EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD

Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives

World Health Organization
Geneva 1993
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Geneva, 9–18 June 1992

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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. 41/5, 1993.

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**INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY**

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

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

A meeting of the Joint FAO/WHO Expert Committee on Food Additives was held at WHO headquarters, Geneva, from 9 to 18 June 1992. The meeting was opened by Dr. J. Rochon, Director, Division of Health Protection and Promotion, on behalf of the Directors-General of the World Health Organization and the Food and Agriculture Organization of the United Nations.

Dr. Rochon noted that, at the four previous meetings of the Committee to consider veterinary drug residues in food, more than 30 veterinary drugs had been evaluated and extensive methodology for the safety assessment of residues in food had been developed. Despite this effort, the assessment of veterinary drugs that had been on the market for many years, and on which information that met contemporary standards did not exist, was still a major problem. One of the important tasks of the Committee at the present meeting would therefore be to consider whether information relating to the long history of use of these substances could be taken into account in establishing Acceptable Daily Intakes (ADIs) and estimating Maximum Residue Limits (MRLs).

The four previous meetings of the Committee to consider veterinary drug residues in food (Annex 1, references 80, 85, 91, and 97) had been held in response to the recommendations of the Joint FAO/WHO Expert Consultation held in 1984 (1). The present meeting\(^1\) was convened in response to the recommendation made at the thirty-eighth meeting of the Committee that meetings on this subject should be held annually (Annex 1, reference 97). The Committee's purpose was to provide guidance to FAO and WHO Member States and to the Codex Alimentarius Commission on public health issues pertaining to residues of veterinary drugs in foods of animal origin. The specific tasks before the Committee were:

(a) to further elaborate principles for evaluating the safety of residues of veterinary drugs in food and for establishing 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);

(b) to evaluate the safety of residues of certain veterinary drugs (see section 3 and Annex 2); and

(c) to discuss matters of interest arising from the report of the Sixth Session of the Codex Committee on Residues of Veterinary Drugs in Foods (2). Issues of concern to the Codex Committee are discussed in sections 2.3 and 2.6.

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\(^1\) As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in 1955 (FAO Nutrition Meeting Report Series, No. 11, 1956, WHO Technical Report Series, No. 107, 1956), there have been 36 previous meetings of the Joint FAO/WHO Expert Committee on Food Additives (Annex 1).
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 account the principles contained in *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference 76) and the general considerations outlined in the thirty-second, thirty-fourth, thirty-sixth, and thirty-eighth reports of the Committee (Annex 1, references 80, 85, 91, and 97) 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**

Isometamidium and ivermectin were added to the agenda for both toxicological and residues evaluation.

2.3 **Evaluation of veterinary drugs with a long history of use**

At its thirty-second meeting in June 1987, the first to be exclusively devoted to veterinary drugs, the Committee established international criteria to be applied in evaluating toxicological and residue data for use in assessing the safety of veterinary products present in human food. Although many such drugs have already been evaluated in accordance with national governments' requirements, their assessment by the Joint FAO/WHO Expert Committee on Food Additives is undertaken both to set international standards for their safe use from the point of view of human food and to promote harmonization so as to avoid unnecessary technical barriers to international trade.

From the Committee's deliberations on a variety of veterinary products, it has become apparent that, for certain products with a long history of use (often referred to as older veterinary drugs), data that do not meet modern criteria may nevertheless be useful in the safety assessment of residues in human food. The scientific literature and experience with human use of such drugs may provide additional relevant information.

The main concern of the Committee in considering alternative sources of information is to ensure that the safety of older veterinary products can be assured to an extent equivalent to that achieved for newer products. To that end, the Committee decided that the following issues relevant to human food safety must be adequately addressed in each submission: pharmacological effects, general toxicity, reproductive toxicity, embryo-toxicity/fetotoxicity, genotoxicity, carcinogenicity, other effects identified as being of importance, metabolism, tissue residues, and analytical methodology.

The Committee therefore developed an approach for evaluating veterinary drugs with a long history of use, as outlined below, that takes into account
each of these concerns. If human food safety can be adequately assessed from a combination of animal studies and alternative sources of information, then an ADI and MRL can normally be established for these veterinary drugs.

2.3.1 General principles

Each submission should contain information on all available and relevant animal studies (pharmacology, toxicology, and residue chemistry) for evaluation by the Committee. Such information could include company and national files.

When all of the areas of concern listed above are not addressed by animal studies, the manufacturer should provide an evaluation report that includes a comprehensive review of the scientific literature, relevant human data, and/or relevant data for the target species. The report may provide information both on the specific compound concerned and the general class of compounds to which it belongs. In the latter case, a structure-activity analysis should be included; this should indicate whether extrapolation from the general class to the specific compound is warranted. In addition, the report should include any necessary toxicological/residue information not provided by animal studies, or give the reasons why such information is not required for the particular compound in question.

The evaluation report should present a logical analysis of the information, rather than merely a summary of the data. The full texts of all references forming the basis of the evaluation report should be provided to the Committee. In addition, the report should contain information on the scale and pattern of use of the compound so that human exposure can be assessed.

2.3.2 Toxicological data

The toxicological data required will depend on the characteristics of the compound concerned. Genotoxicity data should be provided that encompass a range of end-points sufficient to enable the genotoxic potential of the veterinary product to be adequately determined. A teratogenicity study will be necessary for compounds that are suspect teratogens or where a high or significant exposure of humans to residues in food is expected. In addition, any toxicologically significant effects identified in the evaluation report will need to be investigated.

The Committee will consider toxicological studies that do not meet currently established criteria and, where warranted, may compensate for inadequacies by increasing the safety factor applied when determining the ADI. However, a lack of information about a potential toxicological hazard cannot be compensated for in this way.

Information on microbiological risk (Annex 1, reference 9l, section 2.4) will ordinarily be required in the assessment of antimicrobial compounds.
However, given the current level of development of studies designed to assess this risk, the Committee was unable to recommend any particular studies.

2.3.3 Residue data

In addition to information needed to establish an ADI, the Committee requires data to permit it to recommend appropriate MRLs and, in this connection, to identify:

- a suitable analytical marker residue for the residues of toxicological concern;
- at least two target tissues (see section 2.7) on which to base the MRLs, one of which will be liver or kidney to accommodate current practices in national control programmes and the other muscle or fat to facilitate testing in international trade;
- a suitable analytical method for the marker residue that satisfies current scientific criteria.

The Committee will adopt a flexible approach in the evaluation of data submitted and will determine to what extent conclusions may be based on them. In connection with the decision taken at its thirty-sixth meeting (Annex 1, reference 97), in recommending either MRLs or temporary MRLs, the Committee will also consider, for practical reasons, good practice in the use of veterinary drugs and the applicability of analytical methods.

2.4 General safety concerns and pharmacological effects

The Committee evaluates the safety for humans of residues of veterinary drugs ingested in food. The purpose of recommending ADIs and MRLs is to establish international standards that ensure human food safety and to harmonize international trade in animal products. While it is expected that these evaluations will serve as the scientific basis for decisions regarding human food safety, it is recognized that the stringency of procedures used for drug safety management varies widely from one country or territory to another. Other issues of concern to national authorities that do not directly impinge on food safety are the risks of adverse health effects in workers preparing or handling veterinary drugs, and the use of production aids that could have adverse effects on animal health and welfare, or on the environment. While these issues are not directly relevant to the work of the Committee, it recognizes that they are among the additional factors that may be taken into account by national authorities when considering the use of certain veterinary drugs.

At its thirty-eighth meeting (Annex 1, reference 97), the Committee considered the relevance of pharmacological effects in the establishment of ADIs and concluded that the evidence from humans, the routes of administration, data on sensitive populations, and the most sensitive pharmacological end-point should be taken into account whenever
possible and on a case-by-case basis. When appropriate, an ADI could be established on the basis of a pharmacological rather than a toxicological effect.

This general concern about pharmacological effects arose from a discussion of the risk from residues of drugs that might still be present at slaughter, at the injection site (Annex 1, reference 97, sections 2.4 and 2.5). The Committee recognized that such residues could arise as the result both of the recommended and of the improper use of veterinary drugs. However, it is important to stress that, in the evaluation process, the Committee considers only the correct use of the product in accordance with the manufacturer's recommendations, for example concerning dose, route of administration, species, and duration of treatment.

When the active ingredients of drugs that have an approved veterinary therapeutic use are used illegally as production aids, as has happened, for example, with the β-adrenoceptor agonists, the likelihood of residue-dependent adverse health effects occurring in the consumer is increased. Of particular concern in this regard are the widespread use of such drugs, their inclusion in feed at concentrations designed to deliver a dose higher than that necessary for an approved use, and the continuation of in-feed dosing until slaughter. Serious adverse pharmacological effects have been reported in humans who have consumed residues of β-adrenoceptor agonists. The potency of these drugs as pharmacological agents is such that the need to protect consumers from their pharmacological effects will be an important consideration in their evaluation by the Committee.

2.5 ADI and MRL “not specified”

The term ADI “not specified” is used by the Committee for food additives. However, in establishing, at its present meeting, an ADI “not specified” for four recombinant bovine somatotropins and MRLs “not specified” for these substances in bovine milk and edible tissues (section 3.3.1), the Committee has applied these terms to veterinary drugs for the first time. In this context the terms are defined as follows:

ADI “not specified”: 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 has concluded that use of the veterinary drug does not represent a dietary hazard to human health and that there is no need to specify a numerical ADI.

MRL “not specified”: Available data on the identity and concentration of residues of the veterinary drug in animal tissues 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 has 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.

The Committee intends to refer to these definitions whenever the terms are used so as to clarify their meaning.

In applying these terms to certain veterinary drugs, the Committee does not wish to imply that they are of “unlimited” safety. The most important characteristic of such drugs is that the safety margin between the potential intake of their residues in animal products and the level of health concern is so large that it would be extremely unlikely that that level would be exceeded as a result of known uses of the drug, including the use of elevated therapeutic doses. Any extension of uses beyond those covered by the existing MRLs “not specified” should be evaluated by the Committee.

The Committee does not envisage estimating numerical MRLs when an ADI “not specified” has been established. However, the situation may arise in which a numerical ADI is specified but one or more MRLs “not specified” are established because the potential intake by humans of residues in the animal product(s) is so low that their presence is of no significant health concern.

2.6 Food consumption surveys

The Committee reviewed the food consumption data provided by the Sixth Session of the Codex Committee on Residues of Veterinary Drugs in Foods. At the invitation of the Codex Committee, several Member countries had submitted data based on their national surveys. These estimates of food consumption were, however, not comparable, having been derived from different sources, including market basket surveys, dietary recall, and national food disappearance data. Nevertheless, the data indicated that the values established at the thirty-fourth meeting of the Committee for the consumption of edible animal products were realistic but conservative and therefore protected human health. These daily food intake values are 300 g of meat (as muscle tissue), 100 g of liver, 50 g of kidney, 50 g of tissue fat, 100 g of egg, and 1.5 litres of milk.

At its thirty-fourth meeting (Annex 1, reference 85), the Committee concluded that, given the assumptions and variables involved in establishing MRLs, no great effort should be made further to refine food intake estimates. The Committee reaffirmed this position at its present meeting.

2.7 Target tissues and marker residues

At its thirty-eighth meeting (Annex 1, reference 97), the Committee decided to identify at least two target tissues whenever possible in recommending MRLs. As mentioned earlier, one target tissue is liver or
kidney to accommodate current practices in national control programmes and the other is muscle or fat to facilitate testing for international trade in meat.

The term “target tissue” has several different meanings within national control programmes. However, as used by the Committee, it is defined as the edible animal tissue (muscle, fat, liver, or kidney) for which the MRL is recommended and that may be analysed for purposes of the enforcement of the MRL.

The term “marker residue” may also be used in various ways in national programmes. For the Committee's purposes, however, the term refers to the substance that is, or is representative of, the residue of toxicological concern in the target tissue and/or milk/eggs. Identification of a marker residue is extremely important as it is the substance determined for control purposes in the enforcement of MRLs by national governments and industry.

2.8 **Identity and quality of veterinary products**

Evaluations by the Joint FAO/WHO Expert Committee on Food Additives depend on studies performed with a chemical substance or product of defined identity, purity, and physical form. The ADI is valid only for products that do not differ significantly in identity and quality profile from the material used to generate the data used for the evaluation.

3. **Comments on residues of specific veterinary drugs**

The Committee considered for the first time the safety and residues of three anthelmintic agents, two antimicrobial agents, and two production aids. It reconsidered the safety and residues of one anthelmintic agent and one trypanocide and the residues of one anthelmintic agent. The recommendations made with regard to these compounds are given in Annex 2.

Toxicological and residues monographs were prepared for all the substances considered in this section.

3.1 **Anthelmintic agents**

3.1.1 **Closantel**

Closantel was evaluated at the thirty-sixth meeting of the Committee (Annex 1, reference 97), when an ADI was established and MRLs were recommended for sheep tissues. However, only temporary MRLs were recommended for cattle tissues. Since that time, additional residue data and information on the use of closantel in cattle have been provided by the manufacturer. At its present meeting, the Committee reviewed this
supplementary information and amended its recommendations accordingly. Only the new data are considered in this report.

**Residue data**

*Cattle.* A pharmacokinetic study was carried out in cattle using \[^{14}C\]closantel. The radiolabelled drug was administered orally as a single dose of 10 mg per kg of body weight to Friesian heifers and steers. The average maximum concentrations of total radioactivity in blood and plasma were 26.8 and 35.7 μg of closantel equivalents/ml respectively, occurring 48 hours after oral dosing. The half-life of total residues in plasma was 11 days. All the radioactivity in plasma was unmetabolized drug. Within 42 days, 90% of the dose was excreted in the faeces and less than 0.25% in the urine.

Unchanged closantel was found to be the major residue in faeces (82% of the total residue) and in kidney, muscle, and fat (70-80%, 80-100%, and 60-100% of the total residue, respectively). Unmetabolized closantel accounted for only 6-15% of the total residue in liver.

Characterization of the closantel-derived radioactivity in the liver showed that 40-77% could be accounted for by the 3-monooiodoclosantel metabolite. In addition to the unmetabolized drug, only one notable metabolite was detected in extracts of the faeces. This metabolite accounted for approximately 6% of the administered dose in faecal samples collected during the first 2 weeks; it also occurred in the bile. It was determined to be a sulfate conjugate of a hydroxyl-substituted closantel derivative in which one iodine had been removed from the benzamide moiety.

In the metabolism study noted above, residue-depletion data were collected over a 6-week period. The results of assays on tissues for total radioactivity and unchanged closantel are given in Table 1. Of the edible tissues, liver contained the highest amount of total residue (3.7 mg/kg at 14 days withdrawal time and 1.1 mg/kg at 42 days) and kidney the highest concentration of unmetabolized closantel.

<table>
<thead>
<tr>
<th>Withdrawal time (days)</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TR</td>
<td>C</td>
<td>TR</td>
<td>C</td>
</tr>
<tr>
<td>14</td>
<td>0.71</td>
<td>0.57</td>
<td>3.71</td>
<td>0.54</td>
</tr>
<tr>
<td>28</td>
<td>0.34</td>
<td>0.37</td>
<td>2.41</td>
<td>0.17</td>
</tr>
<tr>
<td>42</td>
<td>0.13</td>
<td>0.16</td>
<td>1.13</td>
<td>≤ 0.1</td>
</tr>
</tbody>
</table>

TR: total residues; C: closantel.

\(^a\) For each withdrawal time, values are means for three animals.

\(^b\) This mean does not include one value that was ≤ 0.1 mg/kg.
The tissue:plasma radioactivity ratios for cattle were calculated from the above study. The results suggest that these ratios are independent of time, so that plasma elimination reflects the depletion of residues from tissues. The mean values for all withdrawal times were 0.046 for muscle, 0.321 for liver, 0.171 for kidney, and 0.084 for fat.

