bind to human somatotropin receptors in liver in vitro. Rats displayed a physiological response to parenterally administered pST and rpST, although the hormones are about 250 times less potent in this species than in pigs. The physiological effects of rpST were indistinguishable from those of native pST in pigs or hypophysectomized rats after parenteral
administration. pST and rpST showed similar inhibition of binding of 125I-labelled pST to liver microsomal membrane fractions from pigs or rabbits in vitro.

Peak serum concentrations of rpST in rats occurred 6 hours after an intramuscular injection of 125I-labelled rpST, while pST was no longer measurable 60 hours after treatment. The biological half-life in serum was not determined. Biodegradation appears to be rapid, and parenterally administered rpST at the doses used for growth promotion in pigs did not lead to concentrations of pST in blood or muscle that were greater than physiological levels 27 hours after treatment.

A study in rats given rpST as single doses of up to 5 g/kg of body weight showed no biological or toxicological effects. No adverse effects were observed in a study in which rats were given pST or rpST orally at a dose of 4 mg/kg of body weight per day for 15 days. In two further studies in which rats were given rpST at doses of up to 130 mg/kg of body weight per day for 15 days, no adverse effects were seen, and no pST could be found in serum. In a comprehensive study in rats given rpST at doses of up to 26 mg/kg of body weight per day or pST at 8.8 mg/kg of body weight per day, no clinical signs of toxicity or treatment-related changes in body-weight gain, haematological, clinical chemical or urinary parameters, organ weights or gross pathological appearance were observed. A statistically significant decrease in the serum concentration of glucagon was observed in male rats dosed with rpST at 8.8 or 26 mg/kg of body weight per day or with pST at 8.8 mg/kg of body weight per day. However, the observed effect on serum glucagon concentration was not reproduced in two additional studies of identical design which were conducted in two independent laboratories. In a further study, oral administration of rpST to rats at 25 mg/kg of body weight per day for 21 days did not cause any treatment-related histopathological changes. These studies demonstrated that pST and rpST have no biological activity when administered orally.

When rpST was given intramuscularly to rats for 15 days, decreased concentrations of the thyroid hormones tri-iodothyronine and thyroxine, decreased activities of serum alkaline phosphatase and aspartate aminotransferase, decreased albumin concentrations, and increased serum cholesterol concentrations, platelet counts and weights of the liver, testes and adrenals were observed at doses of 4.4 mg/kg of body weight per day and above.

In several studies, pigs weighing 27–40 kg were treated parenterally with pST or rpST at doses of 0.01–0.07 mg/kg of body weight per day for up to 77 days. The observed effects included increased body-
weight gain, efficiency of feed conversion into edible tissues, serum glucose and triglyceride levels, blood urea nitrogen and IGF-I concentrations, and increased weights of the liver, kidneys and heart. These effects were considered to be due to specific binding of pST and rpST to pST receptors. Doses of 0.035 mg of pST or rpST per kilogram of body weight per day and above affected the physical mobility of the pigs by causing lesions of the bone and cartilage of the major leg joints. Since pST and rpST do not bind to the human somatotropin receptor, the Committee considered that the effects seen in pigs are unlikely to occur in humans. No effect on key immune functional parameters was observed in pigs receiving daily intramuscular injections of rpST at doses of up to 25 mg per animal for 57 days.

No information was available on the reproductive toxicity of pST or rpST in laboratory animals. Reproductive effects in pigs given intramuscular injections of approximately 5 mg of pST or rpST per animal per day were investigated in a number of studies. No effects were observed on the age at onset of puberty, length of estrus or estrus cycle, or ovulation rate in nulliparous pigs, while treatment of pregnant nulliparous pigs increased placental and fetal weights. The lactation performance of sows and the composition of their milk were not affected. Treatment of sows late in gestation and during lactation had no effect on the birth weight of the offspring, the number of live births, or survival up to 21 days.

No information was available on the genotoxic potential of rpST; however, the Committee noted that the structurally related compound recombinant bovine somatotropin did not show evidence of genotoxicity in two assays (Annex 1, reference 135).