Although the dosage regimen in cattle recommended by the manufacturer of closantel is an oral dose of 5 mg per kg of body weight or an intramuscular dose of 2.5 mg per kg of body weight, the most recent residue study — like the metabolism study described above — was conducted using an oral dose of 10 mg per kg of body weight. Again the highest concentrations of total residues in edible tissues were found in the liver, while kidney contained the highest concentrations of unmetabolized closantel. Kidney concentrations decreased from a mean of 2.14 mg/kg at day 14 to 0.33 mg/kg at day 42, muscle concentrations from a mean of 0.41 mg/kg at day 14 to 0.19 mg/kg at day 42, and liver concentrations from a mean of 0.68 mg/kg at day 14 to less than 0.10 mg/kg in three out of four animals at day 42.

In an earlier study on calves, in which a dose of 2.5 mg per kg of body weight of closantel was given by intramuscular injection, the highest concentrations of closantel were seen initially in kidney. Very little, if any, depletion of closantel occurred in the edible tissues of the calves during the first 28 days of withdrawal. In fact, over the time period studied, the concentration of closantel in fat seemed to increase slightly. The concentrations of unchanged closantel in the tissues at withdrawal times of 14, 28, and 42 days were: 0.67, 0.70, and 0.29 mg/kg in muscle; 1.54, 1.43, and 0.56 mg/kg in liver; 2.84, 2.93, and 1.39 mg/kg in kidney; and 2.08, 1.97, and 2.36 mg/kg in fat, respectively.

**Sheep.** Studies on animals treated with closantel according to good practice in the use of veterinary drugs have indicated that residues may occur at concentrations greater than 1.5 mg/kg in kidney tissue. The Committee reviewed these data, since it had previously recommended an MRL of 1.5 mg/kg for all edible tissues of sheep (Annex 1, reference 91). The ADI of 0-30 µg per kg of body weight, established at the thirty-sixth meeting of the Committee, would correspond to a maximum daily intake of 1.8 mg of total drug residue, contributed by 500 g of food-animal meat in a 60-kg person. The Committee estimated that, at 28 days withdrawal time, the intake of residues even at the maximum use limits for closantel would be well below the ADI. On the basis of these data, the Committee recommended that the MRLs for sheep should be amended.

**Appraisal.** Residue depletion in the edible tissues of cattle and sheep treated with closantel is similar. The residue levels in tissues decrease with a half-life of 2-3 weeks. In both sheep and cattle, an oral dose is about half as bioavailable as a parenteral dose.

The studies in which sheep and cattle were treated with [14C]closantel indicate that there is virtually no metabolism in muscle, kidney, or fat. In sheep liver, approximately 61-71% of the radioactivity was parent
closantel, with the remaining residue consisting of 3- and 5-monoiodo-
closantel. No evidence for the existence of other metabolic pathways
was reported. In cattle liver, approximately 10\% of the radioactivity
was unchanged closantel, and 40–77\% was accounted for by 3-mono-
iodoclosantel. In faeces, 6\% of the radioactivity was identified as a sulfate
conjugate of a closantel derivative.

Residue depletion of closantel from plasma parallels that from the edible
tissues. Within a given species, there is a reasonably constant tissue:plasma
ratio which is independent of time. The tissue:plasma ratios in liver and in
fat are, respectively, approximately four times and 12 times as large in
cattle as in sheep. The tissue:plasma ratios for muscle and kidney in sheep
and cattle are comparable.

**Maximum Residue Limits**

Taking into account this information and the specific residue data
discussed on pages 8–10, the Committee recommended amended MRLs
for closantel in sheep (Table 2) and new MRLs for cattle (Table 3). The
recommendations for cattle are based on the studies in which an oral dose
of closantel of 10 mg per kg of body weight and an intramuscular dose of
2.5 mg per kg of body weight were given; from these the Committee
calculated that the theoretical maximum daily intake of closantel residues
at 42 days withdrawal time would be below the ADI of 1.8 mg for a 60-kg
person. It should be noted that the manufacturer recommends an oral dose
of 5 mg per kg of body weight for cattle. Residues from such an oral dose
will be lower than those given in Table 3.

3.1.2 **Flubendazole**

Flubendazole had not been previously reviewed by the Committee. The
compound is used as an anthelminthic in pigs and poultry. It belongs to the
group of benzimidazole carbamates.

**Toxicological data**

A substantial database was available for assessment, including data on
kinetics and metabolism, acute toxicity, short-term and long-term toxicity,
reproductive and developmental toxicity, and genotoxicity.

The absorption, metabolism, and excretion of flubendazole have been
studied using radiolabelled drug. Flubendazole is poorly absorbed and is
metabolized in a qualitatively similar way in all species studied. More than
50\% of the ingested drug is eliminated unchanged in the faeces. The
absorbed drug is rapidly metabolized, so that levels of parent drug in the
blood and urine are extremely low. The main site of metabolism is the liver,
and major metabolic pathways are carbamate hydrolysis and ketone
reduction. It seems probable that flubendazole undergoes enterohepatic
circulation.

Single oral doses of flubendazole were slightly toxic to experimental
animals, the median lethal dose (LD$_{50}$) being greater than 5000 mg per kg
of body weight in mice, rats, and guinea-pigs.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Observed residue (mg/kg parent drug)</th>
<th>Estimated daily intake(^bc) (mg closantel equivalents)</th>
<th>Theoretical maximum daily intake(^d) (mg closantel equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oral dose(^a)</td>
<td>Intramuscular dose(^a)</td>
<td>Oral dose(^a)</td>
</tr>
<tr>
<td>Muscle</td>
<td>&lt; 0.4</td>
<td>1.1</td>
<td>0.12</td>
</tr>
<tr>
<td>Liver</td>
<td>0.8</td>
<td>(1.14)(^b)</td>
<td>0.7</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.7</td>
<td>1.2</td>
<td>0.04</td>
</tr>
<tr>
<td>Fat</td>
<td>0.7</td>
<td>0.4</td>
<td>0.04</td>
</tr>
<tr>
<td>Total</td>
<td>0.31</td>
<td>0.53</td>
<td>1.05</td>
</tr>
</tbody>
</table>

\(^a\) Based on concentrations at 28 days withdrawal time. For the original discussion of residue data for sheep, see Annex 1, reference 91.
\(^b\) Calculated from the observed residue levels.
\(^c\) Based on a daily intake of 0.5 kg of meat made up of 0.3 kg of muscle, 0.1 kg of liver, 0.05 kg of kidney, and 0.05 kg of fat.
\(^d\) 10 mg/kg of body weight.
\(^e\) 5 mg/kg of body weight.
\(^f\) Estimate of total residues; after oral administration, closantel accounted for 70% of the total residues in liver.
\(^g\) Estimate of total residues; after intramuscular administration, closantel accounted for 60% of the total residues in liver.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Observed residue (mg/kg parent drug)</th>
<th>Estimated daily intake&lt;sup&gt;b&lt;/sup&gt; (mg closantel equivalents)</th>
<th>Theoretical maximum daily intake&lt;sup&gt;h&lt;/sup&gt; (mg closantel equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oral dose&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Intramuscular dose&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Oral dose&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.19</td>
<td>0.29</td>
<td>0.06</td>
</tr>
<tr>
<td>Liver</td>
<td>0.16</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.6 )&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(5.6 )&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.16</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.83</td>
<td>1.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.0 )&lt;sup&gt;f&lt;/sup&gt;</td>
<td>(1.7 )&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.05</td>
</tr>
<tr>
<td>Fat</td>
<td>0.7</td>
<td>2.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.0 )&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(3.4 )&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05</td>
</tr>
<tr>
<td>Total</td>
<td>0.32</td>
<td>0.91</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Calculated from the observed residue levels.  
<sup>b</sup> Based on a daily intake of 0.5 kg of meat made up of 0.3 kg of muscle, 0.1 kg of liver, 0.05 kg of kidney, and 0.05 kg of fat.  
<sup>c</sup> 10 mg/kg of body weight, 28 days withdrawal time.  
<sup>d</sup> 2.5 mg/kg of body weight, 42 days withdrawal time.  
<sup>e</sup> Estimate of total residues; closantel accounted for 10% of the total residues in liver.  
<sup>f</sup> Estimate of total residues; closantel accounted for 80% of the total residues in kidney.  
<sup>g</sup> Estimate of total residues; closantel accounted for 70% of the total residues in fat.
Flubendazole was given orally in gelatin capsules to dogs at doses of 2.5, 10, or 40 mg per kg of body weight per day, 6 days a week for 3 months. Some atrophic changes and congestion of the epididymis were observed in the male genital tract at doses of 10 and 40 mg per kg of body weight per day, and atrophic changes occurred in the female genital tract at all doses. The changes in the female genital tract were considered to be within normal limits for dogs of the age of those used in the study. On histological examination of male sex organs, changes in the testes could not be clearly associated with flubendazole treatment. The findings in male dogs may not be compound-related, but because of the lack of conclusive evidence as to the cause of these changes, the Committee concluded that the no-observed-effect level (NOEL) was 2.5 mg per kg of body weight per day.

Carcinogenicity studies were performed in mice and rats at doses up to 30 and 20 mg per kg of body weight per day, respectively; no treatment-related effects were observed. There was no treatment-related increase in any type of neoplasm. The Committee was of the opinion that flubendazole had no carcinogenic potential at the highest doses administered in these studies.

The results from a range of in vitro and in vivo genotoxicity tests were all negative.

The Committee considered data from reproduction, embryotoxicity, and teratogenicity studies. Studies in mice, rabbits, and pigs were negative. Flubendazole was extensively studied in segmented reproduction studies in rats performed as required for human drug regulation purposes and accepted by the Committee in lieu of a multigeneration reproduction study. In several rat developmental studies, doses of up to 40 and 160 mg per kg of body weight per day, given on gestation days 6-15, did not produce any embryotoxic or teratogenic effects. In a rat teratogenicity study published in 1987, using material extracted from a commercial preparation, gross skeletal and internal fetal malformations were recorded at doses of 40 and 160 mg per kg of body weight per day. The NOEL in this study was 10 mg per kg of body weight per day.

An ADI of 0-12 µg per kg of body weight was established for flubendazole, based on the NOEL of 2.5 mg per kg of body weight per day in the 3-month study in dogs and a safety factor of 200. This safety factor was used by the Committee to take account of the fact that the doses were administered only 6 days per week in this study, the precise consequences of which could not be assessed.

The Committee noted that the ADI also provided a safety margin corresponding to a factor of about 1000 with respect to the NOEL of 10 mg per kg of body weight per day derived from the rat teratogenicity study. Furthermore, the Committee considered that further carcinogenicity studies would not be required, since the highest dose used in the negative studies that it had evaluated exceeded the ADI by a factor of approximately 2000.
**Residue data**

The Committee considered data on the metabolism of flubendazole and the depletion of flubendazole residues from the edible tissues of pigs, and on the depletion of flubendazole residues from the edible tissues and eggs of laying hens.

When pigs or poultry are treated with flubendazole, the tissue with the highest residue concentration and slowest depletion rate is the liver. The major metabolite in pig liver is (2-amino-1H-benzimidazol-5-yl)-4-fluorophenylmethanone, which is found at a much higher concentration than parent flubendazole. Residue concentrations are higher and more persistent in egg yolk than in egg white.

**Pigs.** A residue-depletion study was conducted using 18 feeder pigs given a dose of 1.5 mg per kg of body weight of [14C]flubendazole daily for 5 days (Table 4). Total residue concentrations were highest in liver throughout the 30-day withdrawal period.

Three male pigs received flubendazole at 30 mg per kg of body weight in the feed for 5 consecutive days. Flubendazole levels measured by high-performance liquid chromatography (HPLC) were less than 0.01 mg/kg (the sensitivity limit of the method) in plasma, liver, kidney, muscle, and fat at withdrawal times of 16, 30, and 54 hours.

A similar residue study was conducted using single oral doses of 5 mg per kg of body weight in three groups of five male pigs. Tissues and plasma were analysed using a radioimmunoassay with quantification limits of 1 μg/kg in plasma and 5 μg/kg in tissues. Animals were slaughtered in groups of five at 24, 72, and 168 hours after dosing. At 24 hours withdrawal time, the tissues contained 7-12 μg/kg of parent flubendazole. All residues were below the detection limit at 72 hours.

In another study, seven sows were treated with 30 mg per kg of body weight flubendazole in the diet for 10 consecutive days. The sows were

<table>
<thead>
<tr>
<th>Withdrawal time</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 hours</td>
<td>262</td>
<td>3865</td>
<td>2678</td>
<td>212</td>
</tr>
<tr>
<td>5 days</td>
<td>35.5</td>
<td>1863</td>
<td>435</td>
<td>50.1</td>
</tr>
<tr>
<td>10 days</td>
<td>10.5</td>
<td>529</td>
<td>78.2</td>
<td>16.3</td>
</tr>
<tr>
<td>16 days</td>
<td>8.66</td>
<td>433</td>
<td>76.6</td>
<td>15.6</td>
</tr>
<tr>
<td>23 days</td>
<td>8.67</td>
<td>194</td>
<td>49.9</td>
<td>13.5</td>
</tr>
<tr>
<td>30 days</td>
<td>2.51</td>
<td>106</td>
<td>22.5</td>
<td>3.38</td>
</tr>
</tbody>
</table>

For each withdrawal time, values are means for three pigs.
<table>
<thead>
<tr>
<th>Withdrawal time (days)</th>
<th>Plasma (mg/l)</th>
<th>Muscle (mg/kg)</th>
<th>Liver (mg/kg)</th>
<th>Kidney (mg/kg)</th>
<th>Fat (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.007</td>
<td>≤ 0.01</td>
<td>0.210</td>
<td>0.080</td>
<td>≤ 0.01</td>
</tr>
<tr>
<td>2</td>
<td>0.005</td>
<td>≤ 0.01</td>
<td>0.146</td>
<td>0.054</td>
<td>≤ 0.01</td>
</tr>
<tr>
<td>4</td>
<td>0.002</td>
<td>≤ 0.01</td>
<td>0.069</td>
<td>0.010</td>
<td>≤ 0.01</td>
</tr>
<tr>
<td>7</td>
<td>0.001</td>
<td>≤ 0.01</td>
<td>0.073</td>
<td>≤ 0.01</td>
<td>≤ 0.01</td>
</tr>
<tr>
<td>11</td>
<td>≤ 0.001</td>
<td>≤ 0.01</td>
<td>0.080</td>
<td>≤ 0.01</td>
<td>≤ 0.01</td>
</tr>
<tr>
<td>14</td>
<td>≤ 0.001</td>
<td>≤ 0.01</td>
<td>0.016</td>
<td>≤ 0.01</td>
<td>≤ 0.01</td>
</tr>
</tbody>
</table>

* For liver and kidney at 1 day withdrawal time, values are means for three animals; all other values are means for four animals.

Slaughtered 7 days after the last treatment with flubendazole. Mean levels of flubendazole measured by HPLC were 59, 67, 13, and 33 μg/kg for liver, kidney, muscle, and fat, respectively.

**Poultry.** A total of 28 laying hens received [14C]flubendazole at a dose equivalent to 30 mg per kg of body weight in food for 6 consecutive days. At all withdrawal times tested from 1 to 14 days after treatment, the concentration of radioactive equivalents of flubendazole in blood and plasma was less than 0.01 μg/ml, which suggests that absorption was poor. After total radioactivity levels reached a steady state in 5–6 days, eggs contained an average of 0.12 mg of flubendazole equivalents per kg. Radioactivity in the yolks (0.34 mg/kg) was much higher than in the egg white (0.02 mg/kg). The highest observed levels of radioactivity in individual tissues, calculated in terms of flubendazole equivalents, were 0.21 mg/kg in liver and 0.08 mg/kg in kidney 24 hours after the last dose. Table 5 shows residues in plasma and tissue for various withdrawal times.

When chickens were treated with flubendazole at 60 mg per kg of body weight for 7 days, residues were detectable in egg yolk for 11 days after treatment ended. Residue levels were higher in yolk than in white. Eggs and tissues were analysed by an HPLC method sensitive to 0.01 mg/kg. Of the tissues, liver had the greatest amount of residue at zero withdrawal time, although flubendazole could not be detected in any tissue by 6 and 7 days withdrawal time. The residue data are summarized in Table 6.

**Methods of analysis for residues in tissues**

For the studies described on pages 14–15, plasma and tissue levels of flubendazole in pigs were measured by radioimmunoassay or by an HPLC method with ultraviolet detection at 313 or 254 nm, which is sensitive to 0.01 or 0.02 mg/kg, respectively. Another HPLC method has been developed for flubendazole, with ultraviolet detection at 254 nm, that gives excellent separation between flubendazole and the major metabolite.
Table 6

Unchanged flubendazole (mg/kg) in eggs and tissues of chickens given flubendazole in the diet at 60 mg per kg of body weight for 7 days

<table>
<thead>
<tr>
<th>Withdrawal time (days)</th>
<th>Egg yolk</th>
<th>Egg white</th>
<th>Muscle</th>
<th>Kidney</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.592</td>
<td>0.036</td>
<td>0.079</td>
<td>0.173</td>
<td>0.198</td>
</tr>
<tr>
<td>4</td>
<td>NM</td>
<td>NM</td>
<td>0.071</td>
<td>0.236</td>
<td>0.200</td>
</tr>
<tr>
<td>6</td>
<td>NM</td>
<td>NM</td>
<td>≤ 0.01</td>
<td>≤ 0.01</td>
<td>≤ 0.01</td>
</tr>
<tr>
<td>7</td>
<td>0.318</td>
<td>≤ 0.01</td>
<td>≤ 0.01</td>
<td>≤ 0.01</td>
<td>≤ 0.01</td>
</tr>
<tr>
<td>11</td>
<td>0.019</td>
<td>Λ 0.01</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>28</td>
<td>NM</td>
<td>NM</td>
<td>≤ 0.01</td>
<td>≤ 0.01</td>
<td>≤ 0.01</td>
</tr>
</tbody>
</table>

NM: not measured.

resulting from carbamate hydrolysis. However, the method described applies to the analysis of pure substances and does not include extraction procedures for tissues.

An HPLC method that has detection limits of 20-50 μg/kg has been developed for simultaneously determining eight benzimidazoles in tissue. This method might be suitable for measuring flubendazole and the major metabolite found in pig tissue, (2-amino-1H-benzimidazol-5-yl)-4-fluorophenylmethanone. Typical recoveries from spiked samples (0.1 mg/kg) were above 70% for flubendazole in liver, kidney, and muscle.

Maximum Residue Limits

In reaching its decision on MRLs, the Committee took into account the following points:

- An ADI of 0-12 μg per kg of body weight was established. This would result in a maximum ADI of 720 μg for a 60-kg person.
- The marker residue is the parent drug for all tissues and for eggs.
- The total daily intake of flubendazole-related residues in food would be about 620 μg (see Table 7), if assumed to be accounted for by pig tissue and eggs at zero withdrawal time, and calculated on the basis of the data presented in Table 4 and the study in chickens treated with a dose of 30 mg per kg of body weight.

Eggs. The daily intake of flubendazole-related residues will probably remain below the ADI even when flubendazole is given at 60 mg per kg of body weight, although this dose produces a much higher concentration of residues in eggs. The argument that increased doses of flubendazole will not increase residue levels because of the drug's low systemic availability appears not to be valid for eggs: the levels of parent flubendazole in egg yolk found in the study with 60 mg per kg of body weight are double the residue levels of all flubendazole-related residues found in the study with 30 mg per kg of body weight.

An MRL for whole egg of 400 μg/kg of parent flubendazole is recommended.
Table 7
Estimated total daily intake of flubendazole-related residues in food

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Observed residue (mg/kg parent drug equivalents)</th>
<th>Estimated daily intake(^a) (µg parent drug equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>0.262(^b)</td>
<td>79</td>
</tr>
<tr>
<td>Liver</td>
<td>3.865(^b)</td>
<td>386</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.678(^b)</td>
<td>134</td>
</tr>
<tr>
<td>Fat</td>
<td>0.212(^b)</td>
<td>11</td>
</tr>
<tr>
<td>Eggs</td>
<td>0.12 (^c)</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>622</td>
</tr>
</tbody>
</table>

\(^a\) Calculated from the observed residue levels. Based on a daily intake of 0.5 kg of meat (made up of 0.3 kg of muscle, 0.1 kg of liver, 0.05 kg of kidney, and 0.05 kg of fat) and 0.1 kg of eggs.

\(^b\) Based on concentrations at 6 hours withdrawal time in a study in which pigs received an oral dose of [\(^14\)C]flubendazole at 1.5 mg per kg of body weight daily for 5 days.

\(^c\) Average concentration detected in eggs on day 6 of a study in which laying hens received [\(^14\)C]flubendazole in the diet at 30 mg per kg of body weight for 7 days.

*Poultry.* As no withdrawal period is required for poultry, parent flubendazole is an adequate marker residue. MRLs of 500 and 200 µg/kg are recommended for parent flubendazole in poultry liver and muscle, respectively.

*Pigs.* Although edible tissues from pigs require no withdrawal period from a human food safety perspective, a withdrawal period based on good practice in the use of veterinary drugs has been applied.

Parent flubendazole is the only analyte available as the marker residue for pig liver. Methods are available for determining flubendazole, and the residue data indicate that misuse can be detected by monitoring for parent flubendazole in pig tissue.

An MRL of 10 µg/kg is recommended for the parent compound in pig liver and muscle.

### 3.1.3 *Ivermectin*

Ivermectin (a mixture of ≥ 80% 22,23-dihydroavermectin B\(_{1a}\) (H\(_2\)B\(_{1a}\)) and ≤ 20% 22,23-dihydroavermectin B\(_{1b}\) (H\(_2\)B\(_{1b}\))) had previously been evaluated at the thirty-sixth meeting of the Committee (Annex 1, reference 97), when an ADI of 0-0.2 µg per kg of body weight was established, based on a NOEL of 0.1 mg per kg of body weight per day for maternal toxicity in the CF\(_1\) mouse and a safety factor of 500.

*Toxicological data*

The Committee reappraised the developmental toxicity of ivermectin and
reviewed the human data, including a large amount of new information made available since its thirty-sixth meeting. The information clearly demonstrates that the CF₁ mouse is extremely sensitive to the toxicity of ivermectin. In no species are there data to suggest that the drug is a teratogen in the absence of maternal toxicity. Various toxicity studies in rats and rabbits in addition to those in cattle, pigs, sheep, and horses emphasize the particular susceptibility of the CF₁ mouse to ivermectin toxicity. Despite the extremely wide use of ivermectin, there is no evidence of significant incidences of adverse effects on reproductive performance in treated animals and the very limited data on reproductive toxicity in humans indicate that ivermectin does not increase the incidence of birth defects.

The main effects noted in field and community-based trials with ivermectin in humans infected with *Onchocerca* spp. have been those arising from the death of the parasites, the so-called Mazzotti reaction, which is characterized by arthralgia, pruritus, fever, hypertension, tachycardia, headache, and ocular changes. Neither in these studies nor during ivermectin treatment of other parasitic diseases in humans in Africa, South America, the Caribbean, and certain other areas has a subset of atypically sensitive individuals been detected. Furthermore, the adverse effects experienced by the small number of persons accidentally exposed to doses (often of veterinary preparations) higher than customary human doses are in keeping with those noted in several test animal species.

In the light of the above findings, the Committee decided to amend its previous conclusions. Taking into account the absence of major effects in humans, the data indicating that the compound was a developmental toxicant rather than an overt teratogen, and the extreme sensitivity of the CF₁ mouse to these effects, the Committee concluded that a safety factor of 100 was justified. However, as the CF₁ mouse was the most sensitive species studied, the ADI should continue to be based on the NOEL of 0.1 mg per kg of body weight per day for maternal toxicity in that species. On this basis, an ADI of 0-1 μg per kg of body weight was established.

**Residue data**

Levels of ivermectin residues greater than expected after a withdrawal period of 35 days were observed in livers of heavy cattle (weighing approximately 450 kg) treated with injectable ivermectin in New Zealand. The original cattle residue study summarized in the report of the thirty-sixth meeting of the Committee (Annex 1, reference 9/) was conducted with cattle weighing approximately 260 kg. In view of these new data, a tissue-residue study with injectable ivermectin was conducted using cattle weighing 300-400 kg. Cattle in this weight range were considered the most appropriate population in which to assess tissue-residue depletion of anthelmintics given their use pattern in the cattle industry.

A total of 72 cross-bred beef cattle aged 12-14 months and weighing 297-401 kg were given a single subcutaneous injection of ivermectin at a dose of 0.3 mg per kg of body weight, after which 12 animals per
Table 8

Unchanged ivermectin (µg of H₂B₁₅₅ per kg) in tissues of cattle given a single subcutaneous injection of 0.3 mg per kg of body weight

<table>
<thead>
<tr>
<th>Withdrawal time (days)</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
<th>Injection site</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>4</td>
<td>46</td>
<td>4</td>
<td>29</td>
<td>NM</td>
</tr>
<tr>
<td>28</td>
<td>1</td>
<td>27</td>
<td>2</td>
<td>11</td>
<td>1280</td>
</tr>
<tr>
<td>35</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>6</td>
<td>576</td>
</tr>
<tr>
<td>42</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>570</td>
</tr>
<tr>
<td>49</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>231</td>
</tr>
<tr>
<td>56</td>
<td>NM⁹</td>
<td>NM⁹</td>
<td>NM⁹</td>
<td>NM⁹</td>
<td>NM</td>
</tr>
</tbody>
</table>

NM: not measured.

* For each withdrawal time, values are means for 12 animals.

⁹ These tissues were not assayed because the levels at the withdrawal time of 49 days were nearly undetectable.

withdrawal period were killed at 21, 28, 35, 42, 49, and 56 days. As in the previous study, the injection site, liver, and fat contained the greater part of the residues, as shown in Table 8.

The Committee used the residue chemistry data available at its thirty-sixth meeting, together with the new residue data, to recommend MRLs for cattle. The new ADI of 0-1 µg/kg of body weight means that the permitted maximum daily intake of ivermectin is 60 µg of total drug-related residue contributed by 500 g of food-animal meat in the diet of a 60-kg person. At 28 days withdrawal time, the intake of residues of ivermectin, calculated from the study in cross-bred beef cattle, is well below the ADI (see Table 9). Based on the data from the metabolism and residue studies, the Committee recommended MRLs for cattle of 100 µg/kg for liver and 40 µg/kg for fat as H₂B₁₅₅.

The recommended MRLs shown in Table 9 for liver and fat were set at levels high enough to ensure that, at 28 days withdrawal time, the 95% upper confidence limit for the 99th percentile of residue levels in cattle treated at 0.3 mg per kg of body weight of ivermectin would be below the MRL. With these recommended MRLs, therefore, the intake of residues of ivermectin is well below the ADI.

The concentrations of ivermectin at the injection site were considered significant from the point of view of their magnitude; however, the human toxicological data indicate that consumption of an injection site, which is extremely rare, would not result in an adverse health effect. In addition, the theoretical maximum daily intake of residues of ivermectin from muscle, liver, kidney, and fat is well below the ADI.

For species other than cattle, the MRLs allocated at the thirty-sixth meeting of the Committee still apply.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Observed residue(^a) (µg/kg H(<em>2)B(</em>{13}))</th>
<th>Estimated daily intake(^b,,c) (µg H(<em>2)B(</em>{13}) equivalents)</th>
<th>Recommended MRL (µg/kg H(<em>2)B(</em>{13}))</th>
<th>Theoretical maximum daily intake(^e) (µg H(<em>2)B(</em>{13}) equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>1 (1.5)^(e)</td>
<td>0.45</td>
<td>[2]^(e)</td>
<td>0.9</td>
</tr>
<tr>
<td>Liver</td>
<td>27</td>
<td>7.3</td>
<td>100</td>
<td>(270)^(l)</td>
</tr>
<tr>
<td>Kidney</td>
<td>2 (3.7)^(g)</td>
<td>0.19</td>
<td>[4]^(d)</td>
<td>0.4</td>
</tr>
<tr>
<td>Fat</td>
<td>11 (61)^(h)</td>
<td>3.05</td>
<td>40</td>
<td>(222)^(h)</td>
</tr>
<tr>
<td>Total</td>
<td>10.99</td>
<td></td>
<td></td>
<td>39.4</td>
</tr>
</tbody>
</table>

\(^a\) Subcutaneous dose of 0.3 mg/kg of body weight, 28 days withdrawal time.
\(^b\) Calculated from the observed residue levels.
\(^c\) Based on a daily intake of 0.5 kg of meat made up of 0.3 kg of muscle, 0.1 kg of liver, 0.05 kg of kidney, and 0.05 kg of fat.
\(^d\) For calculation purposes only; not a recommended MRL.
\(^e\) Estimate of total residues; H\(_2\)B\(_{13}\) accounted for 67\% of the total residues in muscle.
\(^f\) Estimate of total residues; H\(_2\)B\(_{13}\) accounted for 37\% of the total residues in liver.
\(^g\) Estimate of total residues; H\(_2\)B\(_{13}\) accounted for 54\% of the total residues in kidney.
\(^h\) Estimate of total residues; H\(_2\)B\(_{13}\) accounted for 18\% of the total residues in fat.
3.1.4 **Tiabledazole (thiabendazole)**

Tiabledazole had not been previously reviewed by the Committee, but was evaluated at the 1970 and 1977 joint FAO/WHO meetings on pesticide residues (3, 4). At the 1977 meeting, an ADI of 0-0.3 mg per kg of body weight was established. Tiabledazole is a benzimidazole compound used both as a broad-spectrum anthelminthic in various animal species and for the control of parasitic infestations in humans.

**Toxicological data**

Information from a range of studies on tiabledazole was available for assessment, including data on kinetics and metabolism, acute toxicity, short-term and long-term toxicity, reproductive and developmental toxicity, and genotoxicity and observations made in humans.

Toxicokinetic studies revealed rapid absorption after oral dosing in mice, rats, dogs, and humans, peak plasma levels occurring within 3 hours of drug administration. The drug was excreted in the urine and faeces. Elimination of tiabledazole and its degradation products appeared to be more rapid in humans than in mice, rats, and dogs, but metabolism was similar in mice, rats, and humans. The main urinary metabolites were the glucuronide and sulfate conjugates of 5-hydroxytiabledazole; there were also small amounts of unconjugated tiabledazole and the 5-hydroxy derivative. At very high doses, other minor metabolites were detected in rat and mouse urine.

Single oral doses of tiabledazole were slightly toxic (LD$_{50}$ ≥ 2000 mg per kg of body weight) in mice, rats, rabbits, sheep, and goats. Where deaths occurred, they appeared to be due to respiratory failure.

Following repeated oral administration of tiabledazole to mice, rats, dogs, chickens, sheep, pigs, and cattle, the most common finding was a reduction in food consumption and in body-weight gain. The effects on weight gain appeared to be greater than could be accounted for by the depressed food intake. Anaemia was seen in rats and dogs, and to some extent may have been related to poor nutrition. However, the presence of increased haemosiderosis in lymphoid tissues and reticulocytosis in some studies was suggestive of red cell destruction. The haematological changes occurred at dose levels of 100 mg per kg of body weight per day and above for 3 months in rats and 150 mg per kg of body weight per day and above for 3 months in dogs.

The major target organs for toxicity were the hepatic system, thyroid gland, and lymphoid organs. Liver hypertrophy was seen in a 3-month study in rats at doses of 37 mg per kg of body weight per day and above, and in a 2-year study in dogs minor hepatobiliary changes were observed at 50 mg per kg of body weight per day and above. Thyroid follicular-cell hyperplasia and colloid depletion were noted in a 3-month study in rats at 37 mg per kg of body weight per day and above and in a 16-week study in sheep at 800 mg per kg of body weight per day. The thymus, spleen, lymph nodes, and/or bone marrow exhibited lymphoid depletion or atrophy in a
28-day study in rats at 100 mg per kg of body weight per day and above and in a 16-week study in sheep at 800 mg per kg of body weight per day. Effects on blood cells, the liver, and lymphoid organs are commonly observed with benzimidazoles.