Many of the physiological effects of somatotropin are mediated by IGF-I. The chemical structures of human, porcine and bovine IGF-I are identical. The bioactivity of IGF-I residues in the edible tissues and milk was discussed at the fortieth meeting in relation to use of bovine somatotropin (Annex 1, reference 104). At that time, the Committee concluded that, although the liver is the major site of IGF-I synthesis, IGF-I is also present in human milk, saliva and pancreatic secretions. The Committee further concluded that IGF-I is not biologically active when administered orally to hypophysectomized rats, as dietary IGF-I is almost completely degraded by digestive enzymes and is not expected to contribute significantly to the high endogenous concentration of IGF-I in the intestine. Using data considered at the fiftieth meeting, when recombinant bovine somatotropins were evaluated (Annex 1, reference 134), the Committee concluded at its present meeting that when rpST is used in pigs, the
levels of IGF-I residues in the edible tissues are several orders of magnitude lower than the amount produced endogenously in humans and are therefore extremely unlikely to represent any health risk for consumers.

The Committee noted that recombinant proteins may cause allergy; however, because there was no evidence that pork meat, which contains pST, is allergenic in humans and because rpSTs are antigenically similar to native pST, residues of rpST in food are not likely to cause an allergic response in humans after consumption.

In reaching a conclusion on the safety of rpST, the Committee noted the following:

- The lack of biological activity of rpST in rats after oral administration.
- The lack of biological activity of rpST in humans, as evidenced by the substantial difference in the amino acid sequence of somatotropin from pigs and humans, the absence of binding of rpST to human somatotropin receptors, and the lack of effect in humans injected with either pST or serum plasmin-digested pST.
- The lack of biological activity of orally ingested IGF-I.

From the above, the Committee concluded that rpST can be used in pigs without any appreciable health risk for consumers from the administered rpST or from IGF-I residues in rpST-treated pigs. It established an ADI of "not specified" for rpST, which applies to the three products that were evaluated at the present meeting.

A toxicological monograph was prepared.

Pharmacokinetic data
The Committee considered data in the published literature and reports submitted to the present meeting. The blood concentrations of pST given below represent total pSTs, as the radioimmunoassays used to measure plasma and serum concentrations do not allow a distinction between native and recombinant pST.

Porcine somatotropin. The pharmacokinetics of pST in pigs was investigated after intramuscular injection with quantification by radioimmunoassay, except in one study in which intravenous administration with quantification by radiolabel counting was used.

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

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In one study, two pigs received a single intramuscular injection of pituitary-derived pST at 0.022 or 0.044 mg/kg of body weight. A third pig served as a control. Plasma levels were determined every 30 minutes up to 4 hours after dosing; thereafter, samples were taken hourly until 12 hours after dosing and then every 2 hours until 24 hours after dosing. The plasma concentrations of pST peaked at 36 and 32 ng/ml, respectively, 2 hours after dosing in the animals treated at 0.022 and 0.044 mg/kg of body weight. The higher dose resulted in elevated concentrations for 4 hours, after which a rapid decline was observed. Background concentrations of 2–4 ng/ml were attained 5 hours after treatment with the lower dose and 9 hours after treatment with the higher dose.

In another study, three groups of three pigs received single intramuscular injections of pST at a dose of 0.01, 0.1 or 1.0 mg/kg of body weight. A fourth group served as controls. The plasma concentrations of the drug peaked at 28, 112 and 286 ng/ml in the three treated groups, respectively, between 1 and 2 hours after dosing, but returned to baseline values by 4, 12 and 24 hours after dosing, respectively. The control values ranged between 1.6 and 5.7 ng/ml over the course of the experiment. It should be noted that the highest dose was approximately 20 times the recommended dose.

In three studies, the serum concentrations of pST were measured after 36 daily intramuscular injections. In the first study, three groups of 12 barrows weighing 50 kg (mean) were given daily injections of pituitary-derived pST at doses of 0.01, 0.03 or 0.07 mg/kg of body weight. An additional group served as controls. Serum samples were taken on days 0, 17 and 35. On day 17 of treatment, the concentrations of pST were 0.1 ng/ml for the untreated controls and 9.5, 56 and 116 ng/ml for the three treated groups, respectively, indicating dose-related increases. In a second study, the effects of pST and rpST were compared in groups of 12 barrows. On day 49 after the beginning of treatment (day 13 after drug withdrawal), the serum concentrations were 6.0 ng/ml for controls, 25 ng/ml for barrows treated with 0.035 mg/kg of body weight of pST, 20 ng/ml for animals given the same dose of rpST, 36 ng/ml for animals given 0.070 mg/kg of body weight of pST, and 44 ng/ml for those given rpST at this dose. In a third study, a group of 18 barrows weighing 25 kg (mean) received a single daily intramuscular injection of pituitary-derived pST at 0.1 mg/kg of body weight. A second group of 18 animals served as controls. When the body weight of the animals reached 50 kg, the serum concentrations of pST were 0.8–2.1 ng/ml in the controls and 20–21 ng/ml in the treated pigs.