Less common observations included both renal tubular degeneration and hyperplasia in one 7-day study in mice at 250 mg per kg of body weight per day and above and in one 3-month study in rats at 100 mg per kg of body weight per day and above. Gastric changes, including mucosal degeneration, cytoplasmic rarefaction, and/or necrosis, were produced in a 3-month study in rats at 100 mg per kg of body weight per day and above. Atrial thrombosis was present at high incidence in a 2-year study in mice at doses of 200 mg per kg of body weight per day and above that resulted in the death of the affected animals.

Tumour incidences were not increased in a 2-year carcinogenicity study in mice. The NOEL was 6 mg per kg of body weight per day, and there was increased mortality at the next highest level, 60 mg per kg of body weight per day.

The incidence of preputial or clitorial gland adenomas was increased at a dose level of 200 mg per kg of body weight per day in one of two 2-year studies in rats. Statistical significance was achieved only in males; the animals also displayed poor development due to severe effects on weight gain, which suggests that the maximum tolerated dose was exceeded. The relevance of these tumours to low-dose exposure of humans to thiabendazole is questionable. The NOEL was 10 mg per kg of body weight per day, based on depressed weight gain at the next highest level, 40 mg per kg of body weight per day.

Genotoxicity tests were generally negative. In one of three Salmonella typhimurium reverse-mutation assays, positive results were obtained in strain TA98 only. Further investigation showed that an impurity in some batches was responsible for the mutagenic activity. The manufacturer indicated that no positive results had been obtained in tests on several hundred further batches. In one laboratory, micronuclei were induced in mouse bone marrow and abnormal anaphase-telophase figures were increased in cultured Chinese hamster ovary cells. The effects were seen only at relatively high levels and may be indicative of the tubulin-binding activity characteristic of benzimidazoles (Annex 1, reference 97, section 3.2.4). A range of other assays for mutation, DNA damage, and cytogenetic activity were clearly negative.

A multigeneration reproduction study in rats showed no adverse effects on reproduction parameters up to the highest dose tested, 80 mg per kg of body weight per day. In mice, litter size and survival during the lactation period were reduced at 750 mg per kg of body weight per day, but reproduction was unaffected at lower doses in a five-generation study. The NOEL was 30 mg per kg of body weight per day.

Developmental toxicity was examined in mice, rats, rabbits, and sheep. In a
series of studies in mice, embryotoxicity was observed at 1300 mg per kg of body weight per day and above, a dose that also resulted in the death of 5 of 39 females. Fetal malformations were produced at 240 mg per kg of body weight per day and above, the main abnormalities being a reduction deformity of the limbs, cleft palate, and fusion of vertebral arches and vertebral bodies. Unchanged tiabendazole was implicated as the probable embryotoxic and teratogenic agent.

The only finding in several rat developmental studies was a reduction in fetal weight at 40 mg per kg of body weight per day and above. The NOEL was 10 mg per kg of body weight per day.

In rabbits, abortions occurred at 600 mg per kg of body weight per day in one study on developmental toxicity, but not in the prior range-finding study at higher doses or in a repeat experiment in which similar treatment levels were used. Embryotoxicity and low incidences of domed head, hydrocephaly, and enlarged fontanelles were seen at 120 mg per kg of body weight per day and above in one study, but not in the repeat study at similar dose levels. All effects in rabbits were seen only at maternally toxic dose levels. The NOEL was 24 mg per kg of body weight per day.

No adverse reproductive effects were observed in pregnant ewes given doses of 400 mg per kg of body weight per day.

In a 24-week study in humans, male volunteers received 250 mg of tiabendazole per day; there were no treatment-related effects on a range of physical and biochemical parameters. The NOEL was 3–4 mg per kg of body weight per day. In another study, patients with trichinosis were given up to 3 g of tiabendazole daily for 10 days. Side-effects occurred in 14 of 23 patients, the most common being nausea, retching, and vomiting.

The Committee noted the availability of extensive data in support of the safety evaluation of tiabendazole, and the manufacturer indicated that further studies had been completed or were under way. At the 1977 joint FAO/WHO meeting on pesticide residues, the 24-week study in human volunteers was used to establish an ADI of 0–0.3 mg per kg of body weight. The Committee considered that the investigations undertaken as part of this human study were limited in nature, and that in view of the availability of a number of recent well conducted animal studies, the NOEL should be derived from the animal data.

The overall NOEL was 10 mg per kg of body weight per day based on reduced weight gain in a 2-year dietary study in rats and decreased fetal weight in a developmental study in rats. The Committee decided not to use the NOEL of 6 mg per kg of body weight per day from the 2-year study in mice, as the next administered dose was considerably higher at 60 mg per kg of body weight per day.

Using a safety factor of 100, the Committee established an ADI of 0–100 μg per kg of body weight for tiabendazole. The Committee noted that the ADI provided a safety margin corresponding to a factor of over 1000 with respect to the dose required for fetal malformations in mice.
The Committee asked to see the results from recently completed and ongoing toxicological studies in order to update the database on tiabendazole.

Residue data
The Committee considered data on the metabolism of tiabendazole and the depletion of residues of tiabendazole from the edible tissues of cattle, sheep, goats, and pigs. Residue-depletion studies have shown that tiabendazole is rapidly metabolized and excreted in all animal species and that concentrations of the drug and its metabolites in tissue and excreta decrease rapidly to control levels. The major metabolite is 5-hydroxytiabendazole, generally found as its glucuronide or sulfate ester.

Cattle. No detectable residues of tiabendazole were found in the liver, kidney, and muscle of cattle 3 days after oral doses of 50 or 110 mg per kg of body weight. Residue levels in fat approximated those found in muscle tissue.

In lactating animals, approximately 0.1% of an oral dose was detectable in milk within 60 hours. Tiabendazole and 5-hydroxytiabendazole were not detectable in the milk 60 hours after oral doses of 66, 110, or 220 mg per kg of body weight.

Sheep. No residues of the drug were found in muscle, liver, or kidney 7 days after oral administration of tiabendazole at 82 and 100 mg per kg of body weight to sheep.

Goats. Studies in goats that received radiolabelled tiabendazole showed that no drug residues were present 30 days after oral administration of 50 or 150 mg per kg of body weight. With a dose of 50 mg per kg of body weight, only liver and kidney had detectable residues of 1.1 and 2.7 mg/kg, respectively, 24 hours after dosing. At 17 days withdrawal time, no residue was found in the kidney, while a residue of 0.2 mg/kg remained in the liver.

Pigs. No residues of tiabendazole or related metabolites were found in pig tissues 7 days after a single oral dose of 100 mg per kg of body weight.

When tiabendazole was administered to pigs in the feed at 40 mg per kg of body weight per day for two weeks, no residues of the drug or its metabolites were found in muscle, liver, kidney, or fat at 7 days withdrawal time, and only minute amounts (0, 0.12, 0.19, and 0.17 mg/kg respectively) were detected after a 2-day withdrawal period.

Methods of analysis for residues in tissues and milk
In a spectrofluorometric method, tiabendazole and 5-hydroxytiabendazole were extracted from urine, tissue, plasma, and milk samples into ethyl acetate and then into hydrochloric acid. In liver and kidney samples, the glucuronide and sulfate esters of 5-hydroxytiabendazole were converted into 5-hydroxytiabendazole by means of β-glucuronidase and sulfatase, and then extracted. The percentage recovery of tiabendazole and 5-hydroxytiabendazole was high (89-101%) for values in the range
0.1–5 mg/kg for plasma and tissue. The determination limit in milk was approximately 0.05 mg/kg.

Two chromatographic methods that measure benzimidazoles in tissue are available, of which the first is a highly precise but time-consuming HPLC method with a detection limit of 20–50 µg/kg. The second method is a less precise but quicker liquid chromatographic method with a detection limit of 50 µg/kg.

**Maximum Residue Limits**

In reaching its decision on MRLs, the Committee took into account the following:

- An ADI of 0–100 µg per kg of body weight was established. This would result in a maximum ADI of 6 mg for a 60-kg person.
- The total residues of tiabendazole can be approximated by the sum of the levels of tiabendazole and 5-hydroxytiabendazole and their conjugates.
- The sum of the residue levels of tiabendazole and 5-hydroxytiabendazole generally decreases to below 0.1 mg/kg in all tissues and milk of animals within a few days of withdrawal. For a total daily food intake from 0.5 kg of edible tissues and 1.5 kg of milk containing 0.1 mg/kg of tiabendazole residues, the theoretical maximum daily intake for the veterinary uses of tiabendazole would be:

\[(0.1 \text{ mg/kg} \times 0.5 \text{ kg tissue}) + (0.1 \text{ mg/kg} \times 1.5 \text{ kg of milk}) = 0.2 \text{ mg}\]

This figure is much less than the value of 1.4 mg obtained by calculating theoretical maximum daily intakes based on other agricultural uses of tiabendazole (5) using the *Guidelines for predicting dietary intake of pesticide residues* (6). The theoretical maximum daily intake from all uses of tiabendazole corresponds to 27% of the maximum ADI.

- At the 1975 joint FAO/WHO meeting on pesticide residues, MRLs had been set for the edible tissues of cattle, goats, horses, pigs, and sheep at 0.1 mg/kg for the sum of tiabendazole and 5-hydroxytiabendazole.

The Committee recommended MRLs for tiabendazole plus 5-hydroxytiabendazole of 100 µg/kg for all edible tissues of cattle, goats, pigs, and sheep and milk of cattle and goats. Tiabendazole and 5-hydroxytiabendazole are joint marker residues.

**3.1.5 Triclabendazole**

Triclabendazole had not been previously reviewed by the Committee. It is a benzimidazole used for the control of liver fluke infestation in sheep, goats, and cattle.

**Toxicological data**

A range of studies on triclabendazole was submitted for assessment, including data on kinetics and metabolism, acute toxicity, short-term and
long-term toxicity, reproductive and developmental toxicity, and genotoxicity.

Approximately 40-50% was absorbed following the administration of single oral doses of radiolabelled triclabendazole to rats and dogs. Oral absorption was considerably higher in rabbits but could not be quantified in a human study. Peak plasma levels of radioactivity were generally achieved within 8 hours; elimination (primarily in the faeces) was rapid in rats, rabbits, and humans. In dogs, peak plasma levels of radioactivity were maintained for 2-3 days and significant levels persisted for more than 7 days. Triclabendazole was rapidly and extensively degraded in rabbits, dogs, and humans, the sulfoxide and sulfone being the main metabolites found in plasma. In addition to these oxidation products, rat excreta contained 4-hydroxytriclabendazole and 2-benzimidazolone.

Single oral doses of triclabendazole were slightly toxic (LD₅₀ > 8000 mg per kg of body weight) in mice and rats and moderately toxic (LD₅₀ = 206 mg per kg of body weight) in rabbits, whose greater sensitivity was probably due to a greater bioavailability of oral doses.

Findings after short-term oral dosing in rats and dogs were primarily non-specific. In 3-month studies, decreased weight gain was observed at 7 mg per kg of body weight per day and above in rats and at 37 mg per kg of body weight per day in dogs; anaemia was seen at 68 mg per kg of body weight per day and 37 mg per kg of body weight per day, respectively. Evidence of hepatotoxicity, for example increases in plasma levels of liver enzymes and cholesterol and in liver weight, was seen at approximately 70 mg per kg of body weight per day in rats and 37 mg per kg of body weight per day in dogs. Minor non-neoplastic liver lesions were observed in 3-month study in dogs at 37 mg per kg of body weight per day. The NOELs were 0.7 mg per kg of body weight per day in rats and 0.35 mg per kg of body weight per day in dogs.

Hepatomas were seen in female mice given up to 29 mg per kg of body weight per day for 2 years. The incidences of adenomas were higher than in controls at all dose levels, but a dose-response relationship was not found. Significance at the 99% level, which is widely used to assess the significance of tumours that occur at a high background rate, was not reached for this common benign mouse tumour. In addition, there was no significant increase in the incidence of hepatocellular carcinomas at any dose level. The NOEL was 0.27 mg per kg of body weight per day based on increased liver weight at the next highest dose, 1.4 mg per kg of body weight per day. Tumours were not induced in a 2-year dietary study in rats, the NOEL being 1.2 mg per kg of body weight per day based on reduced weight gain at 4 mg per kg of body weight per day. Several different in vitro and in vivo genotoxicity studies were clearly negative. These results suggest that triclabendazole has no carcinogenic potential.

In a two-generation reproduction study in rats, postnatal survival and growth were reduced during the lactation period of the second generation at 1 mg per kg of body weight per day and above. However, the incidence of
these effects was not dose-related and was similar to that in the first-generation offspring. In addition, statistical significance was not achieved, and it was concluded that the findings were not related to treatment. The NOEL was 5.5 mg per kg of body weight per day.

Developmental studies in rats and rabbits provided no evidence of embryotoxicity. Fetotoxicity, in the form of reduced fetal weight in rats and decreased ossification in rabbits, was observed at 100 and 10 mg per kg of body weight per day, respectively. From a group of 20 female rabbits given triclabendazole at 20 mg per kg of body weight per day, one fetus exhibited omphalocele, a rare abnormality in the strain used. All the above effects were present only at maternally toxic dose levels. Drug treatment during pregnancy in sheep and cattle did not affect growth and development of the conceptus. The NOELs were 50 mg per kg of body weight per day in rats and 3 mg per kg of body weight per day in rabbits.

Special studies with triclabendazole in combination with either fenbendazole (developmental and genotoxicity studies) or levamisole (acute, subchronic, developmental, and genotoxicity studies) did not reveal any synergism between the drugs.

The database in support of the safety evaluation of triclabendazole is substantial. The lowest NOEL was 0.27 mg per kg of body weight per day, based on increased liver weight in the long-term study in mice. Using a safety factor of 100, the Committee established an ADI for triclabendazole of 0–3 μg per kg of body weight.

Residue data
The absorption, distribution, metabolism, and excretion of triclabendazole have been studied and are qualitatively similar in both cattle and sheep. As with several other benzimidazoles, the use of triclabendazole in food-producing animals results in a large portion of the total residue being bound to endogenous tissue, the ratio of bound residue to total residue increasing with increasing withdrawal periods. The marker residue for triclabendazole is 5-chloro-6-(2',3'-dichlorophenoxy)-benzimidazole-2-one and is produced when common fragments of triclabendazole-related residues are hydrolysed under alkaline conditions at 90–100°C. As the marker residue does not represent total residues, the ratio of marker residue to total residue needs to be determined for each species. Marker residue levels can be converted into triclabendazole equivalents by multiplying by a conversion factor of 1.09.

Cattle. Two ruminating heifers were treated with a single oral dose of [14C]triclabendazole of 12 mg per kg of body weight. The animals were slaughtered 28 and 42 days after dosing. Total residue concentrations are shown in Table 10.

In three separate residue-depletion studies, cattle were dosed orally with triclabendazole at 12 mg per kg of body weight. The concentrations of marker residue were determined in the edible tissues at various withdrawal times. A common withdrawal time for all three studies was 28 days. The
average levels ($n = 8$) of the marker residue at this withdrawal time were 0.11, 0.12, 0.07, and 0.05 mg per kg in muscle, liver, kidney, and fat, respectively. These values represent 84, 50, 66, and >100% of the total residue in the respective tissues at that withdrawal time.

_**Sheep and goats.**_ A sheep and a goat were each treated once orally with [14C]triclabendazole at 10 mg per kg of body weight. The animals were slaughtered 10 days after dosing. Residues were extracted after thorough alkaline solubilization of the tissues and partitioning into methylene chloride. Total radioactivity data are given in Table 11.

In two separate residue-depletion studies, sheep were dosed orally with triclabendazole at 10 mg per kg of body weight. The quantities of marker residue in the edible tissues were determined at various withdrawal times. Common withdrawal times in both studies were 7, 14, and 28 days. If the residue levels at 7 and 14 days are averaged to give approximately those
that would have been expected at 10 days in the radiolabel study, the average values (n = 10 for kidney, muscle, and fat; n = 9 for liver) of the marker residue were 0.19, 0.44, 0.25, and 0.04 mg/kg in muscle, liver, kidney, and fat, respectively. These values represent 33, 24, 23, and 51% of the triclabendazole-related total residues observed in the respective tissues in the radiolabel study. With these percentages and concentrations of marker residue at 28 days withdrawal time of 0.116, 0.097, 0.076, and <0.03 mg/kg in the respective tissues, the estimated daily intake of triclabendazole-related residues (based on consumption of 0.5 kg of meat) is approximately 159 μg.

Methods of analysis for residues in tissues
Triclabendazole residues can be measured by an HPLC method combined with ultraviolet absorbance detection. The method measures residues of triclabendazole hydrolysable and oxidizable to 5-chloro-6-(2,3-di-chlorophenoxy)-benzimidazole-2-one. Tissue samples are hydrolysed under alkaline conditions at 90–100°C and the entire hydrolysate is extracted with methylene chloride under acidic conditions. The detection limit for marker residue is 0.027 mg/kg or 0.029 mg triclabendazole/kg (conversion factor = 1.09).

Maximum Residue Limits
In reaching its decision on the MRLs for triclabendazole, the Committee took into account the following:

• An ADI of 0–3 μg per kg of body weight was established. This would result in a maximum ADI of 180 μg for a 60-kg person.
• In view of the vigorous extraction conditions required in the method for the marker residue, it probably measures more residues than those usually defined as extractable residues. However, the method does not measure total residues.

Recommended MRLs for triclabendazole in cattle and sheep are given in Tables 12 and 13, respectively.

The Committee noted that additional data would be required if the MRLs for triclabendazole in sheep were to be increased. More accurate estimates of the total residues in edible tissues of sheep, and of the ratio of total residue concentrations to marker residue concentrations are needed. Since the recommended MRLs account for most of the ADI at 28 days withdrawal time, bioavailability studies on the bound residue of triclabendazole may be needed to estimate the amount of residue of toxicological concern.
Table 12
Recommended MRLs for triclabendazole in cattle

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Observed residue* (µg/kg marker residue)</th>
<th>Estimated daily intakeb,c (µg triclabendazole equivalents)</th>
<th>Recommended MRL (µg/kg marker residue)</th>
<th>Theoretical maximum daily intakec (µg triclabendazole equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>110 (131)d</td>
<td>39</td>
<td>200 (238)d</td>
<td>71</td>
</tr>
<tr>
<td>Liver</td>
<td>120 (240)e</td>
<td>24</td>
<td>300 (600)e</td>
<td>60</td>
</tr>
<tr>
<td>Kidney</td>
<td>70 (106)f</td>
<td>5</td>
<td>300 (454)f</td>
<td>23</td>
</tr>
<tr>
<td>Fat</td>
<td>50</td>
<td>2</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>70</td>
<td></td>
<td>159</td>
</tr>
</tbody>
</table>

* Oral dose of 12 mg/kg of body weight, 28 days withdrawal time.

b Calculated from the observed residue levels.

c Based on a daily intake of 0.5 kg of meat made up of 0.3 kg of muscle, 0.1 kg of liver, 0.05 kg of kidney, and 0.05 kg of fat.

d Estimate of total residues; the marker residue accounted for 84% of the total residues in muscle.

e Estimate of total residues; the marker residue accounted for 50% of the total residues in liver.

f Estimate of total residues; the marker residue accounted for 66% of the total residues in kidney.
Table 13
Recommended MRLs for tricylambendazole in sheep

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Observed residue</th>
<th>Estimated daily intake</th>
<th>Recommended MRL</th>
<th>Theoretical maximum daily intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(µg/kg marker residue)</td>
<td>(µg tricylambendazole equivalents)</td>
<td>(µg/kg marker residue)</td>
<td>(µg tricylambendazole equivalents)</td>
</tr>
<tr>
<td>Muscle</td>
<td>110 (333)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100</td>
<td>100 (303)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>91</td>
</tr>
<tr>
<td>Liver</td>
<td>97 (404)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40</td>
<td>100 (417)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>42</td>
</tr>
<tr>
<td>Kidney</td>
<td>76 (330)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16</td>
<td>100 (435)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>22</td>
</tr>
<tr>
<td>Fat</td>
<td>&lt;30 (59)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3</td>
<td>100 (196)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>159</td>
<td></td>
<td>165</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Oral dose of 10 mg/kg of body weight, 28 days withdrawal time.
<sup>b</sup> Calculated from the observed residue levels.
<sup>c</sup> Based on a daily intake of 0.5 kg of meat made up of 0.3 kg of muscle, 0.1 kg of liver, 0.05 kg of kidney, and 0.05 kg of fat.
<sup>d</sup> Estimate of total residues; the marker residue accounted for 33% of the total residues in muscle.
<sup>e</sup> Estimate of total residues; the marker residue accounted for 24% of the total residues in liver.
<sup>f</sup> Estimate of total residues; the marker residue accounted for 23% of the total residues in kidney.
<sup>g</sup> Estimate of total residues; the marker residue accounted for 51% of the total residues in fat.
3.2 Antimicrobial agents

3.2.1 Furazolidone

Furazolidone had not been previously evaluated by the Committee. It is a nitrofuran derivative used both therapeutically and prophylactically as an antimicrobial agent in poultry, pigs, rabbits, and fish.

Toxicological data

The Committee considered data from pharmacodynamic, pharmacokinetic, metabolism, acute and short-term toxicity, carcinogenicity, genotoxicity, reproductive and teratogenicity studies as well as special studies on endocrine function and some clinical studies in humans.

The distribution, excretion, and biotransformation of radiolabelled furazolidone were studied in rats, chickens, pigs, and humans. After oral administration, furazolidone was rapidly absorbed and the radioactivity was widely distributed, the highest levels being found in liver, kidney, fat, and muscle. It was rapidly metabolized and excreted predominantly in urine. In chicken and human urine, only trace amounts of unchanged furazolidone could be detected, and of the large number of metabolites found only some were identified. In rat and pig urine, the common metabolite appeared to be the open chain cyanometabolite 3-(4-cyano-2-oxobutylideneamino)-2-oxazolidone. The Committee noted that quantitative information on the metabolites was lacking. In pigs, a substantial portion of the metabolites was bound to macromolecules, and it appeared that approximately 15-40% of this bound fraction was bioavailable. However, the Committee questioned the validity of the extraction procedures used to isolate these bound metabolites.

In acute oral toxicity studies in mice and rats, furazolidone was slightly toxic; the LD$_{50}$ values were of the order of 1100 and 1500 mg per kg of body weight, respectively.

No NOEL could be established from short-term studies performed with rats and dogs. Rats receiving furazolidone in the diet at doses in the range 0.5-50 mg per kg of body weight per day showed hypertrophy of liver cells. Palpable mammary tumours and a decrease in body-weight gain were observed at 50 mg per kg of body weight per day. In dogs, oral dose levels of 5-25 mg per kg of body weight per day led to neurological symptoms and histological changes in the basal ganglia, together with testicular degeneration. It was noted that the available information was deficient by current standards and poorly reported.

Two three-generation reproduction studies were performed in rats. In one study, rats were exposed to furazolidone at concentrations up to 100 mg/kg in feed. In the other study, only female rats were treated with diets containing 500 mg/kg, but this concentration was gradually reduced to 250 mg/kg in order to avoid the observed growth depression. No effects on reproductive performance were observed in either study. The NOEL was equivalent to 12.5 mg per kg of body weight per day.
In a special study designed to evaluate the effects on the male reproductive system, rats exposed to a dietary furazolidone concentration equivalent to 33 mg per kg of body weight per day exhibited testicular degeneration. At 16 mg per kg of body weight per day, no effects were observed.

Neither embryotoxicity nor teratogenicity was observed in rabbits after oral administration of furazolidone at a dose of 30 mg per kg of body weight per day.

A carcinogenicity study was conducted in Swiss MBR/ICR mice, which received a diet containing concentrations of furazolidone equal to average daily doses of 12, 24, or 47 mg per kg of body weight per day for 13 months, followed by a control diet for 10 months. In the mid- and high-dose groups, a significant increase in the incidence of bronchial adenocarcinomas was observed in both sexes, and the incidence of lymphosarcomas was significantly increased in male mice.

In two long-term toxicity/carcinogenicity studies, furazolidone was administered in the diet to Fischer 344 and Sprague-Dawley rats at concentrations equivalent to daily doses of 12.5, 25, or 50 mg per kg of body weight per day for 20 months. In Fischer 344 rats, a significant increase in the incidence of mammary gland adenocarcinomas was observed in females in the high-dose group. In addition, an increased incidence of sebaceous gland adenomas and thyroid adenomas was observed in both sexes at 25 and 50 mg per kg of body weight per day and of basal-cell epithelioma and carcinoma in males of the high-dose group. In the high-dose group of Sprague-Dawley rats, significantly increased incidences were reported for mammary adenocarcinomas in females and for neural astrocytomas in males. In both strains of rats, female animals showed a significant increase in the incidence of mammary neoplasms (benign and malignant combined) at all dose levels, but without a dose-response relationship.

Furazolidone has been tested in a wide variety of genotoxicity studies. Positive findings were recorded in bacterial assays with and without metabolic activation, in the sex-linked recessive lethal test in *Drosophila melanogaster*, in a gene-mutation assay with mammalian cells *in vitro*, in a sister chromatid exchange test, and in two DNA-repair tests. Positive as well as negative results were obtained in chromosome aberration assays with mammalian cells *in vitro*, and in tests for unscheduled DNA synthesis. One mouse micronucleus test was negative, while another gave equivocal results.

The majority of *in vitro* genotoxicity tests with postulated metabolites gave negative results; however, nitrofuraldehyde and urine from furazolidone-treated rats gave positive results. It was concluded that furazolidone was genotoxic *in vivo*.

Several studies were performed on the endocrine effects of furazolidone. Furazolidone inhibited the conversion of progesterone into corticosterone in adrenal cells both *in vivo* and *in vitro*. It has been hypothesized that
disturbances of steroidogenesis constitute the underlying mechanism for the increased incidence of tumours caused by furazolidone. The Committee noted that it was unlikely that such a mechanism could account for the increase in neural astrocytomas and uncommon skin tumours in rats. With respect to the occurrence of mammary tumours, no information was available on the effect of furazolidone on plasma progesterone concentrations, and no consistent effects on plasma prolactin concentrations were observed. The Committee therefore concluded that no support had been provided for the hypothesized mechanism.

Furazolidone caused reversible inhibition of monoamine oxidase (MAO) activity in pig hepatocytes in vitro and in liver and brain tissue of rats following in vivo administration. Irreversible MAO inhibition both in vitro and in vivo was observed for the postulated metabolites amino-oxazolidone and hydroxyethylhydrazine.

On the basis of the positive effects of furazolidone in genotoxicity tests in vitro and the increased incidence of malignant tumours in mice and rats, the Committee concluded that furazolidone was a genotoxic carcinogen. Since the drug is rapidly and extensively metabolized, the Committee also considered information on the metabolites of furazolidone. Although a large number of postulated metabolites produced negative results in genotoxicity tests, it was noted that only a few of these had been either identified or quantified in rats and pigs. Furthermore, the Committee concluded that insufficient data were available on the nature and toxic potential of compounds released from the bound residues.

Because of the genotoxic and carcinogenic nature of furazolidone and the above-mentioned deficiencies with respect to the data on the metabolites, the Committee was unable to establish an ADI.

Before considering the compound again, the Committee would wish to have detailed information on the nature, quantity, and toxicity of the metabolites of furazolidone, including the bound residues.

Residue data
Following oral administration to pigs or chickens, furazolidone is partially metabolized in the gastrointestinal tract. The parent drug and its metabolites are well absorbed and extensively metabolized in the tissues. The major route of excretion is via the urine (see also page 32). The parent drug has a short half-life in vivo and continues to degrade in postmortem tissues. It is either not found as a residue in tissues or found at very low concentrations at zero withdrawal time. Nevertheless, the parent drug is occasionally detectable in tissues at slightly longer withdrawal times.

The concentration of total residues of furazolidone in pigs (given 300 mg/kg in the diet for 10-14 days; 14 days withdrawal time) and chickens (given 220 mg/kg in the diet for 4 days; 3 days withdrawal time) is in the mg/kg range, with less than 0.001% being parent drug. Most of the residues are polar compounds in either the extractable or the bound form.
Table 14
Total residues (mg of furazolidone equivalents per kg) in tissues of pigs given \([^{14}C]\)furazolidone at 300 mg per kg of feed for 14 days

<table>
<thead>
<tr>
<th>Withdrawal time (days)</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13.2</td>
<td>41.1</td>
<td>34.4</td>
</tr>
<tr>
<td>21</td>
<td>3.3</td>
<td>4.4</td>
<td>3.4</td>
</tr>
<tr>
<td>45</td>
<td>2.4</td>
<td>2.2</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Numerous metabolites have been either identified or suggested following \textit{in vitro} and \textit{in vivo} studies in pigs, rats, and rabbits. Nevertheless, no major metabolite has been identified that would qualify as a marker residue.

\textit{Pigs.} Several radiometric studies have been carried out in pigs using \([^{14}C]\)furazolidone labelled in either the formyl or the methylene group. The results showed that:

- residues of parent drug were at extremely low levels or absent;
- most of the residues were polar metabolites; and
- a significant portion of the residues was non-extractable.

In the most recent study, pigs were fed \([^{14}C]\)furazolidone at 300 mg/kg in feed for 14 days and killed at 0, 21, and 45 days withdrawal time. The total levels of residues are shown in Table 14.

In another study, residues of parent drug were measured in tissues following treatment of pigs for 8 days with 300 mg/kg furazolidone in medicated feed. At zero withdrawal time, residues were either not detected or were present at low \(\mu g/kg\) concentrations. No residues of the parent drug were detected at longer withdrawal times.

\textit{Cattle.} After treatment of calves with furazolidone-medicated feed at 16 mg per kg of body weight per day for 5 days, residues of parent drug (33 \(\mu g/kg\)) were detected in muscle at zero withdrawal time but not at longer withdrawal times.

\textit{Poultry.} Chickens were given \([^{14}C]\)furazolidone (methylene label) at 220 mg/kg in feed for 4 days. The chickens were killed at various withdrawal times and the total residues determined; the results are shown in Table 15. In another study, chickens were killed during a 21-day period of drug administration and tissue residues were determined. Drug residues reached a maximum after 8 days of furazolidone administration and then remained constant for the duration of the dosing period.

After treatment of chickens for 10 days with furazolidone at 440 mg/kg in medicated feed, residues of parent drug were detected in low \(\mu g/kg\) concentrations for up to 4 days in muscle and 5 days in eggs.

\textit{Bound residues.} The amounts of extractable and bound residues were measured in selected liver and muscle samples taken during the

35
Table 15
Total residues (mg of furazolidone equivalents per kg) in tissues of chickens given [14C]furazolidone at 220 mg per kg of feed for 4 days*

<table>
<thead>
<tr>
<th>Withdrawal time (days)</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.40–6.08</td>
<td>18.6, 21.1</td>
<td>22.1, 20.8</td>
<td>3.53</td>
</tr>
<tr>
<td>1.5</td>
<td>1.58–1.89</td>
<td>20.8, 22.1</td>
<td>4.06</td>
<td>1.11</td>
</tr>
<tr>
<td>3</td>
<td>0.73–1.16</td>
<td>3.34, 3.64</td>
<td>2.31, 2.82</td>
<td>1.09, 1.16</td>
</tr>
<tr>
<td>5</td>
<td>0.70–0.87</td>
<td>2.32</td>
<td>1.39</td>
<td>NM</td>
</tr>
<tr>
<td>8</td>
<td>0.54–0.68</td>
<td>1.08</td>
<td>0.90</td>
<td>1.23</td>
</tr>
<tr>
<td>11</td>
<td>0.44–0.48</td>
<td>0.87</td>
<td>0.58</td>
<td>1.46</td>
</tr>
</tbody>
</table>

NM: not measured.
* The values for muscle are the range for residues in three different muscles taken from two birds at each time point. All other values are single determinations.