In a further study, a group of 11 pigs received intramuscular injections of pituitary-derived pST daily at a dose of 0.022 mg/kg of body weight for 30 days. An additional group of 12 animals served as controls.
Blood samples were taken 3 hours after dosing on days 10, 20 and 30 to determine the concentrations of pST. The mean concentrations in the control animals were 2.5–3.5 ng/ml, whereas the mean concentrations in treated pigs were 20.1 ng/ml on day 10 after treatment, 22.8 ng/ml on day 20, and 15.3 ng/ml on day 30.

In another study, blood concentrations of pST were reported in pigs weighing 60 kg (mean) that were treated with methionyl rpST. Two trials were conducted, in which 14 mg of rpST in bicarbonate buffer solution were injected into shoulder muscle twice weekly for either 6 or 13 weeks (trial 1) or 9 weeks (trial 2). Control pigs received only the buffer solution. Plasma from 47 treated and 47 control pigs in the first trial was assayed at slaughter 4.5 days after the last injection. The mean plasma concentrations of pST were 2.6 ng/ml for control pigs and 1.6 ng/ml for treated animals, which were similar to the background levels. In the second trial, the plasma concentrations of pST in 17 treated and 18 control pigs were measured 1 hour after administration of each dose of rpST and at slaughter 26–27 hours after the last dose. The mean plasma concentration was higher (280 ng/ml) in the treated animals than in the controls (2 ng/ml) 1 hour after dosing, but had returned to background levels of 2–3 ng/ml after 26–27 hours.

The half-life of pST in pigs has been reported to be 7–10 minutes. In one study, six pigs received intravenous injections of approximately 50 ng of ^125^I-labelled pST or rpST. The elimination was biphasic, the half-life for the fast phase being 4 minutes for both pST and rpST and that for the slow phase being 39 and 49 minutes, respectively. The half-life of natural pST in the plasma of pigs in studies of the natural surges and spikes due to endogenous production by the pituitary gland was also 7–8 minutes, indicating that the values derived for ^125^I-labelled pST and rpST reflect the true half-life of the hormone, at least in the initial phase. The baseline concentrations of pST in swine plasma have been reported in a number of studies to be 1.7–6.8 ng/ml, with episodic peaks two to three times higher.

The plasma concentrations of pST were also determined in a residue-depletion study in which three pigs were given a sustained-release implant of 123 mg of rpST. Three control pigs were given daily injections of buffer solution. Samples of plasma were analysed at slaughter 28 days after treatment. The concentrations in plasma were 1.3–2.5 ng/ml for the controls and 7.0–9.3 ng/ml for the treated pigs, showing that the sustained-release preparation continued to release rpST.

*Insulin-like growth factor.* Several studies have demonstrated that treatment with pST results in an increase in the concentration of IGF-
I in serum. In one study, treatment of 11 pigs with pituitary-derived pST at a dose of 0.022 mg/kg of body weight for 30 days caused a significant increase ($p < 0.001$) in the mean concentration of IGF-I, from 197 International Units (IU)/l in the 12 controls to 305 IU/l in the treated pigs 3 hours after the last injection. In a second study, groups of 12 barrows were treated for 49 days with either pST or rpST at a dose of 0.035 or 0.070 mg/kg of body weight, and serum samples taken 3 hours after the last injection. The mean control value was 145 IU/l. The mean serum concentration in pigs that were treated with the lower dose was 161 IU/l with pST and 165 IU/l with rpST, while that in pigs given the higher dose was 227 IU/l with pST and 215 IU/l with rpST. The results at the two doses were significantly different ($p < 0.05$) from that for controls and from each other, although the concentrations of IGF-I were similar after injection of natural and recombinant sources of somatotropin. In another study, IGF-I concentrations were measured in the serum of 12 barrows weighing 58 kg (mean value) 10, 24 and 36 hours after injection of doses of pituitary-derived pST of 0, 0.01, 0.1 or 1.0 mg/kg of body weight. The concentrations of IGF-I in the serum of controls and of pigs treated with pST at 0.01 mg/kg of body weight were 80–180 IU/l throughout the study. Pigs given 0.1 mg/kg of body weight had concentrations of 200–250 IU/l from 10 to 36 hours, and those treated with 1.0 mg/kg of body weight had a mean serum concentration of >200 IU/l at 6 hours, which increased to 380 IU/l at 24 hours and persisted at 340 IU/l 36 hours after dosing. It should be noted that 1.0 mg/kg of body weight is about 20 times the recommended dose of pST.

Plasma samples from pigs treated with 14 mg of methionyl rpST twice weekly for 6 or 13 weeks (trial 1) or for 9 weeks (trial 2) in the study described above (see page 80), were also analysed for IGF-I. In trial 1, the average IGF-I concentration 4.5 days after the last injection was 327 μg/l in control animals and 359 μg/l in treated animals. As the standard deviations were well over 100, these values are not significantly different. In the second trial, the average concentrations of IGF-I were 340 μg/l for controls and 551 μg/l for treated animals 1 hour after the last injection and 271 μg/ml for controls and 941 μg/l for treated animals 26–27 hours after administration. The results in plasma exceed somewhat the values seen in the pharmacokinetic study described in the paragraph below, perhaps due to the more than twofold higher dose given just 1 day prior to analysis.

The plasma concentrations of IGF-I were also determined in the 28-day pharmacokinetic study described above (see page 80), in which three pigs were given a sustained-release implant of 123 mg of rpST and three control pigs were given a daily injection of buffer solution.
The concentrations of IGF-I in plasma from control pigs were 180–
400µg/l, whereas those for the treated pigs were 400–490µg/l, indicating
that the sustained-release preparation continued to release rpST.

Using data considered at the fiftieth meeting, when recombinant
bovine somatotropins were evaluated (Annex 1, reference 134), the
Committee concluded at its present meeting that when rpST is used in
pigs, the levels of IGF-I residues in the edible tissues are several
orders of magnitude lower than the amount produced endogenously
in humans (10^7ng/day) and are therefore extremely unlikely to repre-
sent any health risk for consumers.

Residue data
Porcine somatotropin. Few data are available on the concentrations
of residues of pST and IGF-I in the edible tissues of rpST-treated pigs.
The concentrations of pST and IGF-I in tissues of untreated and
rpST-treated pigs were reported by one sponsor; however, the data
were derived from a limited number of samples of plasma and tissue
in an efficacy study that did not comply with good laboratory practice.
The samples were analysed by radioimmunoassay. Very few data on
validation of the analytical method were submitted to the Committee.

In the efficacy study, three control pigs received daily intramuscular
injections of a buffer solution, and three pigs were given a sustained-
release implant of 123mg of rpST. All animals were slaughtered
28 days after the treated animals received the implant. Because the
treated animals received sustained-release implants, the residues
were measured immediately after slaughter, with no withdrawal time.
The mean basal concentration of pST in the plasma of control pigs
was 1.7±µg/l (range, 1.3–2.5±µg/l), and the mean concentrations in the
edible tissues were 14±µg/kg (range, 12–18±µg/kg) in muscle, 17±µg/kg
(range, 14–22±µg/kg) in liver, 26±µg/kg (range, 22–33±µg/kg) in kidney
and 3.3±µg/kg (range, 2.9–4.2±µg/kg) in fat. In the treated pigs, the
mean plasma concentration of pST was 8.2±µg/l (range, 7.0–9.3±µg/l).
Liver and kidney samples from these pigs contained the highest con-
centrations, as was found in the controls. The mean concentrations
in the edible tissues of treated pigs were 13±µg/kg (range, 12–13±µg/kg)
in muscle, 20±µg/kg (range, 17–24±µg/kg) in liver, 23±µg/kg (range,
19–25±µg/kg) in kidney and 2.8±µg/kg (range, 2.3–3.2±µg/kg) in fat.
Thus, at 28 days, the plasma pST concentration was increased from a
mean of 1.7±µg/l in control pigs to 8.2±µg/l in treated animals, reflecting
the continuing release of the hormone from the implant. However,
the concentrations in the edible tissues of the treated animals were
essentially the same as those in the control group.
In the published study described in the section on pharmacokinetics (see page 80), in which pigs were treated twice weekly with 14 mg of methionyl rpST, residues were measured in the muscle of the shoulder opposite that which had been injected from four out of the 47 animals slaughtered 4.5 days after the last of 12 treatments (trial 1) and three out of the 17 animals slaughtered 26–27 hours after the last of 18 treatments (trial 2). Two animals in trial 1 and one animal in trial 2 served as controls. The concentration of pST residues in the opposite shoulder muscle was reported to be less than 5 μg/kg, which was within the range of concentrations observed in plasma. No increase in the concentrations of pST in tissues from the treated animals was observed. On the basis of these results, the Committee concluded that the concentrations of total pST in the edible tissues from pST-treated pigs were equivalent to those in untreated controls.