Table 16
Concentration and bioavailability of extractable and bound residues (mg/kg) in tissues of pigs given [14C]furazolidone at 300 mg per kg of feed for 14 days

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Withdrawal time (days)</th>
<th>Total residues</th>
<th>Extractable residues</th>
<th>Bioavailable extractable residue</th>
<th>Bound residues</th>
<th>Bioavailable bound residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>0</td>
<td>41.1</td>
<td>18</td>
<td>10.4</td>
<td>23</td>
<td>7.15</td>
</tr>
<tr>
<td>Liver</td>
<td>45</td>
<td>2.2</td>
<td>0.18</td>
<td>0.09</td>
<td>1.97</td>
<td>0.32</td>
</tr>
<tr>
<td>Muscle</td>
<td>45</td>
<td>2.4</td>
<td>0.33</td>
<td>0.22</td>
<td>2.07</td>
<td>0.77</td>
</tr>
</tbody>
</table>

experiments in pigs treated with [14C]furazolidone-medicated feed at 300 mg/kg for 14 days. The non-extractable radioactive residues were measured in the fraction remaining after extraction of the tissues with solvents. The results of these studies are shown in Table 16.

A large portion of the total residues was in the bound fraction and the percentage, although not the absolute amount, of bound residues increased with withdrawal time.

**Bioavailability.** The bioavailability of the bound residues was measured by feeding to rats lyophilized pig tissues from the [14C]furazolidone study mentioned above and measuring the amount of radioactivity absorbed and excreted by the rats. A summary of the results is shown in Table 16.

In another study, bioavailability was similarly determined by feeding to rats the non-extractable fraction of muscle tissues from piglets fed radiolabelled furazolidone for 10 days and slaughtered at zero withdrawal time. Approximately 41% of the residues was bioavailable.
The results for bioavailability indicate that:
- the extractable residues are not all bioavailable; and
- the fraction of bound residues that is bioavailable is in the range 16-41%.

No information was available on the toxic potential of the bioavailable bound residues.

**Methods of analysis for residues in tissues**

At present there is no recognized marker residue for furazolidone residues in animal tissue. The position is further complicated by the degradation of parent drug in postmortem tissues, so that it is rarely found as a residue. The Committee was made aware of results of studies that showed that residues of furazolidone degrade in deep-frozen tissues obtained from both pigs and cattle.

Furazolidone is also unstable on exposure to light. Nevertheless, many countries regulate use of the drug by determining furazolidone as the parent drug. Many satisfactory methods are available for the routine screening or quantification of residues of the parent drug; they have detection limits of about 0.2-2 μg/kg and are well validated for accuracy and precision. Investigations are under way on the possible conversion of drug residues into 3-amino-2-oxazolidone for use as a marker residue. Residues of 3-amino-2-oxazolidone were detected in pig liver at up to 45 days withdrawal time and represented between 15% and 25% of the bound residues.

The Committee did not recommend an MRL because:
- no ADI was established;
- the residue data presented to the Committee were not sufficient for it to identify a marker residue; and
- insufficient information was available on the quantity and nature of the total residues.

### 3.2.2 Nitrofurazone

Nitrofurazone (also known as nitrofurazone) is a broad-spectrum antimicrobial drug used both therapeutically and prophylactically in a number of food-producing species including pigs, sheep, goats, cattle, chickens, and turkeys. It had not been previously reviewed by the Committee.

**Toxicological data**

The results of acute and short-term toxicity studies, carcinogenicity studies, and teratogenicity studies were considered by the Committee, in addition to several genotoxicity studies. Some limited pharmacokinetic data were also available which demonstrated good absorption of the drug in rats and cattle, but no data were available on the extent of biotransformation nor on the identities of any metabolites formed.
comparison with other nitrofurans suggests that nitrofural probably undergoes extensive biotransformation.

In acute oral toxicity studies, nitrofural was slightly toxic to mice (LD₅₀ = 300-600 mg per kg of body weight) and rats (LD₅₀ = 600-800 mg per kg of body weight).

Testicular hypoplasia was noted in rats and mice given dietary nitrofural for 13 weeks. The NOEL for this effect was 15 mg per kg of body weight per day in a 13-week study in rats.

Nitrofural was tested for its ability to induce forward mutations in bacteria, yeast, and mammalian cells in vitro. In the majority of these studies it produced positive results. Positive results were also noted in tests for DNA damage in bacteria and in mammalian cells in vitro, in in vitro studies for clastogenic effects in Chinese hamster ovary or bone marrow cells, and in human lymphocytes. However, in vivo studies of clastogenic potential in Chinese hamster or rat bone marrow were negative. The results suggest that nitrofural is genotoxic in vitro but not in vivo.

Carcinogenicity studies with nitrofural were conducted in rats and mice. In a dietary study in B6C3F₁ mice, the animals were given nitrofurazol equal to daily oral doses of 14 and 29 mg per kg of body weight per day. In female mice, nitrofurural induced a high incidence of ovarian atrophy and of ovarian tubular-cell hyperplasia in both dose groups when compared with controls. There were also increased incidences of ovarian granulosa-cell and benign mixed tumours in low-dose and high-dose groups. There were no elevated incidences of any tumour type in male mice.

In a 2-year carcinogenicity study in rats, testicular degeneration was one of the major non-neoplastic findings. There were also elevated incidences of degeneration of the joint articular cartilages at several sites in both males and females. No-effect levels could not be identified for these findings. There was an increased incidence of mammary fibroadenomas in females given nitrofurinal in the diet at the low and high doses of 11 and 24 mg per kg of body weight per day.

Teratogenicity studies were conducted in mice and rabbits. In mice given nitrofurinal in the diet, the rate of late fetal death was increased in treated animals at maternally toxic doses; the NOEL was 11 mg per kg of body weight per day. Pregnant rabbits were given gavage doses of up to 20 mg per kg of body weight per day. At the highest dose, there was an elevated incidence of minor fetal malformations per litter, but this dose produced maternal toxicity. The NOEL was 15 mg per kg of body weight per day. The data suggest that nitrofurinal is not a direct teratogen but produces fetotoxic effects at maternally toxic doses in mice and rabbits.

Although nitrofurinal was tumorigenic in rats and mice, the tumours it produced were benign and were restricted to endocrine organs and the mammary gland. Moreover, the mutagenicity studies suggest that nitrofural is genotoxic in vitro but not in vivo. Taken together, the data suggest that nitrofurual is a secondary carcinogen producing its effects in
endocrine-responsive organs by a mechanism that remains to be elucidated. In rats, nitrofurantoin has been shown to disrupt the utilization of progesterone and blocks the synthesis of cortisone from deoxycorticosterone. Effects on steroidogenesis may therefore be involved in the process of tumour formation. The closely related compound nitrofurantoin also produced benign ovarian tumours in B6C3F1 mice, which were thought to be secondary to drug-induced ovarian atrophy (7). This type of effect cannot be excluded in the biogenesis of the ovarian tumours produced by nitrofurantoin. However, the Committee recognized that no-effect levels for these effects could not be identified. The Committee noted that nitrofurantoin had been reviewed by the International Agency for Research on Cancer, which concluded that there was limited evidence for the carcinogenicity of nitrofurantoin in animals but inadequate evidence in humans (8).

The Committee concluded that it could not establish an ADI for nitrofurantoin because no-effect levels had not been established for the tumorigenic effects. It noted that, although a no-effect level (15 mg per kg of body weight per day) had been established in rats for testicular hypoplasia in a 13-week study, a slightly lower dose (11 mg per kg of body weight per day) resulted in a high incidence of testicular degeneration in the 2-year carcinogenicity study. There was no NOEL for this effect in the 2-year study and, moreover, no study on reproductive performance was available. The degenerative changes in the joints of rats were seen in both males and females but a no-effect level could not be established.

Before reviewing nitrofurantoin again the Committee would wish to see:

1. Further data from long-term studies in rats which would allow the identification of no-effect levels for the effects on joint articular cartilages and for testicular degeneration.
2. Data to support the view that tumour formation in rodents following nitrofurantoin administration has an endocrine origin. If this were demonstrated, no-effect levels for suitable end-points would need to be identified.
3. Additional data on the identity, quantity, and biological characteristics of nitrofurantoin metabolites.

Residue data
Information on nitrofurantoin residues is very limited. In common with other nitrofurans, it is well absorbed from the gut and is almost certainly extensively metabolized, although information on the metabolites and total residues is not available because a radiometric study has not yet been done. Furthermore, there is no information on the amounts of bound and extractable residues. There is some evidence that the 5-nitro group is reduced to the amine by a xanthine oxidoreductase system.

In a recent study, residues of the parent drug were measured in tissues of chickens at zero withdrawal time after administration of a commercial dose of nitrofurantoin at 55 mg/kg in the feed for 42 days. The levels were highest in liver (113 μg/kg) and lowest in muscle tissue (0.7–9 μg/kg). At 2
days withdrawal time, residues of parent drug were not detectable (<1 µg/kg). In an older study on a group of 16 chickens given nitrofural at 150 mg/kg of feed for 14 days, residues were found in only two birds: at the 2 µg/kg level in the kidneys of one chicken at 3 days withdrawal time and in another bird at 5 days withdrawal time. In a study in which four pigs were fed a commercial dose of nitrofural no residues were detected at the 0.1 mg/kg level at zero withdrawal time.

There are several well validated methods for measuring residues of the parent drug at the 1 or 2 µg/kg level.

The Committee did not recommend an MRL because:

- no ADI was established;
- the residue data available to the Committee were not sufficient for it to identify a marker residue; and
- no information was available on the quantity and nature of the total residues.

3.3 Production aids

3.3.1 Bovine somatotropins

Bovine somatotropins had not been previously reviewed by the Committee. They are used to increase milk production in lactating dairy cows. A combined data package was reviewed by the Committee in order to assess four analogues of natural bovine somatotropin (bST) that are produced by recombinant DNA techniques (rbSTs): somagrebove, sometribove, somavubove, and somidobove.

Toxicological data

Somatotropins and insulin-like growth factors (which mediate many of the physiological effects of somatotropins) are found in all mammalian species. Pharmacological studies on the four compounds show that their physiological effects are indistinguishable from those of naturally occurring bST in dairy cows. The somatotropins display species specificity and pituitary-derived bST is inactive even when administered parenterally to humans. However, rats display a physiological response to parenterally administered bST and rbSTs.

Somatotropins, including rbSTs, are degraded by enzymes of the gastrointestinal tract and should therefore be inactive when administered orally. Acute oral toxicity studies in rats with rbST doses up to 5 g per kg of body weight failed to show any biological or toxicological effect. No adverse biological effects were observed in rats in two 2-week oral feeding studies with doses of rbSTs up to 10 mg per kg of body weight per day, or in two 4-week oral feeding studies with doses up to 50 mg per kg of body weight per day. In two 90-day oral feeding studies in rats with rbSTs at doses up to 100 mg per kg of body weight per day, no drug-related changes were observed, and in a 90-day oral feeding study in dogs at doses up to 10 mg per kg of body weight per day, rbST treatment caused no adverse
toxicological effects. Two 9-day oral feeding studies in hypophysectomized rats with rbST doses up to 4 mg per kg of body weight per day demonstrated that ingested rbSTs are degraded and are not absorbed in a biologically active form. These studies confirm that rbSTs have no biological activity when administered orally.

The genotoxic potential of the rbSTs was evaluated in two assays, both of which were negative.

Many of the physiological effects of rbSTs are mediated by bovine insulin-like growth factor-I (IGF-I), which is structurally identical to human IGF-I. The liver is the major site of IGF-I synthesis, but it is also present in human milk, saliva, and pancreatic secretions. Two feeding studies, one in intact and one in hypophysectomized adult rats, confirmed that IGF-I at doses up to 2 mg per kg of body weight per day has no biological activity when administered orally. Because human and bovine IGF-I are structurally identical, the effect of dietary IGF-I on the gastrointestinal tract was evaluated in several studies. These showed that IGF-I is degraded by digestive enzymes and is not active in the upper gastrointestinal tract.

Residue data

Treatment of lactating dairy cows with rbSTs causes an increase in plasma somatotropin concentrations and milk production which is physiologically indistinguishable from the changes induced with pituitary-derived bST. The analytical methods used to determine the concentration of the hormone in plasma, milk, or tissues do not differentiate between rbST and endogenous bST. Figures for somatotropin concentrations therefore relate to total bovine somatotropins.

Milk residue studies demonstrate that, even at exaggerated doses, the proposed use of rbSTs will not lead to any detectable concentrations of somatotropins in milk greater than those normally present in milk from untreated cows (0.9–1.6 μg/l). In addition, the major metabolite identified in the serum is the same as the bST fragment cleaved by thrombin, between amino acids 132 and 133.

As far as tissue residue data are concerned, rbST treatment of cows leads, at most, to a twofold increase in somatotropin concentrations to levels of 4.2 μg/kg in muscle and 25 μg/kg in liver.

Some studies suggest that rbST treatment may produce a slight increase in average milk IGF-I concentration. However, the most definitive and comprehensive studies demonstrate that IGF-I concentrations are not altered after rbST treatment; the same applies to IGF-II concentrations in milk.

While rbSTs are denatured by pasteurization, IGF-I is not destroyed by it. However, the heating of milk for the production of infant formula reduces the amount of IGF-I by at least 50%. Human breast milk contains IGF-I concentrations similar to those found in milk from control and rbST-treated cows.
IGF-I concentrations in the biopsied muscle and liver of rbST-treated cows are increased at most twofold. However, the elevated IGF-I concentrations in muscle may be related to wound healing after biopsy rather than to rbST treatment.

The effects of rbSTs on the major components of milk, if any, are minor and mainly occur early in the treatment period before adjustments in dry matter intake by the cow. Furthermore, the composition of the milk from treated cows is well within the normal variation observed during the course of lactation. It appears, therefore, that rbST treatment does not significantly affect the nutritional and processing qualities of milk.

**Acceptable Daily Intake and Maximum Residue Limits**

The lack of oral activity of rbSTs and IGF-I and the low levels and non-toxic nature of the residues of these compounds, even at exaggerated doses, results in an extremely large margin of safety for humans consuming dairy products from rbST-treated cows. In view of the lack of impact on human food safety, the Committee established an ADI “not specified” for rbSTs which applied to somagrebove, sometribove, somavubove, and somidobove. MRLs “not specified” were established for these rbSTs in bovine milk and edible tissues (see section 2.5).

### 3.3.2 Ractopamine

Ractopamine, a phenylethanolamine β-adrenoceptor agonist, had not been previously reviewed by the Committee. The compound is used in pigs for the improvement of weight gain, carcass leanness, and feed efficiency.

**Toxicological data**

Results of various studies were reviewed by the Committee, including pharmacokinetic, biotransformation, acute and short-term toxicity, reproductive, teratogenicity, and genotoxicity studies and a limited number of studies in humans.

Studies with [14C]ractopamine in several species have indicated rapid absorption following oral administration. The drug is rapidly excreted, mostly via the urine (more than 80% of the dose within 24 hours). In pigs, labelled ractopamine was excreted almost quantitatively; approximately 88% was recovered in urine and 9% in faeces during a 7-day period. Studies in pigs, rats, and dogs fed [14C]ractopamine showed three major metabolites, identified as monoglucuronides of ractopamine.

In acute studies, ractopamine was substantially more toxic orally to the rat (LD$_{50}$ approximately 4500 mg per kg of body weight) than to the mouse (LD$_{50}$ approximately 3000 mg per kg of body weight).