**Insulin-like growth factor.** Residues of IGF-I were measured in the residue-depletion study of pST reported by the sponsor (see page 81), in which three pigs were given a sustained-release implant of 123 mg of rpST. Three pigs served as controls. The highest mean concentration of IGF-I in the control animals was found in plasma, at 300 μg/l (range, 175–401 μg/l). The mean concentrations of IGF-I in tissues from the control pigs were lower than those in plasma, and were less variable. The concentrations were 2.7 μg/kg (range, 1.7–3.5 μg/kg) in muscle, 20 μg/kg (range, 9.5–29 μg/kg) in liver, 45 μg/kg (range, 26–64 μg/kg) in kidney and 6.7 μg/kg (range, 3.8–8.8 μg/kg) in fat.

In the rpST-treated pigs, the mean plasma concentration of IGF-I was 430 μg/l (range, 400–485 μg/l), which was the highest concentration measured in any biological sample. However, less variation between individual pigs was seen than in the untreated group. The mean concentrations in tissues were 7.1 μg/kg (range, 5.7–8.5 μg/kg) in muscle, 45 μg/kg (range, 37–57 μg/kg) in liver, 65 μg/kg (range, 47–75 μg/kg) in kidney and 12 μg/kg (range, 9.5–16 μg/kg) in fat. Thus, at 28 days, the concentrations of IGF-I in the rpST-treated pigs were up to 2–3 times those in the control group. These values were used in determining the potential exposure to IGF-I of consumers of edible tissues from pST-treated pigs.

**Analytical methods**

Radioimmunoassays were used to determine pST and IGF-I residues in plasma and tissues. The methods were described only minimally and appeared to have undergone little validation. They were therefore of limited use for research. In a radioimmunoassay for IGF-I reported by one sponsor, the recoveries were 83% for plasma and 73–110% for tissues; the recovery from liver was 48%. In a
radioimmunoassay for pST reported by the same sponsor, the recoveries were 89% for plasma and 75–128% for tissues. In the protocol of the latter assay, recoveries were determined concurrently in each assay matrix as a quality control. No validated methods were available for the analysis of pST or IGF-I after administration of any of the three recombinant products.

**Maximum Residue Limits**

Porcine somatotropin. The very limited information presented on the kinetics and residues of injected pSTs show that the natural and recombinant compounds have similar, rapid depletion characteristics in plasma. The half-lives of pST and rpST in pigs were 4–8 minutes for the initial phase and 38–49 minutes for the terminal phase, and the peptide hormone is destroyed by gastric and intestinal proteases after oral ingestion. Furthermore, the concentrations of pST in edible tissues after administration of sustained-release or injectable forms of rpST were not significantly elevated. pST has also been shown to be biologically inactive in humans. The Committee concluded that there was no need to recommend MRLs for residues of rpST.

Insulin-like growth factor. Although the concentrations of IGF-I in tissues of pigs treated with the sustained-release recombinant product were low, they were 2–2.5 times those in control animals. The smallest amounts of IGF-I were observed in muscle, while the highest levels were seen in kidney; however, this tissue is consumed in much smaller amounts than muscle. Table 11 shows the concentrations of IGF-I derived from the study in which pigs were treated with a sustained-release form of rpST described above (see page 80). It is presented to illustrate the theoretical exposure to IGF-I of consumers of edible tissues from pST-treated pigs.

Table 11 shows that the maximum theoretical difference between the concentration of total IGF-I in untreated pigs and that in pigs treated with rpST is 5.2 μg, which is exceedingly small (0.05%) in comparison with the estimated production of 10000 μg/day in humans. Since the calculated amount of IGF-I secreted daily into the gastrointestinal tract is 380 μg (Annex 1, reference 135), the maximum theoretical intake of IGF-I from the diet is insignificant in comparison with the amount produced endogenously. Furthermore, IGF-I was not absorbed to any significant extent in studies with several neonatal animal models and IGF-I is inactive after oral administration (Annex 1, reference 135). Although the amount of IGF-I in tissues may be increased slightly by the use of rpST products, the Committee concluded that use of these products would not result in any biologically
Table 11
Mean concentrations of IGF-I in edible tissues from untreated and rpST-treated pigs

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mean concentration of IGF-I (µg/kg)</th>
<th>Estimated intake of IGF-I (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated pigs</td>
<td>Treated pigs</td>
</tr>
<tr>
<td>Muscle</td>
<td>2.6</td>
<td>7.1</td>
</tr>
<tr>
<td>Liver</td>
<td>20.2</td>
<td>44.8</td>
</tr>
<tr>
<td>Kidney</td>
<td>44.7</td>
<td>65.5</td>
</tr>
<tr>
<td>Fat</td>
<td>6.4</td>
<td>12.2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Based on a daily intake of 300 g of muscle, 100 g of liver, and 50 g each of kidney and fat.

significant increase in the concentration of IGF-I in humans who consume edible tissues from treated animals.

In reaching its decision on MRLs for rpST, the Committee noted the following:

- The lack of increased concentrations of pST residues in the edible tissues of treated animals.
- The lack of a biologically significant increase in the intake of IGF-I by humans who consume the edible tissues of treated animals.
- The lack of toxicological concern with regard to the levels of residues of rpST and exogenous IGF-I likely to occur in rpST-treated pigs.

From the above, the Committee concluded that rpST can be used in pigs without appreciable risk to the health of consumers. The Committee recommended MRLs “not specified” for edible tissues in pigs for the three products that were evaluated at the present meeting.

3.6 Tranquillizing agent

3.6.1 Azaperone

Azaperone was evaluated by the Committee at its thirty-eighth, forty-third and fiftieth meetings (Annex 1, references 97, 113 and 134). At its fiftieth meeting, the Committee established an ADI of 0–6 µg/kg of body weight for azaperone and recommended MRLs of 60 µg/kg for

1 MRL “not specified” means that available data on the identity and concentration of residues of the veterinary drug in animal tissues indicate a wide margin of safety for consumption of residues in food when the drug is used according to good practice in the use of veterinary drugs. For that reason, and for the reasons stated in the individual evaluation, the Committee concluded that the presence of drug residues in the named animal product does not present a health concern and that there is no need to specify a numerical MRL.
muscle and fat and 100 μg/kg for liver and kidney in pigs, expressed as the sum of the concentrations of azaperone and azaperol.

Analytical methods
New information was available on a method of analysis for azaperone, indicating that the HPLC method had been further developed and optimized for quantification of both azaperone and its metabolite azaperol in muscle, liver, kidney, fat and skin of pigs. The method involves an extraction of the sample matrix with n-heptane and isoamyl alcohol and a further extraction involving changes of solvent and pH to remove interfering materials. Reverse-phase HPLC is used for final separation and quantification, with gradient elution and detection by single-wavelength ultraviolet absorbance. An analogue of azaperone in which chlorine replaces the fluorine atom is used as the internal standard in the quantification procedure.

The performance of the method for azaperone and for azaperol did not differ significantly. The accuracy of the method was tested in each of the five porcine tissues and plasma at four levels of fortification, representing 1–8 times the limit of quantification. The limit of quantification for both compounds was 0.025 mg/kg. The recovery was 93–114% with coefficients of variation of 1–15%. No data were supplied for incurred residues in tissues.

The specificity, accuracy and reproducibility of the analytical method were insufficiently characterized. The Committee was therefore unable to fully assess the suitability of the method for the quantification of azaperone and azaperol in porcine tissues. The Committee recommended that the method be further improved, with more complete characterization of its specificity, accuracy and reproducibility, and forwarded to the Codex Committee on Residues of Veterinary Drugs in Foods for consideration.