The genotoxic potential of ractopamine was evaluated in a limited series of *in vitro* and *in vivo* studies, all of which were reported to be negative. However, in the absence of a carcinogenicity study, and as human exposure is likely to be extensive, the Committee concluded that additional genotoxicity testing would be desirable.
The short-term toxicity of ractopamine has been evaluated in mice, rats, dogs, and monkeys. Ractopamine was fed to B6C3F1 mice for 3 months at doses of 25, 175, or 1250 mg per kg of body weight per day. The most significant effect noted was a dose-dependent decrease, both absolute and relative, in testicular weights. In both males and females in the highest-dose group, absolute and relative heart weights were increased. However, no histopathological changes were observed in either the heart or the testes. A clear NOEL could not be established in this study.

Fischer 344 rats were fed doses up to approximately 155 mg per kg of body weight per day for 3 months. The highest of the three dose groups showed decreased body-weight gain, increased food consumption, decreased efficiency of food utilization, and an increase in serum potassium concentration. There was a decrease in uterine weight in rats of this group and a slight reduction in spleen weight in the top two groups. The NOEL was 1.3 mg per kg of body weight per day in this study.

Beagle dogs were given three doses daily, 6 hours apart, totalling 0.112, 0.224 or 5.68 mg per kg of body weight per day of ractopamine in gelatin capsules for 1 year. Treatment-related minor histopathological findings were limited to the high-dose group and to the liver. The occurrence of mild nocturnal bradycardia, most prominent during the first 6 months, meant that a clear no-effect level was not observed in this study. The Committee noted that the quantity of ractopamine residues consumed in 500 g of meat from animals slaughtered without a withdrawal period would closely approach an ADI derived from the lowest dose of 0.112 mg per kg of body weight per day and a safety factor of 100.

In a further study of cardiovascular effects, rhesus monkeys were given either vehicle or ractopamine at 0.125 mg per kg of body weight per day for 90 days by gavage. This dose was 2.5 times the single dose known to produce tachycardia and peripheral vasodilation in the dog. In addition, the selected dose exceeded the total daily dose (0.112 mg per kg of body weight per day) associated with nocturnal bradycardia in the 1-year dog study. The NOEL for this study was 0.125 mg per kg of body weight per day.

Rhesus monkeys were given ractopamine at doses of 0.25, 0.5, or 4 mg per kg of body weight per day once daily by gavage for 6 weeks to determine the doses to be used in a 1-year study. Monkeys given 4 mg per kg of body weight per day developed daily tachycardia, which was maximal by 30 minutes after dosing, and continued for 16 hours. Monkeys in the high-dose group did not demonstrate the significant slowing of the nocturnal heart rate seen in the other groups. The NOEL for this study was 0.5 mg per kg of body weight per day.

The effect of ractopamine on reproductive and developmental performance in Sprague-Dawley rats was evaluated at dosage levels of up to 2000 mg/kg in the diet. Significant effects, which included a reduction in mean litter size and an increase in the total number of resorptions, were restricted to the high dose, which was also maternally toxic. The NOEL
was 200 mg/kg, equal to 15 mg per kg of body weight per day in this study. The Committee considered the teratogenicity segment of this study to be adequate to assess developmental toxicity. A minor teratogenic response was observed only at the highest dose (2000 mg/kg) tested, at which maternal toxicity was also noted.

The bronchodilator and inotropic effects of ractopamine were evaluated in pilot clinical trials in humans. The four patients with chronic bronchial asthma who were studied showed little evidence of bronchodilator activity, central nervous system stimulation, or an increase in pulse rate. Two patients showed a mild elevation of blood pressure lasting for about 1 hour. An infusion study showed inotropic and chronotropic enhancement in both healthy volunteers and heart patients.

The Committee concluded that, on the basis of the short-term studies available, residues of ractopamine appeared to have little toxic potential for the consumer. The effects recorded were in the main those to be expected from a \( \beta \)-adrenoceptor agonist. It might therefore be appropriate to assess ractopamine on the basis of a NOEL for pharmacological effects that are relevant to its ingestion by humans as a residue in edible meats. However, because such a NOEL could not be determined in the 1-year study in dogs, the Committee was unable to establish an ADI.

The Committee noted that: (a) some \( \beta \)-adrenoceptor agonists were carcinogenic; (b) no long-term studies had been conducted in rodents; and (c) there were no data relating to the long-term exposure of humans to ractopamine. Therefore, before reviewing the compound again, the Committee would wish to see evidence and arguments in at least the following areas:

1. Genotoxicity:
   - a further in vivo study such as a micronucleus test.

2. Pharmacology:
   - investigations that fully explore the pharmacological properties of ractopamine;
   - the relative contribution of \( \beta_1 \) and \( \beta_2 \)-adrenoceptor activation to the spectrum of effects produced by ractopamine;
   - a sufficient basis from which to establish the most sensitive indicator (test and species) of the pharmacological effects of ractopamine;
   - validation of the utility of this indicator in the setting of a pharmacological NOEL for humans;
   - a survey of the pharmacokinetic parameters of \( \beta \)-adrenoceptor agonists in humans and laboratory species, including those relevant to oral administration;
   - determination of appropriate timing for observations in animal studies to reveal both the onset and the peak values of all relevant effects.

3. Human data:
   - a survey of all non-therapeutic effects that follow long-term \( \beta \)-adrenoceptor agonist use in humans, to assist in the prediction of
Table 17

<table>
<thead>
<tr>
<th>Residue</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pig(^a)</td>
<td>Dog(^b)</td>
</tr>
<tr>
<td>Parent drug</td>
<td>0.12</td>
<td>0.59</td>
</tr>
<tr>
<td>A</td>
<td>0.03</td>
<td>0.46</td>
</tr>
<tr>
<td>B</td>
<td>0.04</td>
<td>0.77</td>
</tr>
<tr>
<td>C</td>
<td>0.02</td>
<td>1.76</td>
</tr>
</tbody>
</table>

A, B, and C: ractopamine glucuronides.
\(^a\) Withdrawal time 12 hours.
\(^b\) Withdrawal time 6 hours.

The consequences of the long-term intake of residues of ractopamine by consumers of animal meat.

Depending on the results of the above investigations, it may be necessary to perform other studies to explore further the potential carcinogenicity of ractopamine.

**Residue data**

The metabolism of ractopamine is similar in rats, dogs, and pigs (see page 42). The major metabolites are the ractopamine glucuronides (see Table 17), which together with parent drug account for almost all of the extractable residues.

Most of the available residue-depletion studies are radiometric studies in pigs, in which the radiolabel (carbon-14) was positioned in one of the rings. In some studies, the radiolabelled drug was a mixture of the two ring-labelled forms. The position of the label had no effect on the results since in almost all the identified residues the parent drug molecule remained intact.

In five of the reported studies, the dose was 30 mg/kg in the feed. This is 1.5 times the highest anticipated dose level. In the sixth study, the dose was 20 mg/kg ractopamine in the feed for 7 days. The results of the five studies in which the dose was 30 mg/kg are summarized in Table 18 for various withdrawal times.

In the residue-depletion study in pigs in which the dose was 20 mg/kg in feed, the concentration of radiolabelled residue was determined in liver, kidney, muscle, and fat, and the parent drug (expressed as ractopamine hydrochloride equivalents) was determined in liver and kidney tissue. Muscle and fat did not contain detectable residues at any of the withdrawal times. The results are summarized in Table 19.
Table 18  
**Total residues in tissues of pigs given 
$[^{14}$C]ractopamine hydrochloride at 30 mg per kg of feed**

<table>
<thead>
<tr>
<th>Withdrawal time</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 hours$^a$</td>
<td>0.03</td>
<td>0.18</td>
<td>0.74</td>
<td>0.02</td>
</tr>
<tr>
<td>12 hours$^b$</td>
<td>0.02</td>
<td>0.42</td>
<td>0.60</td>
<td>0.02</td>
</tr>
<tr>
<td>2 days$^b$</td>
<td>0.00</td>
<td>0.10</td>
<td>0.06</td>
<td>0.00</td>
</tr>
<tr>
<td>3 days$^a$</td>
<td>0.01</td>
<td>0.09</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>4 days$^b$</td>
<td>0.00</td>
<td>0.05</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td>5 days$^a$</td>
<td>0.00</td>
<td>0.05</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>7 days$^b$</td>
<td>0.00</td>
<td>0.06</td>
<td>0.02</td>
<td>0.00</td>
</tr>
</tbody>
</table>

$^a$ Drug administered for 7 days; values are means (mg of ractopamine equivalents per kg) for two animals.

$^b$ Drug administered for 4 days; values are means (mg of ractopamine hydrochloride equivalents per kg) for three animals.

Table 19  
**Total residues and unchanged ractopamine (µg of ractopamine hydrochloride equivalents per kg) in tissues of pigs given $[^{14}$C]ractopamine hydrochloride at 20 mg per kg of feed for 7 days$^a$**

<table>
<thead>
<tr>
<th>Withdrawal time (days)</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total residues</td>
<td>Ractopamine residues</td>
</tr>
<tr>
<td>1</td>
<td>106</td>
<td>14.8</td>
</tr>
<tr>
<td>2</td>
<td>73</td>
<td>3.7</td>
</tr>
<tr>
<td>3</td>
<td>56</td>
<td>1.7</td>
</tr>
</tbody>
</table>

$^a$ For withdrawal times of 1, 2, and 3 days, values are means for six, six, and four animals, respectively.

Parent drug as a percentage of total residues in liver declined from 14% at 1 day to 3% at 3 days withdrawal time. Similarly, parent drug as a percentage of total residues in kidney declined from 28% at 1 day to 9% at 3 days.

Two studies were reported in which HPLC with electrochemical end-point detection was used to measure the residues of parent drug in pigs. However, this does not measure residues of the glucuronide metabolites, which constitute a major fraction of the total residue (see Table 17), since no hydrolysis step is incorporated into the assay. It was not possible to estimate the percentage of the total residues accounted for by the parent drug in these studies since the ratio of parent drug to glucuronides appears to be highly variable.
Table 20

Unchanged ractopamine hydrochloride (µg/kg) in tissues of pigs given ractopamine hydrochloride at 20 mg per kg of feed for 14 days *

<table>
<thead>
<tr>
<th>Withdrawal time (days)</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
<th>Skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>5.4</td>
<td>11.1</td>
<td>31.8</td>
<td>&lt; 2.0</td>
<td>7.5</td>
</tr>
<tr>
<td>1</td>
<td>1.9</td>
<td>5.8</td>
<td>12.7</td>
<td>&lt; 1.0</td>
<td>NM</td>
</tr>
<tr>
<td>2</td>
<td>NM</td>
<td>3.4</td>
<td>6.7</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>3</td>
<td>NM</td>
<td>1.7</td>
<td>3.0</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>4</td>
<td>NM</td>
<td>1.2</td>
<td>2.2</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>5</td>
<td>NM</td>
<td>ND</td>
<td>&lt; 1.0</td>
<td>NM</td>
<td>NM</td>
</tr>
</tbody>
</table>

NM: not measured; ND: not detected by HPLC.
* For each withdrawal time, values are means for eight animals.

Table 21

Unchanged ractopamine hydrochloride (µg/kg) in tissues of pigs given ractopamine at 20 mg per kg of feed for 9 days *

<table>
<thead>
<tr>
<th>Withdrawal time (days)</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.1</td>
<td>24.6</td>
</tr>
<tr>
<td>2</td>
<td>3.2</td>
<td>7.9</td>
</tr>
<tr>
<td>3</td>
<td>2.2</td>
<td>2.0</td>
</tr>
<tr>
<td>4</td>
<td>1.2</td>
<td>2.3</td>
</tr>
<tr>
<td>5</td>
<td>1.4</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* For each withdrawal time, values are means for six animals.

At 12 hours withdrawal time, the residues of parent drug in pigs given oral doses of 31 mg/kg in feed for 7 days are 5.2 times as high in liver and 3.7 times as high in kidney as those in pigs fed 20 mg/kg ractopamine hydrochloride. The results for pigs fed unlabelled ractopamine hydrochloride at 20 mg/kg in feed for 14 days are summarized in Table 20.

In the most recent study on ractopamine tissue-residue depletion in pigs, 30 cross-bred pigs were fed ractopamine at 20 mg/kg in the diet for 9 days. Tissues collected at each withdrawal time were subjected to quantitative analysis by HPLC with electrochemical detection. The results are summarized in Table 21.

In one of the radiometric studies, in which pigs were fed [14C]ractopamine at 30 mg/kg in the diet for 4 days, non-extractable residues were determined. The mean residue concentrations, calculated as ractopamine hydrochloride equivalents, remaining in the liver and kidney after extraction with perchloric acid (1 mol/l) and ethanol (1:1) are summarized in Table 22. Neither the nature of the non-extractable residues nor that of the bound residues and their bioavailability were investigated.
<table>
<thead>
<tr>
<th>Withdrawal time (days)</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.12 (29%)</td>
<td>0.08 (13%)</td>
</tr>
<tr>
<td>2</td>
<td>0.06 (60%)</td>
<td>NM</td>
</tr>
<tr>
<td>4</td>
<td>0.04 (80%)</td>
<td>NM</td>
</tr>
<tr>
<td>7</td>
<td>0.04 (67%)</td>
<td>NM</td>
</tr>
</tbody>
</table>

NM: not measured.

* Values in parentheses are the non-extractable residues as a percentage of total residues. For each withdrawal time, values are means for three animals.

**Methods of analysis for residues in tissues**

A well characterized HPLC method with electrochemical detection is available for the detection and quantification of ractopamine residues measured as parent drug in the edible tissues of pigs. The method measures parent drug residues as the sum of the four possible stereoisomers. The detection limit is 0.5 μg/kg and the quantification limit 5 μg/kg. The method does not measure the ractopamine glucuronide metabolites. The hydrolysis of glucuronide residues in kidney has been attempted but was only partially successful. In liver, the process requires extensive purification procedures that make it impractical.

**Appraisal**

Adequate information from six radiometric studies is available on the total residues of ractopamine in the edible tissues of pigs. The highest amounts are found in the liver and kidney. Total residue concentrations decline rapidly after drug withdrawal, the decline over 7 days being associated mainly with the extractable residues. The non-extractable residues appear to have a long half-life.

The drug is both rapidly absorbed from the gut and rapidly excreted, mainly via the urine. The parent drug and its major metabolites, the ractopamine glucuronides, account for almost all the extractable residues, the former constituting by itself a significant fraction of them.

The Committee concluded that the establishment of an MRL would have to be based on the total residues because there was no information on the nature of the bound residues. The HPLC assay for parent drug was well characterized but might not be appropriate for determining compliance with an MRL because, as previously mentioned, it does not measure the extractable glucuronides.

Taking into account the toxicological data and an appropriate safety factor, the Committee noted that safe levels of residues would probably be observed only after a withdrawal period of several days.
The Committee was not able to recommend an MRL because:
- no ADI could be determined; and
- sufficient information was not available for the Committee to establish
  a marker residue in animal tissues.

3.4 Trypanocide

3.4.1 Isometamidium

Isometamidium had been previously evaluated at the thirty-fourth meeting
of the Committee (Annex 1, reference 85). The compound has a long
history of use as a trypanocide in animals. At the thirty-fourth meeting, the
Committee had not been able to establish an ADI because the results of
adequate toxicity studies and information on absorption and the nature of
the metabolites were not available. Accordingly, in its thirty-fourth report,
the Committee suggested that certain additional studies should be
performed before it reviewed the compound again, although it recognized
that the data from these studies might still not permit a full evaluation.

The commercial preparations of isometamidium (Samorin®, Trypa-
midium®) used in the studies reviewed by the Committee contained four
isomers and one bis-species; the isometamidium content was 55-65% and
the product contained less than 1% homidium. Because of the nature of the
synthetic process, the manufacturer has not been able to prepare a product
of greater purity, but the composition is controlled within stated limits and
this stable mixture has been used in all studies.