4. Recommendations

1. Recommendations relating to specific veterinary drugs, including ADIs and MRLs, are given in section 3 and Annex 2.
2. 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 annually for this purpose.

Acknowledgement

The Committee wished to thank Mrs E. Heseltine, Lajarthe, Saint Léon-sur-Vézère, France, for her assistance in the preparation of this report.
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 Joint FAO/WHO Expert Committee on Food Additives). 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).


76. Principles for the safety assessment of food additives and contaminants in food. Geneva, World Health Organization, 1987 (WHO Environmental Health Criteria, No. 70) (out of print).\(^1\)


82. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41, 1988 (out of print).


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\(^1\) The full text is available electronically on the Internet at http://www.who.int/pcs.


Annex 2

Recommendations on compounds on the agenda and further information required

β-Adrenoceptor-blocking agent

Carazolol

ADI: 0–0.1 μg/kg of body weight (established at the forty-third meeting of the Committee (WHO Technical Report Series, No. 855, 1995, and corrigendum)).

Acute RfD: 0–0.1 μg/kg of body weight.

Residue definition: Carazolol.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (μg/kg)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
<td>Liver</td>
<td>Kidney</td>
<td>Fat/skin</td>
</tr>
<tr>
<td>Pigs</td>
<td>5</td>
<td>25</td>
<td>25</td>
<td>5</td>
</tr>
</tbody>
</table>

*a These MRLs were recommended at the forty-third meeting of the Committee (WHO Technical Report Series, No. 855, 1995, and corrigendum). At its present meeting, the Committee noted that residues of carazolol at the injection site 2 hours after treatment could result in an intake that exceeds the acute RfD. Therefore, unless appropriate measures can be taken to ensure that the concentrations of residues at the injection site do not result in intake exceeding the acute RfD, use of carazolol during the transport of animals to slaughter is not consistent with safe use of the drug.

Anthelmintic agent

Doramectin

ADI: 0–0.5 μg/kg of body weight (established at the forty-fifth meeting of the Committee (WHO Technical Report Series, No. 864, 1996)).

Residue definition: Doramectin.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (μg/kg)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Cattle</td>
<td>10b</td>
<td>100b</td>
<td>30b</td>
</tr>
<tr>
<td>Pigs</td>
<td>5</td>
<td>100</td>
<td>30</td>
</tr>
</tbody>
</table>

* These MRLs were recommended at the forty-fifth meeting of the Committee (WHO Technical Report Series, No. 864, 1996).
Antimicrobial agents

Dihydrostreptomycin and streptomycin

ADI: 0–50 μg/kg of body weight (group ADI for the combined residues of dihydrostreptomycin and streptomycin, established at the forty-eighth meeting of the Committee (WHO Technical Report Series, No. 879, 1998)).

Residue definition: Sum of the concentrations of dihydrostreptomycin and streptomycin.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>Cattle</td>
<td>600</td>
</tr>
<tr>
<td>Pigs</td>
<td>600</td>
</tr>
<tr>
<td>Sheep</td>
<td>600</td>
</tr>
<tr>
<td>Chickens</td>
<td>600</td>
</tr>
</tbody>
</table>

a The Committee was aware of the existence of more sensitive analytical methods for dihydrostreptomycin and streptomycin in edible tissues and requested that descriptions of these methods be made available to the next session of the Codex Committee on Residues of Veterinary Drugs.

b Temporary MRL, pending the receipt of information on a validated analytical method to quantify low concentrations of both compounds in milk. This information is required for evaluation in 2001.

Neomycin

ADI: 0–60 μg/kg of body weight (established at the forty-seventh meeting of the Committee (WHO Technical Report Series, No. 876, 1998)).

Residue definition: Neomycin.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>Cattle</td>
<td>500a</td>
</tr>
<tr>
<td>Pigs</td>
<td>500a</td>
</tr>
<tr>
<td>Sheep</td>
<td>500a</td>
</tr>
<tr>
<td>Goats</td>
<td>500a</td>
</tr>
<tr>
<td>Turkeys</td>
<td>500a</td>
</tr>
<tr>
<td>Ducks</td>
<td>500a</td>
</tr>
<tr>
<td>Chickens</td>
<td>500a</td>
</tr>
</tbody>
</table>

a These MRLs were recommended at the forty-third meeting of the Committee (WHO Technical Report Series, No. 855, 1995, and corrigendum).
b These MRLs were recommended at the forty-seventh meeting of the Committee (WHO Technical Report Series, No. 876, 1998).
Thiamphenicol

ADI: 0–5 µg/kg of body weight.

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>Cattle</td>
<td>Withdrawn(^a)</td>
</tr>
<tr>
<td>Pigs</td>
<td>50(^c)</td>
</tr>
<tr>
<td>Chickens</td>
<td>Withdrawn(^f)</td>
</tr>
<tr>
<td>Fish</td>
<td>50(^f)</td>
</tr>
</tbody>
</table>

\(^a\) The previous temporary MRLs for the edible tissues of cattle and chickens were withdrawn, as the data required by the Committee at its forty-seventh meeting (WHO Technical Report Series, No. 876, 1998) were not provided.
\(^b\) Temporary MRLs, pending the receipt of the following information:
- The results of a residue-depletion study in pigs given radiolabelled drug to determine the proportions of the total residues accounted for by free thiamphenicol and thiamphenicol conjugates in all tissues.
- A validated analytical method for use with all animal tissues, which includes an enzymatic hydrolysis step to allow determination of the sum of thiamphenicol and thiamphenicol conjugates as free thiamphenicol.

\(^c\) The MRL applies to muscle with naturally adhering skin.

Insecticides

Deltamethrin

ADI: 0–10 µg/kg of body weight (established by the 1982 Joint FAO/WHO Meeting on Pesticide Residues (FAO Plant Production and Protection Paper, No. 46, 1983)).

Residue definition: Deltamethrin.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>Cattle</td>
<td>30(^a)</td>
</tr>
<tr>
<td>Sheep</td>
<td>30(^a)</td>
</tr>
<tr>
<td>Chickens</td>
<td>30(^a)</td>
</tr>
<tr>
<td>Salmon</td>
<td>30(^a)</td>
</tr>
</tbody>
</table>

\(^a\) The Committee noted that the concentrations of residues were very low in muscle, milk and eggs. These MRLs are for guidance only and are based on twice the limit of quantification of the analytical methods.
Phoxim

ADI: 0–4µg/kg of body weight.

Residue definition: Phoxim.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (µg/kg)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>Cattle</td>
<td>50</td>
</tr>
<tr>
<td>Pigs</td>
<td>50</td>
</tr>
<tr>
<td>Sheep</td>
<td>50</td>
</tr>
<tr>
<td>Goats</td>
<td>50</td>
</tr>
</tbody>
</table>

¹ Temporary MRLs, pending the receipt of the following information:
- The results of studies using radio-labelled phoxim to determine the proportion of the total residues accounted for by the marker residue in ruminants and pigs following topical application of the formulated product,
- The results of residue-depletion studies conducted in accordance with good laboratory practice in cattle and sheep given the currently recommended treatments,
- Validation of the available analytical methods for the detection of phoxim residues in tissues of cattle, sheep and goats, and in cows’ milk.

This information is required for evaluation in 2002.

Production aids

Estradiol-17β, progesterone and testosterone

ADI: 0–0.05µg/kg of body weight for estradiol-17β, 0–30µg/kg of body weight for progesterone and 0–2µg/kg of body weight for testosterone.

MRLs: “Not specified”¹ for edible tissues in cattle (applies to estradiol-17β, progesterone and testosterone).

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

ADI: “Not specified”¹ (applies to Grolene®, Reporcin® and Somagrepor®).

MRLs: “Not specified”² for edible tissues in pigs (applies to Grolene®, Reporcin® and Somagrepor®).

Tranquilizing agent

Azaperone

New information on a method of analysis for azaperone and its metabolite azaperol in the edible tissues of pigs was reviewed. The specificity, accuracy and reproducibility of the method were insufficiently characterized. The Committee recommended that the method be further improved, with more complete characterization of its specificity, accuracy and reproducibility, and forwarded to the Codex Committee on Residues of Veterinary Drugs in Foods for consideration.

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

² MRL “not specified” means that available data on the identity and concentration of residues of the veterinary drug in animal tissues indicate a wide margin of safety for consumption of residues in food when the drug is used according to good practice in the use of veterinary drugs. For that reason, and for the reasons stated in the individual evaluations, the Committee concluded that the presence of drug residues in the named animal product does not present a health concern and that there is no need to specify a numerical MRL.