Toxicological data

The Committee considered data from studies of the uptake of
radiolabelled isometamidium from lyophilized bovine tissues, short-term
and teratogenicity studies in rats, and a range of genotoxicity assays.

Isometamidium was not bioavailable in rats either after oral dosing or after
feeding animals bovine tissues containing residues of the drug (see section
on residue data).

Rats were given isometamidium by gavage at doses up to 200 mg per kg of
body weight per day for 21 days or intravenously at a single dose of 2 mg
per kg of body weight. The results showed that isometamidium was not
absorbed after oral administration and was rapidly cleared from the
plasma when given intravenously.

The oral LD50 of a combined group of male and female New Zealand white
rabbits was found to be 455 mg per kg of body weight, which was of the
same order of magnitude as the values previously reported for the rat. In a
study reviewed at the thirty-fourth meeting, deaths were reported in rabbits
at oral doses of 12.5 mg per kg of body weight per day and above. A
re-examination of this study revealed significant pre-existing lung and liver
lesions in the rabbits used, which contributed to the abnormally high
toxicity reported.

In a short-term study in rats, isometamidium was administered at doses up
to 1000 mg per kg of body weight per day by gavage for 13 weeks.
Immediately after treatment all animals showed salivation and respiratory distress. There were deaths in the highest-dose group, with diarrhoea and emaciation, which resulted in discontinuation of this dose. Apart from the acute post-dosing clinical signs, which were of pharmacological origin, and caecal distension at necropsy, no other treatment-related effects were seen at 50 mg per kg of body weight per day, which could be regarded as a non-toxic dose level.

A teratogenicity study was carried out in rats in which isometamidium was administered by gastric intubation at doses up to 540 mg per kg of body weight per day. The maternal toxicity and fetotoxicity seen at the highest dose affected pup survival, but neither effects on fertility nor developmental abnormalities were seen. The NOEL was 60 mg per kg of body weight per day. Data from a rat teratogenicity study reviewed at the thirty-fourth meeting indicated that intravenous administration of 2 mg per kg of body weight per day produced vertebral abnormalities. A reassessment of these data, including an examination of historical control data and a reappraisal of the statistics, suggested that the effects were not significant.

The genotoxic potential of isometamidium was investigated in a range of studies. It induced frame-shift mutations in Salmonella typhimurium in the presence of metabolic activation, but there was no evidence of genotoxicity in any in vitro mammalian test systems. When given by intraperitoneal injection, isometamidium increased the frequency of numerical chromosomal aberrations in rat bone marrow in a cytogenetic assay, but oral administration produced no genotoxic effects on the cells of the rat gastrointestinal tract. All other in vivo genotoxicity tests were negative. The Committee concluded that isometamidium was not genotoxic when administered by the oral route.

An ADI of 0-100 μg per kg of body weight was established for isometamidium based on the non-toxic dose level of 50 mg per kg of body weight per day in the 13-week rat study and a safety factor of 500. The Committee chose this safety factor because of the marginal pharmacological effects seen at the lowest dose in the rat study and the limited extent of the data available, although it recognized that neither the drug nor its metabolites were bioavailable when given by the oral route.

Residue data
New residue data for isometamidium were evaluated by the Committee. In a metabolism study in lactating cows injected with 14C isometamidium, it was shown qualitatively that the compounds found in plasma, urine, liver, and kidney and at the injection site were the same as those present in the parent drug, but no homidium was detected. Metabolic profiles in milk and muscle could not be determined because of the low levels of radioactivity.

1 Radiolabelled isometamidium consists of parent isometamidium (60%) plus several radiolabelled isomers and a bis-species.
Table 23

Total residues (mg of isometamidium equivalents per kg) and unchanged isometamidium (mg/kg) in tissues of dairy cows given a single intramuscular injection of [14C]isometamidium at 1 mg per kg of body weight

<table>
<thead>
<tr>
<th>Withdrawal time (days)</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
<th>Injection site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>3</td>
<td>0.013</td>
<td>4.72</td>
<td>1.22</td>
<td>3.38</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.017</td>
<td>422</td>
</tr>
<tr>
<td>10</td>
<td>0.017</td>
<td>2.53</td>
<td>0.30</td>
<td>2.53</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.007</td>
<td>233</td>
</tr>
<tr>
<td>30</td>
<td>0.012</td>
<td>2.28</td>
<td>0.23</td>
<td>2.02</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.011</td>
<td>96</td>
</tr>
</tbody>
</table>

TR: total residues; I: isometamidium; NM: not measured.

* For each withdrawal time, values represent single animals.

* Unchanged isometamidium was not determined in these tissues.

Isometamidium was the main component found in kidney and at the injection site, but not in liver; at 30 days withdrawal time, it accounted for about 10% and 12% of the total residue in liver and kidney, respectively (Table 23).

The bioavailability of isometamidium residues was measured by feeding lyophilized calf tissues containing [14C]isometamidium to rats and measuring the amount of radioactivity absorbed and excreted by them. There were no detectable residues in the rats’ urine, serum, blood, kidney, liver, spleen, muscle, stomach, or small intestine. Cumulative excretion of radioactivity in rat faeces was approximately 90% after oral dosing with calf tissues containing isometamidium residues and 93% after oral dosing of the drug in an aqueous solution. The results indicate that isometamidium residues in tissues are not bioavailable to any significant extent. The poor bioavailability of the residues may be due to the cationic nature and high affinity of the drug for macromolecules.

In a study of residues in young bulls given an intramuscular injection of isometamidium at 1 mg per kg of body weight, plasma levels dropped to below the quantification limit by 72 hours after treatment. Tissue samples were collected 1, 3, and 6 months after treatment and analysed for parent isometamidium by HPLC (see Table 24). Isometamidium levels in muscle and fat were below the quantification limit at all three withdrawal times. At 3 months after treatment, isometamidium was detected in only one out of five livers, while at 6 months after treatment, no isometamidium was detected in any of them. By 3 months, the isometamidium concentration in kidney had dropped below the quantification limit. At 6 months, two out of five injection sites had isometamidium levels below the quantification limit.

In lactating cows given a single intramuscular injection of [14C]isometamidium at 1 mg per kg of body weight, total residue levels in milk peaked on day 2 at 6.8 μg/l and remained below 3 μg/l after day 7.

51
Table 24
Unchanged isometamidium (mg/kg) in tissues of young bulls given a single intramuscular injection of 1 mg per kg of body weight*

<table>
<thead>
<tr>
<th>Withdrawal time (months)</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
<th>Injection site</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BQ</td>
<td>0.25</td>
<td>0.39</td>
<td>BQ</td>
<td>129.5</td>
</tr>
<tr>
<td>3</td>
<td>BQ</td>
<td>BQ</td>
<td>BQ</td>
<td>BQ</td>
<td>38.7</td>
</tr>
<tr>
<td>6</td>
<td>BQ</td>
<td>BQ</td>
<td>BQ</td>
<td>BQ</td>
<td>1.35</td>
</tr>
</tbody>
</table>

BQ: below the quantification limit (0.1 mg/kg).
* Animals in which isometamidium levels were below the quantification limit were excluded from the calculations; otherwise, for each withdrawal time, values are means for five animals.

Methods of analysis for residues in tissues and milk
An improved HPLC method for measuring isometamidium residues in milk and tissue has been developed. The manufacturer has validated the method to 0.1 mg/kg, and the percentage recoveries, linearity, intraday precision and accuracy, and quantification limit reported are acceptable.

Maximum Residue Limits
Based on the ADI of 0–100 µg/kg of body weight established by the Committee at its present meeting, the permitted daily intake of isometamidium would be 6 mg of total drug-related residue contributed by 500 g of food-animal meat together with 1.51 of milk in the diet of a 60-kg person. From the study in bulls given isometamidium intramuscularly at 1 mg per kg of body weight, the Committee concluded that, at 30 days withdrawal time, the intake of residues of isometamidium would be well below the ADI. It therefore recommended an MRL of 0.1 mg/kg for parent isometamidium in muscle and fat, 0.5 mg/kg in liver, 1.0 mg/kg in kidney, and 0.1 mg/l in milk (see Table 25).

Concentrations of total residues at the injection site at 30 days withdrawal time averaged 96 mg/kg in the study in dairy cows; however, the Committee concluded that these residues would not adversely affect human food safety for the following reasons:
- isometamidium residues in tissues are not bioavailable to any significant extent;
- consumption of an injection site would be extremely rare; and
- the maximum theoretical intake of residues from muscle, liver, kidney, fat, and milk of isometamidium at 30 days withdrawal time is well below the ADI.
Table 25
Recommended MRLs for isometamidium in cattle

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Observed residue&lt;sup&gt;a&lt;/sup&gt; (mg/kg parent drug)</th>
<th>Estimated daily intake&lt;sup&gt;b,c&lt;/sup&gt; (mg isometamidium equivalents)</th>
<th>Recommended MRL (µg/kg parent drug)</th>
<th>Theoretical maximum daily intake&lt;sup&gt;d&lt;/sup&gt; (mg isometamidium equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>&lt; 0.1</td>
<td>0.03</td>
<td>100</td>
<td>0.03</td>
</tr>
<tr>
<td>Liver</td>
<td>0.25</td>
<td>0.25</td>
<td>500</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>(2.5)&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td>(5000)&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.39</td>
<td>0.16</td>
<td>1000</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>(3.25)&lt;sup&gt;g&lt;/sup&gt;</td>
<td></td>
<td>(8333)&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>&lt; 0.1</td>
<td>0.01</td>
<td>100</td>
<td>0.01</td>
</tr>
<tr>
<td>Milk</td>
<td>0.0068&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.01</td>
<td>100&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.15</td>
</tr>
<tr>
<td>Total</td>
<td>0.46</td>
<td></td>
<td></td>
<td>1.11</td>
</tr>
</tbody>
</table>

<sup>a</sup> Intramuscular dose of 1 mg/kg of body weight, 30 days withdrawal time.
<sup>b</sup> Calculated from the observed residue levels.
<sup>c</sup> Based on a daily intake of 0.5 kg of meat, made up of 0.3 kg of muscle, 0.1 kg of liver, 0.05 kg of kidney, and 0.05 kg of fat, and 1.5 l of milk.
<sup>d</sup> Estimate of total residues; isometamidium accounted for 10% of the total residues in liver.
<sup>e</sup> Estimate of total residues; isometamidium accounted for 12% of the total residues in kidney.
<sup>f</sup> This value represents the highest concentration of total isometamidium residues found in milk. It occurs 2 days after dosing.
<sup>g</sup> The MRL for milk is based on the quantification limit of the analytical method.
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.

**Acknowledgements**

The Expert Committee wished to acknowledge the valuable contributions made to its work by: Mr D. Byron, Food Standards Officer, Food Quality and Standards Service, Food Policy and Nutrition Division, FAO, Rome Italy; and Dr H. Galal-Gorchev, Scientist, International Programme on Chemical Safety, WHO, Geneva, Switzerland.

**References**


Annex 1

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


13. Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances (Tenth


63. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 18, 1983.
64. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 28, 1983.
Annex 2

Recommendations on compounds on the agenda

<table>
<thead>
<tr>
<th>Substance</th>
<th>Acceptable Daily Intake (ADI) and other toxicological recommendations</th>
<th>Recommended Maximum Residue Limit (MRL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthelmintic agents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Closantel</td>
<td>Not evaluated&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Muscle and liver (sheep): 1500 µg/kg&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kidney (sheep): 5000 µg/kg&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td>Fat (sheep): 2000 µg/kg&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td>Muscle and liver (cattle): 1000 µg/kg&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kidney and fat (cattle): 3000 µg/kg&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flubendazole</td>
<td>0–12 µg per kg of body weight</td>
<td>Muscle and liver (pigs): 10 µg/kg&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Muscle (poultry): 200 µg/kg&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td>Liver (poultry): 500 µg/kg&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td>Eggs: 400 µg/kg&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ivermectin</td>
<td>0–1 µg per kg of body weight</td>
<td>Liver (cattle): 100 µg/kg&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fat (cattle): 40 µg/kg&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Thiabendazole (thiabendazole)</td>
<td>0–100 µg per kg of body weight</td>
<td>Edible tissues&lt;sup&gt;d&lt;/sup&gt; (cattle, pigs, goats, and sheep) and milk (cattle and goats): 100 µg/kg&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triclabendazole</td>
<td>0–3 µg per kg of body weight</td>
<td>Muscle (cattle): 200 µg/kg&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td>Liver and kidney (cattle): 500 µg/kg&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td>Fat (cattle): 100 µg/kg&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Edible tissues&lt;sup&gt;d&lt;/sup&gt; (sheep): 100 µg/kg&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Antimicrobial agents</strong></td>
<td></td>
<td></td>
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<tr>
<td>Furazolidone</td>
<td>Not allocated&lt;sup&gt;g&lt;/sup&gt;</td>
<td>No MRLs allocated&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nitrofurall (nitrofurazone)</td>
<td>Not allocated&lt;sup&gt;g&lt;/sup&gt;</td>
<td>No MRLs allocated&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Not evaluated
<sup>b</sup> Maximum tolerable daily intake in the diet
<sup>c</sup> Tolerable daily intake
<sup>d</sup> Edible organs (muscle, fat, and liver)
<sup>e</sup> Edible organs (muscle, fat, liver, and kidney)
<sup>f</sup> Edible organs (muscle, fat, and liver)
<sup>g</sup> For the purpose of this report, furazolidone is considered to be an antimicrobial agent.
<sup>h</sup> No MRLs have been allocated for furazolidone in the EU.
<table>
<thead>
<tr>
<th>Substance</th>
<th>Acceptable Daily Intake (ADI) and other toxicological recommendations</th>
<th>Recommended Maximum Residue Limit (MRL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Production aids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine somatotropins</td>
<td>Not specified(^c)</td>
<td>Milk and edible tissues(^d) (cattle): Not specified(^h)</td>
</tr>
<tr>
<td>Ractopamine</td>
<td>Not allocated(^i)</td>
<td>No MRLs allocated(^m)</td>
</tr>
<tr>
<td><strong>Trypanocide</strong></td>
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</tr>
</tbody>
</table>
| Isometamidium     | 0–100 µg per kg of body weight                                        | Muscle, fat, and milk (cattle): 100 µg/kg\(^b\)  
                        |                                                                       | Liver (cattle): 500 µg/kg\(^b\)       |
                        |                                                                       | Kidney (cattle): 1000 µg/kg\(^b\)       |

Notes to Annex 2

\(^a\) An ADI of 0–30µg per kg of body weight was established at the thirty-sixth meeting of the Committee (WHO Technical Report Series, No. 739, 1990).
\(^b\) Expressed as parent drug.
\(^c\) Expressed as 22,23-dihydroavermectin B\(_1\) (H\(_2\)B\(_1\)).
\(^d\) Edible tissues are defined as muscle, fat, liver, and kidney.
\(^e\) Expressed as the sum of tiabendazole and 5-hydroxytiabendazole.
\(^f\) Expressed as 5-chloro-6-(2',3'-dichlorophenoxyl)-benzimidazole-2-one.
\(^g\) An ADI could not be established because of evidence of genotoxicity and carcinogenicity and lack of information on the nature of the metabolites.
\(^h\) MRLs were not allocated because:
- no ADI was established;
- data on residues were not sufficient for the Committee to identify a marker residue; and
- insufficient information was available on the quantity and nature of the total residues.
\(^i\) An ADI could not be established because no-effect levels were not observed for the tumorigenic effects.
\(^j\) The ADI applies to somagroine, sometribine, somavubine, and somidobine. 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 has 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.
\(^k\) The MRLs apply to somagroine, sometribine, somavubine, and somidobine. MRL "not specified" means that available data on the identity and concentration of residues of the veterinary drug in animal tissues 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 has 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.
\(^l\) An ADI could not be established because a clear no-effect level was not observed for pharmacological effects and the issue of carcinogenicity has not been resolved.
\(^m\) MRLs were not allocated because:
- an ADI was not established; and
- sufficient information was not available for the Committee to establish a marker residue in animal tissues.